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Potential Human Health and Environmental Risks Index of Organic Pollutants with References to Anthropogenicity Sources

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ABSTRACT

The sorption of nonionic compounds in sediments influences by organic matters can be used to ascertain the hygienic condition of an abattoir and the surrounding aquatic habitat. Hence, the microbial loads and heavy metals in smoked cattle hide and *Clarias gariepinus* from a major abattoir in South-South Nigeria was investigated and correlated with their metallic loads in the surrounding water body. This is to establish the health implications of the consumers and the aquatic organisms. Roasted catfish, cattle hide, and sediments from the Tombia River were collected for six months and a total of fifty-four samples were examined. Morphological and biochemical characterization was used in identifying the bacterial isolates, while the microscopic depiction of hyphal mass and spore's morphology was used in fungi identification. The heavy metal was analyzed using Atomic Absorption Spectrophotometer. Microbials characterized by sampled fish and cowhide from the abattoir are *Pseudomonas* spp, *Enterobacter* spp, *E. coli, Bacillus subtilis*, *Streptococcus* spp, *Citrobacter* spp, *Micrococcus* spp, and *Acinetobacter* spp. Four heavy metals; Cu, Cd, Zn, and Pb were assessed and observed at different concentrations in all the matrixes. The risk index was 475.05 B grade (high risk). Strong positive and negative correlations were also observed in the fish, hide, and sediments for all the metals. Using these pollution indicators, it was found that the river bed sediment is highly contaminated by toxic metals during the study, hence proper management strategies are required to control the direct dumping of wastewater in the river.

Keywords: Abattoir, Roasted catfish, Roasted cattle hide, Tombia River, Ecological Risk Index

1. INTRODUCTION

One of the greatest attractions in the Niger Delta Region apart from oil and gas is the availability of a wide array of special delicacies of roasted seafood, among the favorite, are meat and fisheries (crustaceans, shellfish, oysters). A major problem reported to be associated with the consumption of this food is the prevalence of pathogenic microorganisms due to unwholesome handling and impartation of

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mutagenic substances due to pollution by various media in the course of production. Also, the smoke emissions from firewood, tyres, and other organic fuel sources used in the processing of meat constitute a striking and potentially toxic substance like furans and benzene which could contaminate the hide and fisheries and render them unfit for human consumption (Obiri-Danso et al. 2008). Tyres for fish and meat processing also contain metals such as lead, mercury, cadmium, chromium, zinc, and arsenic which could contaminate meat and fisheries products, and the consumption of such potentially contaminated meat products can be a great source of health risk to the ultimate consumers (Costa, 2000). The International Panel on Climate Change (IPCC) has proven that particles created by burning wood and other biomass—so-called "black carbon"—are a major contributor to global warming (Kumar et al. 2006).

Common to any industrial process, the meat and fisheries processing industries are associated with significant effluent management issues that could negatively affect the environment. These issues include high water consumption, the generation of high organic strength effluent streams, high energy consumption, odors, high nutrient load, and the management of solid wastes (Rajendra, 2007).

Despite the rudimentary nature of the process of traditional methods, lack of control over the drying rate sometimes results in under-drying or over-drying and expose the meat and fish to unexpected winds, dust, dirt, insect infestation, and contaminants such as flies (Akinola et al. 2006). Traditional methods of preserving meat and fisheries must be improved upon in Nigeria and to improve their quality. Based on this understanding that this study sought to; investigate the consequential health/hygiene status of Tombia abattoir with references to the microbiological and the heavy metal contamination in the roasted catfish, cowhide, and the ecological risk index of the Tombia River, that receives wastes from the abattoir.

2. MATERIALS AND METHODS

Samples used for this study are two food samples; smoked fish *(C.gariepinus*), cowhide, and one environmental sample of the River's sediments. Fish are mainly roasted with firewood, and we limit our sampling to that. Methods employed for cowhide roasting include tyres, firewood, and waste plastics. The environmental samples were collected from the Tombia River in Yenagoa Local Government Area of Bayelsa. Sampling was done on monthly basis with three replicates per sample for six months.

Smoked *C. gariepinus* samples of average size (500g) were randomly sampled and purchased from people carrying out fish roasting within the abattoir. Only the cowhide roasted with tyres were collected for this investigation, is to the fact that it is the most common practice in the region (Fig.1). The sediment samples were collected using Ekman dredge. Bottom smooth sediments from natural depositional zones were collected from the three stations during low-flow conditions. Composites samples from several depositional zones were pooled together. All the samples were packed in sterile bags and transported to the laboratory in ice-packed coolers and analyzed within 24 hours to avoid contamination.

Microbiological Analysis

All glasswares used were carefully washed with detergent, rinsed with tap water, and de-ionized water. Glass wares such as conical flasks, beakers, Petri dishes e.t.c. were sterilized at 160^{0}C for 2 hours using Gallen Kamp Hot Air Oven. The media were sterilized at 121^{0}C for 15 minutes in the autoclave. The direct flaming method as described by Cheesbrough (2000) was adopted for the sterilization of inoculation needles, loops, and glass rods using Bunsen burner flame.

Prescott et al. (2005) method was used in determining the microbial loads in the samples. For total coliforms, aliquots 0.1 milliliters of the serially diluted samples were each inoculated onto different sterile MacConkey agar plates in duplicates, the inoculums were then spread evenly on the surface of the media using a sterile bent glass rod. This was followed by incubation at 37^{0}C for 24 hours, after which the colonies were counted and the mean total coliform count expressed as CFU/ml and CFU/g.

Total Vibrio count was determined by inoculation on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar using the spread plate technique. Aliquots (0.1ml) of the serially diluted samples were inoculated onto sterile pre-dried TCBS agar plates in triplicates and then spread evenly with a sterile bent glass rod. The plates were incubated at 37^{0}C for 18 - 24 hours, after which the colonies that developed were counted and the mean recorded accordingly for surface wastewater, and soil samples

Pure bacteria isolates were identified by the method described by Collins *et al.* (1989) and Cheesebrough (2002). The isolates were subjected to biochemical tests; oxidase test, catalase test, indole test, methyl red test, Voges Proskauer test, starch hydrolysis test, urease test, citrate test, sugar fermentation test, and Triple Sugar Iron agar test.

Potato Dextrose Agar (PDA**)** and the Vapour Phase Transfer method of Mills and Colwell were adopted to determine the population of fungi (Okerentugba and Ezereonye, 2003) using the spread plate technique as described by Odokuma (2003).

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Fungal isolates were identified using their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol-cotton blue and reference made to a fungal identification atlas by Barnett and Hunter (1998). However, yeast isolates wet mount were prepared with normal saline and were further identified using Gram-staining, oxidation, and fermentation tests.

Figure 1: The crude method of roasting of Cowhide with burning Tyre at Tombia, Niger Delta Nigeria

Heavy Metal Analysis

The fish and hide samples were further dried in the laboratory using the oven at 110 °C to constant weight, thereafter homogenized and macerated in a metallic blender, and passed through 160 μm sieve. 5 g sample was weighed and transferred to the glass tube and mixed with 5 mL of HNO_3 (analytical grade) and 3 mL of concentrated hydrochloric acid (HCl). After 10 minutes of mixing, 1 mL of H_2O_2 was added. Samples were heated at 95^{o}C on a hot plate for five minutes and allowed to cool to room temperature. The solution was filtered and quantitatively transferred into a 50ml volumetric flask while diluting to volume with distilled water.

The sediment samples were air-dried in the laboratory at room temperature for a week. They were sieved through 63- m mesh nylon-sieve cloth as described by Ikpesu and Ezenwaka (2018). Thirty (30) ml of condensed water was added followed by 10 ml of HNO_3 (analytical grade) and 3 ml of concentrated hydrochloric acid (HCl). The suspension was filtered (Whatman filter Merck, 0.45 μm) and the filtrate was diluted by distilled water to a final volume of 50 mL and stored in a cleaned sample bottle for analysis

A hollow cathode lamp for the desired metal was installed in the atomic absorption Spectrophotometer and the wavelength dial property set. The slit width was set for the element being measured. The instrument was turned on and allowed to warm up until the energy source is stabilized. The current was readjusted as required after warm-up and wavelength was optimized by adjusting the wavelength dial until optimum energy gain was obtained, the lamp was aligned accordingly. Ground-state atoms are formed by de-solvation by the chemical flame and the particles absorb the light beam from the light source while the population of ground-state atoms in the flame is directly proportional to the concentration of elements of interest.

Statistical Analysis

Data were expressed as means ± standard error. The Student's *t*-test and analysis of variance (ANOVA) were used to test for significance at the 0.05 and 0.01 levels of probability for the fish and cattle hide concentrations. Pearson correlation (r) was used to establish the degree of relationship between the analyzed metals across the abattoir and the surrounding aquatic habitat, using a statistical software package (IBM SPSS version 20). Correlation depicts how two sets of data are related, and has a value – 1 to 1, representing a strong negative and a strong positive correlation respectively. While 0 means no correlation between the set data.

3. RESULTS

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The water quality parameters (pH, temperature, dissolved oxygen, turbidity, and hardness) of the Tombia River monitored during the investigation were not significantly different from the recommendation set by various bodies ($p > 0.05$).

The frequency of occurrence of microbial isolates from the abattoir is represented in Figures 2 and 3. Microbials characterized by sampled fish and cowhide from the abattoir are *Pseudomonas* spp, *Enterobacter* spp, *E. coli*, *Bacillus subtilis*, *Streptococcus* spp, *Citrobacter* spp, *Micrococcus* spp, and *Acinetobacter* spp.

The most abundant bacterium is in *C.gariepinus* is *B. subtilis*, while *Enterobacter* spp was the least. In the hide, *Pseudomonas* spp and *Citrobacter* spp were the most abundant and least occurrence bacteria respectively.

Four heavy metals; Cu, Cd, Zn, and Pb were assessed in roasted catfish, cowhide, and the river's sediment samples. The concentrations obtained and FEPA and WHO recommendation limits are presented in Tables 1 and 2. In the fish, values of Cu ranged from 293 – 315 μg/gdw, Zn (4077 – 4123) μg/gdw, Cd (0.08 – 0.13) μg/gdw, and Pb (8.60 – 9.13) μg/gdw, while in the hide, Cu ranged from 483 – 517 μg/gdw, Zn (5103 – 5117) μg/gdw, Cd (0.32 – 0.43) μg/gdw and Pb (11.60 – 13.10) μg/gdw. The concentrations of the metals in the bottom sediments of the river with their respective indices and risk index are shown in Table 2. The sediment's concentrations (μg/gdw) were; Cu (690), Cd (2.13), Zn (5130), and Pb (15.20). The level of metals was significantly higher ($p < 0.01$) in the sediments than in the hide and the fish, and the hide was ($p < 0.05$) significantly higher than the fish

Table 1: Heavy metal (μg / gdw) load in *C. gariepinus* and cattle hide from Tombia abattoir, Niger Delta Nigeria, and the recommendation limits

	Cu	**Zn**	**Cd**	**Pb**
C. gariepinus	1302 ± 2.80^{a}	4110 ± 03.40^{a}	4.10 ± 0.01^{a}	9.10 ± 0.50^{a}
Cattle hide	1513 ± 0.50^{b}	5110 ± 4.20^{b}	6.40 ± 0.30^{b}	12.30 ± 1.40^{b}
Recommendation Limit				
FEPA	1000	3000	03	10
WHO	2000	-	03	10

Means with the same superscript within the column are not significantly ($p > 0.01$) different

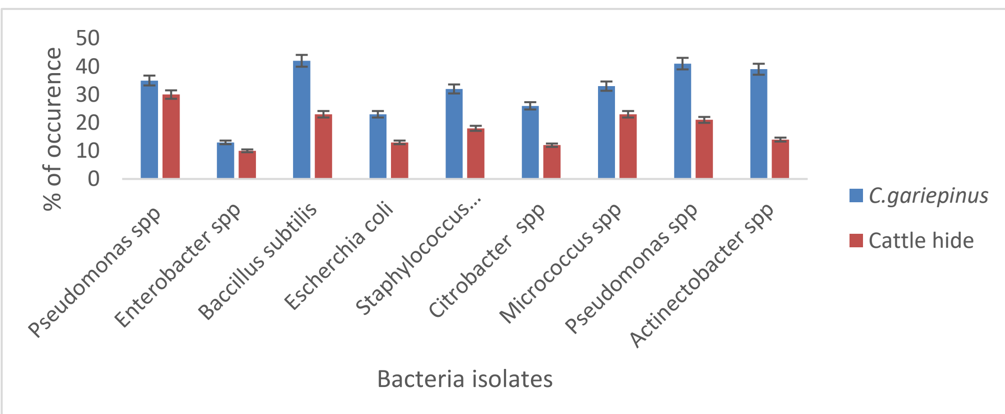

Figure 2: Frequency of occurrence of bacteria isolates in *C.gariepinus* and Cattle hide from Tombia abattoir, Bayelsa State Nigeria.

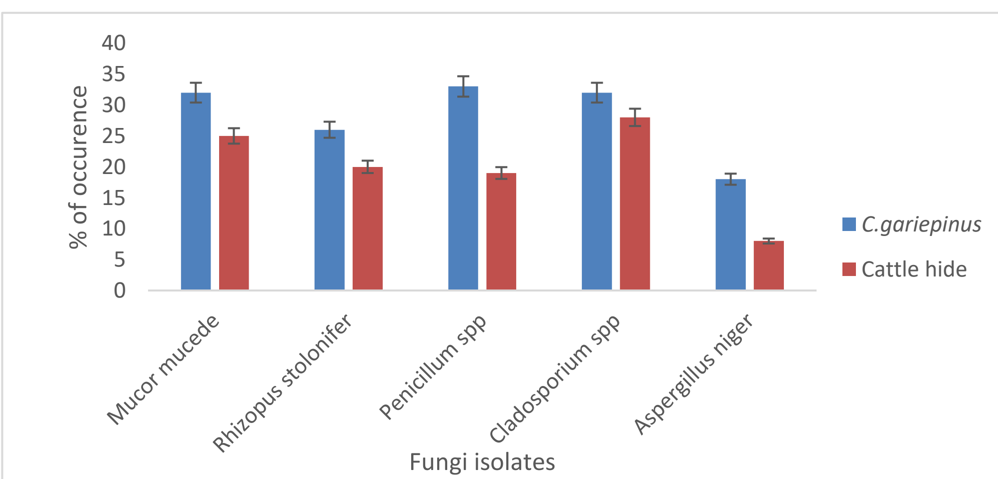

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Figure 3: Frequency of fungi isolates in *C. gariepinus* and Cattle hide from Tombia abattoir, Bayelsa State Nigeria.

Table 2: Potential ecological risk index of Tombia River, Niger Delta Nigeria

	Cu	**Zn**	**Cd**	**Pb**
C^i_o (µg/gdw)	1690	5130	7.13	15.20
C^i_n	30	80	0.5	25
C^i_f	23	26.63	0.26	0.608
T^i_r	5	1	30	5
E^i_r	115	26.63	7.8	3.04
RI ($\sum E^i_r$)	152.47			

where:

C^i_o = the concentration of the investigated metals in sediment

C^i_n = a reference value for metals; Cu = 30, Zn = 80, Cd = 0.5 and Pb = 25

T^i_r = toxic-response factor for the metals; Cu = 5, Zn = 1, Cd = 30, and Pb = 5 (Hakanson, 1980)

$C^i_f = C^i_o / C^i_n$; the contamination factor

$E^i_r = T^i_r \times C^i_f$; the monomial potential ecological risk factor of the investigated sample

$RI = \Sigma E^i_r$ is the sum of all the risk factors for heavy metals in the sediment

The risk index assessment: When RI < 150 (low risk index) D grade; RI > 150 < 300 (moderate risk) C grade; RI > 300 < 600 (high risk) B grade; RI > 600 (very risky) A grade

4. DISCUSSION

The microbes isolated from roasted catfish and the cattle hides; Bacteria (*Pseudomonas* spp, *Enterobacter* spp, *E. coli, Bacillus subtilis*, *Streptococcus* spp, *Citrobacter* spp, *Micrococcus* spp, and *Acinetobacter* species), fungi (*M. mucede*, *R. stolonifera*, *Penicillium* spp, *Cladosporium* spp, and *A. niger*) could be attributed to the hygiene status of the butchers, the fish handlers and the degree of contamination of the fish source. It can also be deduced that the fungi reported in this study could be as a result of favorable environmental conditions that allow the growth of spores into a vegetative state.

Conversely, the microbial loads can be ascribed to the fact that abattoirs and fish processing methods in developing countries are poorly managed, the slaughter's house and fishery are always soiled with animal and fish fluids, gut contents, and other wastes from previously dressed animals and fish which increase the risk of contamination of subsequent meat and fishery. Equally, the sanitation and hygiene status of the butcheries are generally poor and invariably did not meet the sanitary requirements (WHO/UNECE, 2006).

In the slaughterhouse and fishery at Tombia, flies were uncontrollable and more than expected. It is important to note that, the flies are vectors of various diseases causing agents. Similarly, the abattoir does not take cognizance of slaughtering integrity as animals were always slaughtered and gutted on the floor because of the absence of mechanical or manual hoists, a factor that contributed to the main source of pollution.

The abundance of *B. subtilis* in the fish can be attributed to its eco-adaptability (Sudhagar and Reddy, 2017) and one of the main gut bacteria (Wu et al. 2014), while a few *Enterobacter* spp reported could be as a result of its viability (survival) in the environment. Surface contamination depends on many factors related to microorganisms and their viability in the environment favored by the formation of biofilms (Shi and Zhu, 2009). The dominance of *Pseudomonas* spp in cattle hide could be their facultative nature, which

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means that they can thrive in both aerobic and anaerobic environments. Similarly, it could be associated with the cattle bye-products, as they have been isolated from different dairy products, such as cheeses, refrigerated raw milk, and pasteurized milk, (Martins et al. 2006, Arslan et al. 2011). The few *Citrobacter* spp observed could be there not significant etiological agents, though few *Citrobacter* has been isolated from a range of foods (Saba and Gonzalez-Zorn, 2012 and Kouame et al. 2013).

Penicillium spp has the highest percentage of the occurrence. This could be attributed to their mesophilic nature as they adapt easily to the environment while *A. A niger* commonly called black mold of onion was the least reported in this investigation. Though, It is ubiquitous in soil, but commonly found in indoor environments (Cairns and Meyer, 2018), not thriving well under external exposure.

Various concentrations of the four heavy metals; Cu, Cd, Zn, and Pb were observed in roasted catfish and cowhide samples. The concentrations of these metals correlated with the previously reported cases (Avenant-Oldewage and Max 2000; Etesin and Benson, 2007).

Comparing the matrixes, the River's sediment had the highest concentration of the metals, while the fish had the least. Correspondingly, the level of metals was significantly higher ($p < 0.05$) in the sediments than in the hide and the fish, and the hide was significantly higher ($p < 0.05$) than the fish. The high concentrations in the sediments showed that the pollution state of the river is not limited to the abattoir, other factors that may be either natural or anthropogenic sources (industrial effluent, sewage sludge, erosion) could increase the environmental concentrations coupled with the fact that the sediment act as the sink of all the pollutant entering the aquatic environment

(ATSDR, 2012; Ikpesu and Uzoekwe, 2020).

The disparity in the hide and fish concentrations of the heavy metals showed that the organic fuel sources being used to roast them has a great influence on contamination levels and the fat contents in the fish samples. This is similar to the findings of Kazerouni et al. (2001). The concentrations of the metals were slightly above the permissible limits set by various regulatory bodies (Table 1).

The toxic and bioaccumulative properties of these heavy metals in roasted foods call for caution. Lead has no safe concentration (ATSDR, 2007), and is known to interfere with body processes. It is toxic to organs such as the heart, kidney, and bones, it also interferes with zinc in heme synthesis and inhibiting the function of heme-synthesizing enzymes (Goyez and Clarkson, 2001). Zinc is known to be neurotoxic at very high concentrations causing cell death in a dose-dependent manner (Chen et al.2011). Cadmium on the other hand is detrimental to the kidneys and causes kidney impairment, affects reproductive capacity, causes hypertension, and increases the risk of hepatic dysfunction (Waalkes, 2000). Normally, Copper is not that toxic but its oxidative state of Cu^{2+} is very toxic and dominantly in the fish (UNEP, 2006).

Table 1: Heavy metal (μg / gdw) load in *C. gariepinus* and cattle hide from Tombia abattoir, Niger Delta Nigeria, and the recommendation limits

Inland fisheries are of high socio-economic importance to the people of the Niger Delta, Nigeria. Tombia River provides numerous benefits to the fishermen that live along the River, make their living via traditional fishing. The fish process at the abattoir including *C. gariepinus* examined in this investigation were gotten from the River. The fishes may be reserved by the fishermen for personal consumption, sold fresh, or preserved and sold to the public. It becomes imperative to know the risk index of the River. The ecological risk for a single regulatory index for the River (Eir) showed that Cu risk is moderate, Zn, Cd, and Cd were at a very low, while Cu was moderately high. Meanwhile, the general risk factor for the metals revealed that the River is in B grade (475.05), hence at high risk.

Correlating the metal residues in the abattoir fish with the bottom sediment of the River revealed positive correlations for Cu and Pb and a strong negative correlation for Cd and Zn. Similarly, strong positive and negative correlations were also observed in the cattle hide and the sediment for all the metals. This demonstrated a characteristic origin of these components in the waterway sediments, and the abattoir effluents are a suspect (Suresh et al.2011). Though anthropogenic source plays a significant role in contaminating natural water bodies, the normal weathering and disintegration are likewise to input contaminants into the aquatic ecosystem.

5. CONCLUSION

Inland fisheries are of high socio-economic importance to the people of the Niger Delta, Nigeria. Tombia River provides numerous benefits to the fishermen that live along the River, make their living via traditional fishing. The fish process at the abattoir including *C. gariepinus* examined in this investigation were gotten from the River. The fishes may be reserved by the fishermen for personal consumption, sold fresh, or preserved and sold to the public. It becomes imperative to know the risk index of the River. The

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ecological risk for a single regulatory index for the River (Eir) showed that Cu risk is moderate, Zn, Cd, and Cd were at a very low, while Cu was moderately high.

The unhygienic condition and practices observed in the Tombia abattoir likely to expose slaughtered livestock and traditional fish processing techniques to microbes. The main hygiene principle in processing is that clean and unclean operations are efficiently separated. This requires a well-planned plant layout, where the purpose of any structure should be to protect the products against unintended contamination.

Considering the processing technique, the animal roasted with tyres had more metal loads than firewood as in the case of the cowhide and the fish in this investigation. Likewise, the abattoir does not take cognizance of slaughtering integrity as animals were always slaughtered and gutted on the floor because of the absence of mechanical or manual hoists, a factor that contributed to the main source of pollution, and the input of heavy metals from the abattoir to the River was established by the correlation. It must be impressed with everybody employed in the abattoir or food processing factory, that hygiene concerns; process hygiene, environmental hygiene, personal hygiene, cleaning, and sanitation must be strictly observed.

Author's Contribution Statement

ITO: Develop the concept and participate in the samplings and analysis of the data

AAB: Liaised with the communities where samples were collected, analyzed and interpreted data, and take care of the correspondence.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Data and materials availability:

All data associated with this study are present in the paper.

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