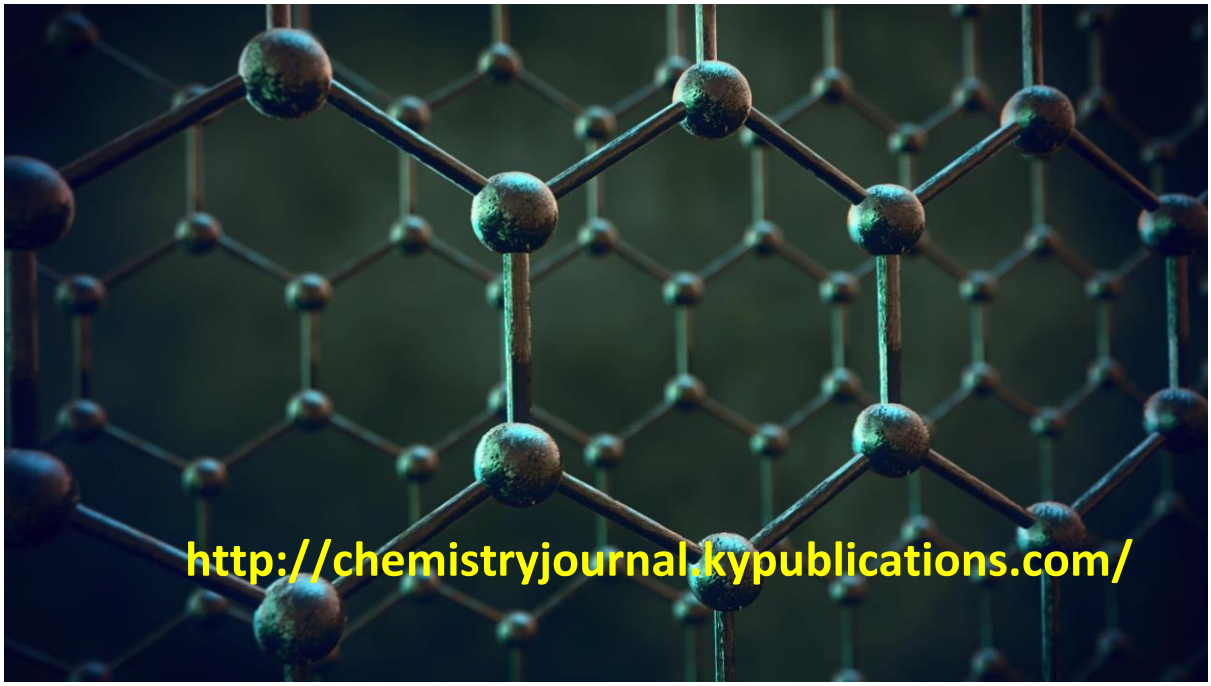


# International Journal of Chemistry and Aquatic Sciences (IJCA)

http://www.chemistryjournal.kypublications.com/

e-ISSN: 2455-040X

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**International Journal of Chemistry and Aquatic Sciences(IJCA)**
**Volume: 2, Issue 4, 2016; Page 1-9**
**e-ISSN: 2455-040X**
**Research Article**

# Assessment of water, sediment pollution on environment and aquatic biota in mangrove areas of Krishna river basin-A Study

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**ABSTRACT**
The long-term effects of sediment exposure on aquatic organisms are poorly understood, yet it is critical for determining threshold effects and exposure limits to mitigate potential impacts with regard to population dynamics. Mangrove ecosystem is a unique coastal wetland habitat formed at the transition zone of marine and freshwater ecosystem and also having close interaction with adjacent ecosystems. In recent years Intern mangrove swamp of river Krishna estuarine region is subjected to severe degradation owing to human intervention such as rapid development in aquaculture activities, cutting of mangrove trees for timber/coal, lesser inflow of freshwater , hyper salinity, upland industrial pollutants and development of coastal corridor. The causative aspects for decline of the mangrove swamp ecosystem and necessary steps for conservation are discussed. Heavy metals analysis was performed by Atomic Absorption Spectrophotometer. The results showed that variable levels of some of the metals in the sediments and crab samples. Some of the metals were higher than others, in some stations and on the different body components. Differences in heavy metal among waters could have resulted from diverse feeding habitats and dissimilar foraging stations.

**Study Area**

The mangroves of Krishna Basin in Andhra Pradesh are located in the coastal plains of Krishna delta. According to the Forest Department, the total area under mangroves is 5,000 ha. The Krishna mangroves lie between 15°2' N and 15° 55' N in latitude and 80° 42'- 81° 01' E [Figure 1]. in longitude spread across Krishna and Guntur districts. The Krishna wildlife sanctuary has been established in a part of the mangrove wetland - the total area of this sanctuary is 19,481 ha (194.81) sq.km.; it includes ***Sorlagondi Reserve Forest (RF), Nachugunta RF, Yelichetladibba RF, Kottapalem, RF, Molagunta RF, Adavuladivi RF and Lankivanidibba RF.*** They occupy the islands of the delta and the adjacent mainlands of both districts. A part of the mangroves is located far from the main mangrove area; it's near Machilipatnam on its eastern side and Nakshatranagar on its western side. Fishermen in surrounding areas use the mangrove resources for fishing, house construction and firewood and to obtain fencing material for their houses. A devastating cyclone that hit the Machilipatnam coast during 1977 led to large areas of the forest getting degraded. The important soil types found in the basin are black soils, red soils, laterite and lateritic soils, alluvium, mixed soils, red and black soils and saline and alkaline soils.

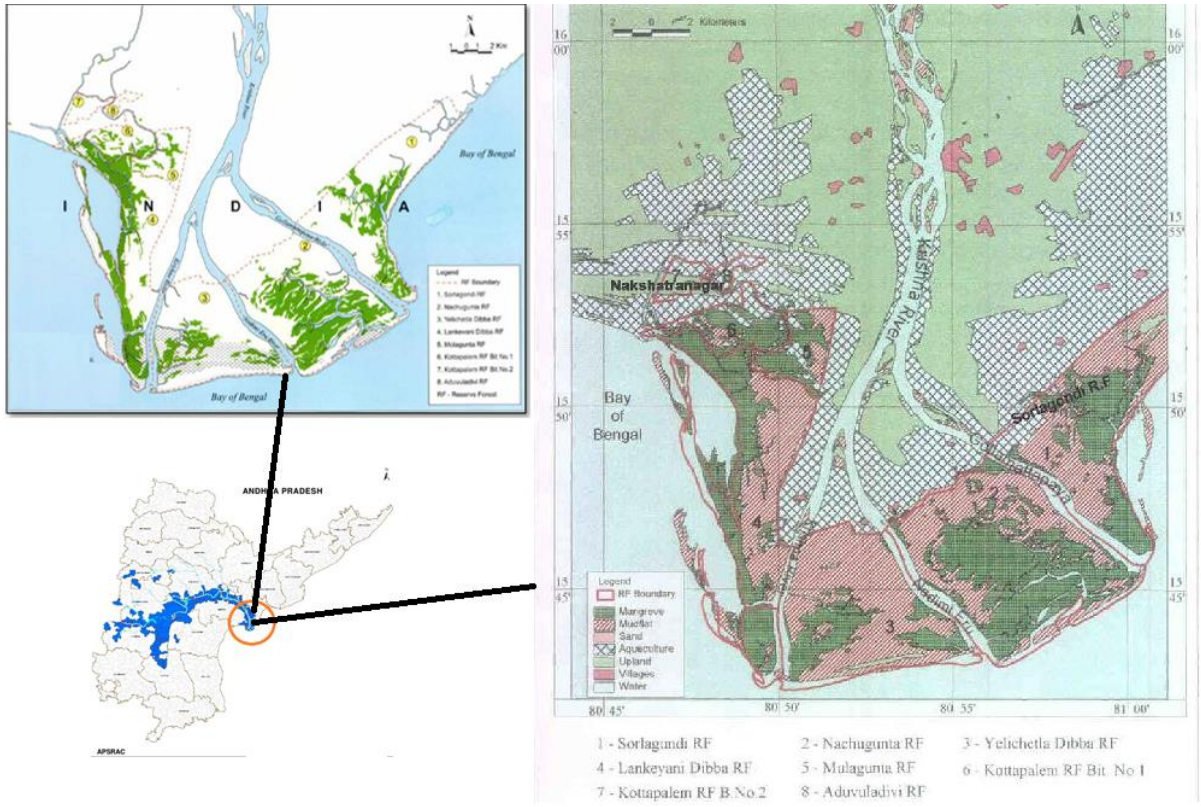



Figure-1: Mangrove forest area in Krishna River A.P., India

Sediment Areas

**Sampling**

For each sampling point, one subsurface liquid sample (depth about 5 to 10cm, 150ml) was collected by immersing a pre-cleaned polyethylene bottle at arm's length from the water's edge. One millilitre of 10% nitric acid was added to each liquid sample within 5 days. This was done to adhere to accepted protocols for the preservation of trace elements in water samples by lowering sample pH to two pH units and below. (U.S. Environmental Protection Agency, 1979). This provided the benefits of minimizing precipitation of heavy metal cations and adsorption of trace elements onto the container wall of the samples. A flaw with this procedure is that the added acid may solubilise certain elements found in the container, as it has been for glass ampoules . Liquid samples were filtered with a 0.45 micron syringe filter to remove suspended particulate, with 10 to 15 of the filtrate collected in centrifuge tubes.

At each sampling point, two sediment samples, 30 cm and 4 m from the water's edge, were collected using a pre-cleaned hand auger. Sediments were taken from a soil depth of 30 cm, as the inability to perform detailed soil analysis and the lack of a standard protocol led us to choose the depth where

roots of mangrove trees and many essential bacteria were situated. Extracted sediment was mixed in a bucket, ignoring sediment characteristic. For each bucket of mixed sediment, 3 hand-scoops of the sediment were stored in clean zip lock bags, compressed to limit the gaseous exchange of samples. Sediment samples were air-dried, aggregates and lumps were broken up and samples were sieved to obtain true soil component. 10 to 15 ml of each remaining sample was collected in centrifuge tubes. Soil samples were treated with hot-plate digestion, before both soil and liquid samples were analysed with ICP-MS.

Mangrove samples: matured Leaves of *Jatropha glandluifera, Crotalaria verrucosa*, *Acacia nilotica, Pithecellobium dulce, Azadirachta indica, Ficus religlosa and Ficus bengalensis* plants were collected in the October 2014 & January 2015 and washed thoroughly, shade-dried and powdered. Samples were digested for heavy metal analysis with a 90$^{o}$C mixture of concentrated nitric acid and hydrogen peroxide, after the method by MacFarlane et al. (2003)[1], and made to 25 ml volume. Digested samples were stored in labelled, acid-washed glass vials. Metal analysis was carried out immediately on ICP-MS with standard procedure.

Sample collection by the Principal Investigator (Right side Standing)

**Table 1a: Sampling Stations with Geographical boundaries**

| Station NO | Reserve forest | Longitude | Latitude |
|---|---|---|---|
| 1. | Sorlagondi RF | 15° 51' 50.3604'' N | 80° 58' 1.4700'' E |
| 2. | Nachugunta RF, | 15° 47' 16.8324'' N | 80° 53' 17.8008'' E |
| 3. | Yelichetladibba RF, | 15° 46' 14.3245'' N | 80° 57' 16.2654'' E |
| 4. | Kottapalem RF, | 15° 46' 37.3008'' N | 80° 51' 40.2588'' E |
| 5. | Molagunta RF | 15° 53' 14.0712'' N | 80° 50' 14.7588'' E |
| 6. | Adavuladivi RF | 15° 54' 55.2384'' N | 80° 46' 19.2504'' E |
| 7. | Lankivanidibba RF | 15° 53' 14.3245'' N | 80° 49' 40.2588'' E |

**Collection of fish sample**

Two different species of fish namely: *Katla Katla* is a commonly seen in the reserve forest waters was collected at Nachugunta RF. The fish samples were put into a pre-cleaned polythene bag and placed inside oven for drying in readiness for digestion process. The water sample collected at two different points were also placed in pre-cleaned container and preserved by adding 5 ml of nitric acid ($HNO_3$) to it thus, preventing microbial growth in the water sample. The sediment samples were collected

from two different point and stored in a pre-cleaned polythene bag and later spread on a flat tray inside the laboratory to be air dried for three days at room temperature (25°C) after which it was been grinded and sieved for further digestion process.

**Digestion of fish samples**

The fish samples were wash with distilled water and dried for 24 hours to constant weight in an oven at 105°C. After drying the fish sample in the oven, the bones and scales of the dried fish samples were removed and remaining only the muscle, head, tail, eyes, gills of the fish sample. The remaining parts of the fish sample were milled with a mortar and pestle. They were put in a dry labeled crucible and stored until digestion. This involves digesting 2 g of the grounded samples with 5 ml of $HNO_3$ and 2 ml of $HCLO_4$ and was heated on a hot plate for 30 minutes at 85°C. After completing the digestion, the residue were allowed to cool and filtered into a 50 ml volumetric flask. Distilled water was added to it to fill up to the mark. The filtrate was transferred into a pre-cleaned sample bottle and stored under cool temperature until it is taken for further Atomic Adsorption Spectrophotometer (AAS) analysis.

**Metal determination procedure using AAS**

A black model 200A flame Atomic Absorption Spectrometer was used in the metal analysis of the sample. The major underlined principle of AAS is that the ground state atoms are capable of absorbing radiant energy of their own specific resonance wavelength when passed through a solution containing the atoms in question, then part of the light will be absorbed. The extent of absorption is proportional to the number of ground state atoms present in the flame.

**Results and Discussion**

**Concentration of heavy metals in fish parts: *Katla Katla***

Concentration of heavy metals in different parts of *Katla Katla* adult fish from Krishna Reserve forest area (Nachugunta RF) is shown in Table 1 below. Concentrations of Iron (Fe), Copper (Cu), Zinc (Zn), Lead (Pb), Cadmium (Cd), Chromium (Cr) and Manganese (Mn) in the Muscle, head, eye, tail and gills of the fish samples were determined. Although there was no significant difference in metal concentration in each part of the fish for each metal but the results showed higher levels of Zn (13.08 ± 0.45 mg/g) and Fe (2.10 ± 0.56) in comparison with other heavy metals examined. Cadmium and Copper were not detected in some parts of the fish, indicating their low concentrations in the fish. Several studies have indicated that metal accumulation in fish depends on numerous factors such as food habits of the fish[2], tropic status, source of a particular metal, distance of the fish from the contamination source and the presence of other ions in the environment [3]. Also, reported that metal accumulation in the tissues of fish varied according to the rates of uptake, storage and elimination. The high levels of iron and zinc in this study could be attributed to their high demand as essential elements in blood haemoglobin and as a dietary essential trace metals [4]. Chromium has exceeded the toxicity threshold in the muscle of the fish in line with W.H.O standards in food.

**Table 1:** Heavy Metals concentrations (mg/g) in Fish Parts (*Katla Katla*) with the W.H.O Allowable Limits in food.

| Sample | Cd | Cr | Zn | Cu | Pb | Fe | Mn |
|---|---|---|---|---|---|---|---|
| Head | 0.01 ± 0.00 | 0.08 ± 0.02 | 13.08 ± 0.45 | ND | 0.30 ± 0.04 | 2.30 ± 0.56 | 0.10 ± 0.01 |
| Muscles | 0.01 ± 0.00 | 0.20 ± 0.05 | 11.65 ± 0.40 | 0.03 ± 0.00 | 0.10 ± 0.05 | 1.90 ± 0.45 | ND |
| Eye | ND | 0.14 ± 0.05 | 12.19 ± 0.42 | ND | 0.10 ± 0.05 | 0.90 ± 0.50 | ND |
| Tail | 0.07 ± 0.02 | 0.11 ± 0.03 | 12.22 ± 0.40 | ND | 0.10 ± 0.03 | 1.08 ± 0.46 | 0.13 ± 0.02 |
| Gills | 0.01 ± 0.00 | 0.12 ± 0.02 | 12.78 ± 0.41 | ND | 0.10 ± 0.05 | 2.10 ± 0.44 | 0.13 ± 0.01 |
| W.H.O | 2.00 | 0.05 0.15 | 10 -75 | 1-3 | 0.1 -0.2 | 1-3 | - |

ND- Not Detected

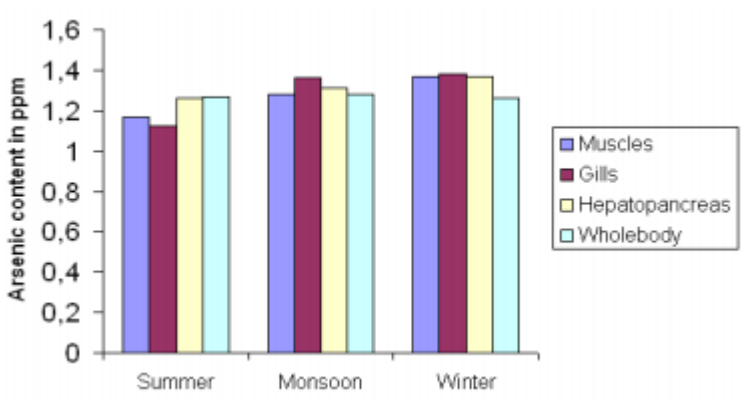
**Impact assessment in Crabs from Krishna Reserve forest**

Healthy uniform size adult crabs, *Barytelphusa guerini* were collected from the **Krishna Reserve forest near** Kottapalem RF . The crabs were collected every season for a period of one year from February-2014 to January-2016.The crabs were brought to the laboratory and placed in fresh river water for few hours to remove mud. *Hepatopancreas, Gills* and Muscle were dissected out from these crabs. These tissues separated from crabs were taken into petridishes and kept in a hot air oven maintained at 60$^{o}$C for a period of 48 –72 hours. The heavy metals concentrations were analyzed from these tissues and whole body. Heavy metal like Arsenic and Chromium were estimated by using standard methods as described by APHA (1998)[5].

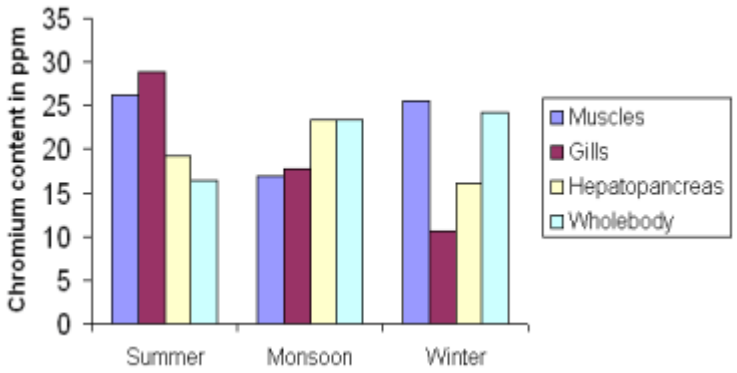
Digestion of Crab Tissues The samples were digested in open beakers on a hot plate. 0. 5 gm of each organ was weighed out in an open beaker and allowed to digest by adding nitric acid and perchloric acid in (4:1) ratio. Kept on hot plate and the temperature gradually allowed to rise to 60o C continue adding both acids in (4:1) ratio till the sample become colourless. The digested sample were allowed to cool and transferred to 25 ml volumetric flasks and made up to mark with deionised water. The digests were kept in plastic bottles and later the heavy metal concentrations were determined using an atomic absorption spectrophotometer (AAS). The actual concentration of each metal was calculated using the formula

$$\text{Actual concentration of metal} = \frac{\text{Reading of digest}}{\text{Weight of sample digested}} \times \text{Dilution factor}$$

The estimation of heavy metal in different tissue like muscle, gills, hepatopancreas and whole body of crab, *Barytelphusa guerini* was carried out different seasons summer, monsoon and winter in a year. The samples were analysed and contents of heavy metals were detected in order to know the concentration of these metals in crab tissues. The results are given in Table 1& 2 and illustrated in Figure (1 and 2). In the present investigation it was found that Arsenic (As) in muscle it varied from 1.37 ppm in winter to 1.17 ppm in summer. In gills it varied from 1.38 ppm in winter to 1.12 ppm in summer. In hepatopancreas it varied from 1.37 ppm in winter to 1.31 ppm in monsoon. In whole body it varied from 1.28 ppm in monsoon to 1.26 ppm in winter. From all the tissue, maximum concentration i.e. 1.38 ppm of arsenic was recorded in gills in winter. However minimum concentration was observed i.e. 1.12 ppm in gills in summer. In all the tissues arsenic content in crab sample from Krishna Reserve Forest exceeded from Indian standard limit of 1.1 mg/kg in food Awashthi (2000)[6]. Arsenic biogeochemical cycle occurs mostly in the aquatic environment and its bioaccumulation is an important ecotoxicological aspects. Inorganic arsenic produce acute, sub acute and chronic toxic effects, which may be either local or system.




Frequency histogram showing arsenic content (ppm) in different tissue of *Barytelphusa guerini*




Frequency histogram showing chromium content (ppm) in different tissue of *Barytelphusa guerini*

**Table 1**

Seasonal variation in average arsenic content (ppm) in different tissues of *barytelphusa guerini*

| Seasons | Muscles | Gills | Hepatopancreas | Whole body |
|---|---|---|---|---|
| Summer | 1.17 | 1.12 | 1.26 | 1.27 |
| Monsoon | 1.28 | 1.36 | 1.31 | 1.28 |
| Winter | 1.37 | 1.38 | 1.37 | 1.26 |

**Table 2**

Seasonal variation in average chromium content (ppm) in different tissues of *barytelphusa guerini*

| Seasons | Muscles | Gills | Hepatopancreas | Whole body |
|---|---|---|---|---|
| Summer | 26.25 | 28.80 | 19.25 | 16.50 |
| Monsoon | 17.00 | 17.83 | 23.33 | 23.33 |
| Winter | 25.50 | 10.62 | 16.12 | 24.20 |

Acute toxic effects include abdominal cramping, hypersthesia in extremities, abdominal patellar reflexes and abdominal electrocardiograms. Other effects of arsenic include peripheral vascular disturbances resulting in gangrene and a disease termed Black foots disease Rajesh and Agrawal (2005)[7]. In the present study it was found that Chromium (Cr) in muscle it varied from 26.25 ppm in summer to 17.00 ppm in monsoon. In gills it varied from 28.75 ppm in summer to 10.62 ppm in winter. In hepatopancreas it varied from 23.33 ppm in monsoon to 16.12 ppm in winter. In whole body it varied from 24.20 ppm in winter to 16.50 ppm in summer. From all the tissue, maximum concentration i.e. 28.75ppm of chromium was recorded in gills in summer. However minimum concentration was observed i.e. 10.62 ppm in gills in winter. Chromium (Cr) level in muscle and gills in summer seasons, hepatopancreas and whole body in monsoon and muscle and whole body in winter season were higher than the recommended maximum allowable standards in food. The level of Cr in muscle (monsoon), gills (monsoon and winter), hepatopancreas (summer and winter) and whole body (summer) were lower than the Indian standard limit of 20.00 mg/kg.

Obasohan et al. (2006)[8] studied the heavy metals concentrations in two tropical fish species, Malapterurus electricus and Chrysichthus nigrodigitarus from Ogba river in Benin city, Nigeria. It was found that the level chromium in both fishes were higher than the world health origination (WHO) recommended maximum allowable standards in food fish. The harmful effects of chromium to human are mostly associated with its hexavalent form. Chromium toxicity includes liver necrosis, nephrites and gastrointestinal irritation Athar and vohora (1995)[9]. Therefore, special attention should be given to the water quality and bioaccumulation of metals (arsenic and chromium) in crab, Barytelphusa guerini. In present investigation it was found that the level of arsenic and chromium in some tissues crab, Barytelphusa guerini were higher than the recommended maximum allowable standards in food. These results suggested that the Godavari river system was contaminated with

heavy metals and the consumption of crab of the river could pose heath hazards to man. It is also suggested that *Barytelphusa guerini* may be useful as a potential indicator of metal pollution, but it should be remembered that there is no certainty that the metal concentration in the environment will be accurately reflected in the tissue of the crab, for there exists a degree of regulation and elimination of metal in the body of *Barytelphusa guerini.*

**Samples collection**

**River sediment collection**: Sediments sample were directly collected from the four locations (Table 1a) along the Krishna Reserve forest. The samples were collected in a nylon cellophane bag and kept air tight condition to avoid further exposure to air. Six sediment samples were collected at the different locations.

After collection, some portions of sediment samples were dried in a vacuum oven at 105°C until constant weight. The sediments were sieved using a 2 mm sieve, lightly ground in an agate mortar for homogenization and prepared for analysis of heavy metal test.

Physical and chemical parameters determination: The following physical and chemical parameters (temperature, dissolved oxygen, pH, electrical conductivity and total dissolved solid) were taken and recorded at the different locations using a multipurpose water parameters test kit. The transparency of the River at the different locations was determined with the use of Secchi Disc which is an instrument for determining the transparency of any water body. The depth of the River was determined using a straight iron rod which was lowered into the River to reach the bottom, the point at which water cut the rod was marked and measured with the use of measuring tape and recorded in situ. Manufacturers' instruction for the water test kits was strictly adhered to Data Analysis/Laboratory Procedure.

**Digestion procedure**

Materials Used for sample digestion

1. Heating source (e.g., block digester, hotplate, water bath) capable of maintaining a sample extract temperature of 95 ± 5°C, Fume cupboard, Gloves and nose mask, Conical flask, Measuring cylinder, Standard flask, Beakers, Electronic weighing balance, Filter paper.

2. Reagents Used: Conc. Hydrochloric Acid (HCl), Distilled water, Conc. Trioxonitrate (V) acid ($HNO_3$), Conc. Tetraoxosulphate (VI) acid.

**Sample preparation for sediment analysis**

1 g of the soil sample was weighed and put into a digestion vessel (conical flask) after which 10 ml of concentrated nitric acid and 5 ml of concentrated sulphuric acid were added to it. This was repeated for all the soil samples including the control samples. The samples were digested for about 2-3 minutes at 95 ± 5°C and then allowed to cool for about 20-24 hours Soil samples were filtered using Wattman no 40 filter paper. The digest was made up to 50 ml using a 50 ml standard flask and stored for analysis. A blank sample was also prepared. The digest was analyzed for heavy metals using Atomic Absorption spectrophotometer (AAS).

**Data analysis**

The data collected were statistically analyzed using the statistical package Social Sciences 16.0. A variance analysis ($p<0.05$) of total metal concentrations among different sampling sites was performed using one-way ANOVA test and mean differentiated using least Significant Difference (LSD) at $p<0.05$.

Physical and chemical parameters

The result of the physical and chemical parameters as presented in Table 5 shows that the mean values of all the parameters tested for were highest in Location 1. The lowest mean temperature (28.6°C) and pH (7.00) were recorded at Location 2 ) while the lowest mean conductivity and TDS (198 mg/l) were recorded at Location 3. The lowest mean transparency (0.54 m) was recorded at Location 4. The lowest and highest (5.24 mg/l and 7.27 mg/l) mean dissolved oxygen however was recorded at Location 2 and 1 respectively. Location 1 presented the highest value (0.92 m) in term of

depth while the lowest value (0.67 m) was recorded at Location 3. There was however no significant difference (P<0.05) in the mean of all the parameters tested for from the four locations.

**Table 5:** Mean values of Physical and chemical Parameters at the different locations Values are expressed in means.Means having same superscript on the same column are not significantly different at p>0.05.

| Locations | Temp (°C) | pH | DO (mg/l) | Transparency(cm) | Conductivity (mμ) | TDS (mg/l) | Depth (m) |
|---|---|---|---|---|---|---|---|
| 1. | 30.5a | 8.01a | 7.27 | 0.67a | 537a | 289a | 0.92 |
| 2. | 28.6abc | 7.00abc | 5.24 | 0.65ab | 511ab | 256ab | 0.73 |
| 3. | 28.7abc | 7.24abc | 6.86 | 0.55abc | 395abc | 198abc | 0.67 |
| 4. | 29.4ab | 7.32ab | 5.76 | 0.54abc | 412abc | 205abc | 0.69 |
| Total mean | 29.3 | 7.39 | 6.283 | 0.6 | 463.75 | 237 | 0.753 |
| Minimum | 28.6 | 7 | 5.24 | 0.54 | 395 | 198 | 0.67 |
| Maximum | 30.5 | 8.01 | 7.27 | 0.67 | 537 | 289 | 0.92 |

Discussion

**Physical and chemical parameters**

There were difference in mean temperature values at the different locations; there was however, no significant difference in the mean of the temperature value. The minimum and maximum temperature of 28.6-30.5°C according to Okayi[10] is normal for tropical waters for optimal growth of organisms. This also agrees with the mean temperature range recommended by WHO for optimal growth of fish. Result of this finding is also in line with the findings of Olalekan et al.[11] who reported similar temperature range in Ogun River between the dry and wet season.

The dissolved oxygen (DO) range observed in this study falls within the acceptable limit for fish survival. The lower level was expected owing to high level of organic matter being introduced and undergoing decomposition thus resulting in oxygen uptake. This is similar with the result of Umunakwe et al.[12] who reported that DO level in Aba River where within the acceptable limit prescribed by WHO and Federal Ministry of Environment.

This study also revealed that hydrogen ion concentration (pH) was generally higher than 7.0 at all sampled locations. The only exception was at Location B, where mean value obtained was 6.89. In similar studies, Tetsola et al[13]. reported that Krishna river which is the source of alkaline in nature (pH above 7.0). The Lower pH values recorded at Location B could be linked with the influx of humic substances into the River which were made available by proliferation of markets, sawmills and massive rural to urban drift and other anthropogenic activities there by making that source point acidic. However, the pH range at all points was still within the acceptable limit for fish survival.

Moreover, the levels obtained for total dissolved solid (TDS) in the study area was higher at Sokori source point and lowest at Location C source point reason could be due to the type of discharge entering into the River at these different points. However, the range falls within the acceptable limits as prescribed by WHO[14] and thus, do not pose any form of threat to the aquatic organism. The result of this study corroborates the finding of Ayobahan et al.[15] who recorded similar high level of TDS in two stations along the Benin River in their study when compared to other stations and attributed reason to the nature of activities going on in those stations.

The mean Electronic Conductivity values were observed to be highest at Location B source point and lowest at Location A source point reasons could be due to the high levels of TDS at this station. This range is however acceptable for optimal growth of organisms in tropical waters as recommended by WHO . Result is similar to that observed by Samuel et al.[16] who reported similar trend in the TDS with the electrical conductivity of River Galma in Zaria, Kaduna State. Also, Ayobahan et al.[17] observed similar trend in their study but attributed reason to the industrial activities at the locations with highest conductivity.

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