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Microbial and Physicochemical Characteristics of Marine Water and Paints Collected from Onne, Rivers State, Nigeria

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ABSTRACT: The objective of this paper was to evaluate the microbial and physicochemical characteristics of marine water and paints collected from Onne, Rivers State, Nigeria using appropriate standard methods. Data obtained show that the paint samples recorded no heterotrophic fungal count, hydrocarbon utilizing bacterial count, and hydrocarbon utilizing fungal count. The water sample recorded counts of 1.23 x 102 CFU/g, 8.4 x 103 CFU/g, and 3.4 x 103 CFU/g for heterotrophic fungal count, hydrocarbon utilizing bacterial count, and hydrocarbon utilizing fungal count. The mean lethal concentration of the paint to *Staphylococcus* sp was recorded as 8.23%, 9.83%, and 9.56%, respectively. During the biodeterioration study, the heterotrophic bacterial count of Jianbang increased from 2.09×10^2CFU/g on day 14 to 4.4×10^4CFU/g on day 42 compared to the marine paint, International, which recorded a bacterial count of 2.20×10^2CFU/g to 3.8×10^5CFU/g on day 42. The fungal count of Jianbang increased from 1.17×10 CFU/g on day 14 to 2.81×10^2 on day 42, with a decreased growth count of 4.4×10^2 on day 48. A decrease in physicochemical parameters, conductivity, viscosity, and pH was observed during the deterioration study. The study revealed that marine paint can biodeteriorate accompanied by changes in their physical and chemical properties after exposure to the marine environment.

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Paint is one of the oldest synthetic materials that humans have ever encountered, having a history that dates back to the prehistoric era. It's a great option for many surfaces because of its evenly distributed mixtures, which fluctuate between a thin liquid and a semi-solid (Karigar *et al*., 2017). In essence, paint is an amalgam of various constituents, comprising of a binder, solvent, pigment, thinner, drier, extender, and additives (Fichera *et al*., 2015; Hayashi *et al*., 2019; Hewlett *et al*., 2019; Monico *et al*., 2016; Fraga *et al*., 2018). Coatings with paint and varnish are applied for both decorative and protective uses. They consist of pigments, each composed of a diverse range of chemical constituents which are mostly organic materials (Kurowski *et al*., 2017). Paints contain Volatile Organic Compounds (VOCs), high mercury content and other heavy metals can significantly harm the environment and humans (Ashwini and Anchana, 2018). Paints used in marine structures protect surfaces from corrosive elements and prevents surface fouling (Sharma and Constantino, 2006). The word, "biodeterioration", refers to the process of degradation of materials that have commercial value. Several variables influence

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the rate at which paints and paint products disintegrate, including the absence of oxygen in the paint, the organic composition of the paint's components, the microbiological quality of the packing materials, and the cleanliness of the production plant processing areas. According to Yamuna *et al*. (2021) the paint industry faces significant financial consequences due to the microbial deterioration that results in unpleasant odors, loss of viscosity, discoloration, and apparent surface growth. Numerous paint chemicals and their corresponding additives, such as thickeners, glues, and surfactants, are biodegradable. Those greenish-black, fuzzy-looking growths that result from too much moisture (or inadequate air circulation) are mildew and/or mold. In addition to having a negative impact on health, these growths compromise the building's fundamental structural integrity. According to Maduka and Igwilo (2019), microorganisms cause harm to painted surfaces by discoloring them, increasing their porosity, decreasing their physical resistance, and making it easier for moisture to permeate the surface. The coating's ingredients have an impact on microbial growth as well; certain ingredients are growth-stimulating, while others are inhibiting. While organic solvents and heavy metals in pigments can negatively impact fungal cells, materials like cellulose derivatives might serve as nutrients for them (Gaylarde *et al*., 2011). Microbial growth accumulation on surfaces causes biofouling, which eventually results in the creation of biofilm (Ibrahim *et al*., 2019).

Therefore, the objective of this paper was to evaluate the microbial and physicochemical characteristics of marine water and paints collected from Onne, Rivers State, Nigeria.

MATERIALS AND METHODS

Sample collection: Two (2) samples of marine paints used in marine structures and were bought from a local market and marine water was collected from Federal Ocean Terminal (FOT) in Onne and analysed for microbial, heavy metals and physicochemical parameters, toxicity and biodeterioration.

Microbiological Analyses: The presence of total heterotrophic bacteria (THB), total fungi (TF), hydrocarbon-utilizing bacteria (HUB), and hydrocarbon-utilizing fungi (HUF) were examined in the paint and water samples using serial dilution, and microbial count was ascertained. The isolates were identified based on cultural morphology, microscopy and biochemical tests.

Physicochemical Analysis: The paint samples were examined for physical-chemical characteristics such as viscosity, colour, pH, electrical conductivity, BOD, COD, TPH while the marine water sample was examined for BOD, COD and TPH.

BOD Determination: Dilution water was prepared by adding phosphate buffer, calcium chloride, magnesium sulfate, and ferric chloride to distilled water. The initial dissolved oxygen was measured in a 300ml BOD bottle and BOD dilutions with different sample volumes were prepared. Samples were incubated at 28°C for 5 days in a BOD incubator. Final dissolved oxygen after 5 days was measured and BOD_5 was calculated using equations 1 and 2:

$$t - day\ BOD = \frac{[DOt - DO0]}{P} \quad (1)$$

Where P = Dilution factor

$$Dilution\ factor\ (P) = \frac{300mL}{sample\ vol\ in\ mL} \quad (2)$$

COD Determination: COD was analyzed using the Closed Reflux method. Culture tubes were washed with 20% sulfuric acid to prevent contamination. The samples were added to the culture tube followed by the digestion solution. Sulfuric acid was carefully lowered under the sample solution and tubes were tightly covered. Digestion was carried out in a block digester followed by titration with 0.10M ferrous ammonium sulfate (FAS) until a colour change was observed.

The COD was calculated using equation 3:

$$COD(mg/L) = \frac{(A - B)\ x\ M\ x\ 8000}{Vsample} \quad (3)$$

Where: A = Volume of FAS used for the blank (mL)
B = Volume of FAS used for the sample (mL)
M = Molarity of FAS
8000 = Milliequivalent weight of oxygen x 1000 mL/L

TPH Determination: The TPH of the samples were measured using gas chromatography.

Heavy Metal Analysis: The marine paint and water were heavy metal elements such as lead (Pb), lithium (Li), chromium (Cr), copper (Cu), and zinc (Zn). Flame Atomic Absorption Spectrometry was used to measure lead, lithium and zinc. Chromium was analyzed by spectrophotometry using diphenylcarbazide reagent. Copper ions were determined by electrochemical method.

Toxicity Test: The toxicity method of Nrior *et al*. (2023) was adopted for the toxicity test of each of the marine paint. Five (5) different concentrations of the paint were prepared using the marine water sample. In each set a total of 5 conical flasks for the concentrations (0.01%, 0.1%, 1%, 10%, and 100%) and the control (0%) were prepared from the stock paint sample. The mixture was homogenized for 2 minutes before use. An aliquot (0.1ml) from each of the setups was inoculated onto nutrient agar plates by spreading. The inoculated plates were then incubated for 24 hours at 37°C. The process was repeated after 24h, 48h, 72 and 92 hours. After incubation, the microbial count was determined and expressed in Log10. The least mortality concentration was used in the biodeterioration study.

Table 1: Experimental setup for the toxicity test using marine water

Percentage (%)	Toxicant (ml)	Marine (ml)	Total volume	Bacteria (ml)
Control (0%)	0	100	100	10
0.01	0.01	99.99	100	10
0.1	0.1	99.9	100	10
1	1	99.0	100	10
10	10	90	100	10
100	100	0	100	10

Biodeterioration Test: Adopting the method of Etim and Antai (2014), the paints were analyzed for the microbial growth and deterioration for a period of 48 days (7 days interval) using Mineral Salt medium. The least concentration of the paint that can deteriorate, which was determined by the toxicity test, was used for the biodeterioration study. One milliliter (1.0ml) of a pure culture of each of the microorganisms identified was added to the 100 ml of mineral salt medium together with the known concentration of paint. Aliquot (0.1ml) from there was inoculated by spreading on the Nutrient agar plates in duplicate, incubated for 24 hours at room temperature (30±1^{o}C). This was repeated on days 7, 14, 21, and 48. The total viable count (TVC) during the duration of the investigation was examined and indicated the extent of bacterial destruction.

Statistical Analysis: Analysis of variance (ANOVA) was used to compare means using the computer system SPSS. Analysis of variance was used to see whether there was any variation in the microbiological development of the paint samples over the course of the experiment.

RESULTS AND DISCUSSION

Physicochemical Parameters of Marine Water and Paint Samples: Chemical Oxygen Demand (COD) is essential for controlling surface water and lowering water pollution (Li *et al*., 2022). The COD value varied between 3.75±0.00 ppm and 72.0±0.00 ppm, with the water samples exhibiting the greatest COD value and the marine paint, Jianbang, recording the lowest. The water sample's COD measurement reveals that every sample was within the 200 mg/l surface water World Health Organization permitted limit. Chemical oxygen demand (COD) concentrations between the marine paints in this study did not differ significantly, indicating that COD is not influenced by paint type. However, the COD of the marine paint from International was marginally higher than that of Jianbang marine paint. Biochemical oxygen demand (BOD) is the amount of dissolved oxygen that is needed for stabilization of organic matter that are biodegradable through the action of aerobic microorganisms and the oxidation of certain inorganic materials (Tikariha and Sahu, 2014). The quantity of BOD levels was not influenced by the type of paint, since there were no significant changes ($P \leq 0.05$) in the amounts. The BOD levels of the marine paints and water samples included in this study, however, are under the WHO and NUPRC allowed limit of 50 mg/kg for surface water. The samples' BOD values varied from 0.114 ppm to 16 ppm, with the marine water sample having the highest BOD and the Jianbang paint having the lowest. Lead, zinc, copper, and lithium in the Jianbang and international paint samples were higher than in the marine water samples. Chromium was not determined in the paint samples however, 0.068±0.00 mg/kg of Chromium was recorded in the marine water sample (Table 2). The study revealed that there was no statistically significant disparity ($P \leq 0.05$) in the concentrations of heavy metals between Jianbang and International marine paints. This suggests that the presence of heavy metals is not influenced by the type of paint used. The concentration of lead (Pb), zinc (Zn), and other heavy metals in each sample exceeded the permissible limits of 5 mg/kg and 0.1 mg/l, respectively. However, the concentration of copper (Cu) in the marine water sample was shown to fall within the permitted limit of 0.5 mg/l. There was no significant difference ($P \leq 0.05$) in the total petroleum hydrocarbon (TPH) content among the paints, Jianbang and International. The amount of total petroleum hydrocarbon (TPH) of the water samples were observed to be above the Directorate of Petroleum Resource acceptable limit of 10mg/l.

Table 2: Physicochemical parameters of the marine water and paint samples

Physicochemical parameters	Marine Water sample	Paint 1 (Jianbang)	Paint 2 (International)	p-value
COD (ppm)	72±0.00	3.75±0.00	10.17±0.00	0.135
BOD (ppm)	16±0.00	0.114±0.00	0.120±0.00	0.135
TPH (mg/kg)	<0.001±0.00	252.525±0.00	246.845±0.00	0.261
Heavy metals				
Pb (mg/kg)	1.176±0.00	922.756±0.00	19.821±0.00	0.261
Zn (mg/kg)	0.126±0.00	53.966±0.00	8.344±0.00	0.261
Cr (mg/kg)	0.068±0.00	ND	ND	0.261
Cu (mg/kg)	0.38±0.00	252.525±0.00	8.137±0.00	0.261
Li (mg/kg)	1.36±0.00	39.398±0.00	8.12±0.00	0.261

*Significant different at p<0.05

Microbial Analyses: The bacteria isolated based on the macroscopic and microscopic characteristics, included *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Enterobacter* sp., *Serratia* sp., *Proteus* sp., and *Pseudomonas* sp. and Fungi, were, *Aspergillus niger, Fusarium, Penicillium, Chryseognum,* and *Candida* sp. A study by Meddeb-Mouelhi *et al*. (2016) revealed that *Bacillus* sp. strains produced many enzymes of relevance for biodeterioration. Some of the fungal isolates (*A. niger*, *A. flavus*, *Candida* sp., and *Fusarium* sp.) in this study possess the capacity for spore production, *Aspergillus* sp. is found could facilitate the discoloration and subsequent structural damage of the painted walls by virtue of their mycelial production (Obidi and Okekunjo, 2017). Asthma, pharyngitis, and allergic rhinitis can all be brought about by fungus spores in indoor air (Stanley *et al*., 2019).

Microbial Count of the Water and Paint Samples: The heterotrophic bacteria count varied between 1.1 x 10^3 and 3.4 x 10^3 CFU/g, with international paint having the lowest count and marine water having the highest count. The water sample recorded counts of 1.23 x 10^2 CFU/g, 8.5 x 10^3 CFU/g, and 3.4 x 10^3 CFU/g for heterotrophic fungal count, hydrocarbon utilising bacterial count, and hydrocarbon utilising fungal count respectively (Table 3).

Table 3: Microbial counts of the samples

	Marine water	International paint	Jianbang paint
Total Heterotrophic bacterial Count (THBC)			
THBC (CFU/ml)	1.5 x 10^5	1.1 x 10^3	3.4 x 10^3
LogCFU/ml	5.17	3.04	3.53
Total Fungi count (TFC)			
TFC (CFU/ml)	1.23 x 10^2	**0**	**0**
Log CFU/ml	2.08	**0**	**0**
Hydrocarbon Utilizing Bacterial (HUB) Count			
HUB (CFU/ml)	8.5 x 10^3	**0**	**0**
LogCFU/ml	3.92	**0**	**0**
Hydrocarbon Utilizing Fungal (HUF) Count			
HUF (CFU/ml)	3.4 x 10^3	**0**	**0**
LogCFU/ml	3.53	**0**	**0**

Mean Lethal Concentration (LC_{50}) of the Paint to the Microorganisms in the Marine Water: The toxicity analysis as expressed in LC_{50} (figure 1) showed that the marine paint was more toxic to *Aspergillus niger* with the LC_{50} of 1.95% followed by *Fusarium* sp. with the LC_{50} of 2.16% followed by *Penicillium* sp. with LC_{50} of 5.7% followed by *Staphylococcus* sp. with the LC_{50} of 8.2% followed *Pseudomonas* sp. with the LC_{50} of 8.68% followed by *Micrococcus* with the LC_{50} of 9.53 followed by *Serratia* sp. with the LC_{50} of 9.56% and followed by *Bacillus* sp. with the LC_{50} of 9.83%. In the case of their consortium, the LC_{50} of the marine paint to a consortium of fungi was 9.12% < 9.5% recorded in the consortium of bacteria < 12.34% recorded in the consortium of bacteria and fungi. The result of this study showed that the marine paint was more toxic compared to the findings of Shaala *et al*. (2015) in which LC_{50} range of 6% to 23.7% of the paint antifouling agent.

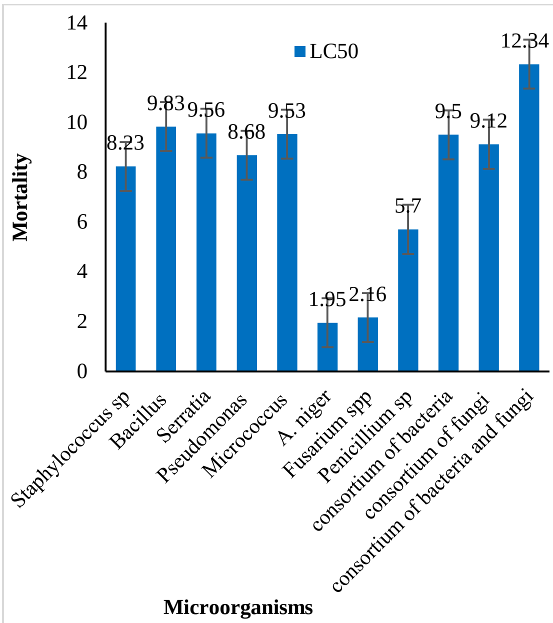

Fig. 1: The LC_{50} of the paint to the microbes in the marine water

Change in the Bacterial Count of the Paints during the Biodeterioration Study: There was no count of bacterial growth during the first 7 days of the biodeterioration study. Growth was observed between the 14th and 48th day of the study. There was a noticeable increase in the growth of microbes in the international paint compared to the Jianbang marine paint. The heterotrophic bacterial count of Jianbang increased from 2.09×10^{2}CFU/g on day 14 to 4.4 $\times10^{4}$CFU/g on day 42 compared to the marine paint, International with recorded bacterial count which varied from 2.20×10^{2}CFU/g to 3.8×10^{5}CFU/g on the day 42 (Figure 2).

Change in the Fungal Count of the Paint during the Biodeterioration Study: There was no fungal growth during the first seven days of the biodeterioration study. Growth was observed between day 14 and day 41 of the research and later recorded decrease in the count of fungal to the day 48 of the study. It was observed that there was more fungal growth in the international paint to day 34 compared to the Jianbang marine paint however on the day 41 and 48, the Jianbang pant recorded higher fungal growth compared to the marine paint, international. The heterotrophic fungal count of Jianbang increased from 1.17×10 CFU/g on the day 14 to 2.81×10^{2} on day 41 after which a decreased fungal growth count of 4.4×10^{2} was observed on day 48. The fungal count of the marine, International ranged from 1.38×10^{2} CFU/g on day 14 to 4.8×10^{2}CFU/g recorded on day 41 after 4.1×10^{1} CFU/g (Figure 3). The widespread presence of microbes in the paints suggests that they were especially rich in organic nutrients which support microbial growth. The high bacterial counts in the paint samples, particularly in the marine water used indicate that the natural microorganisms in the marine ecosystem may have a negative impact on the marine paints' shelf life.

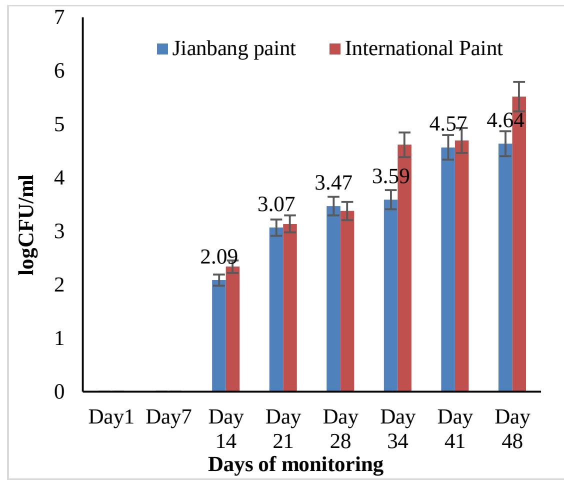

Fig. 2: Bacterial count of the paint during the biodeterioration study

Change in Physicochemical Parameter during the Biodeterioration Study: A decrease in the physicochemical parameter, conductivity, viscosity, and pH was observed, in the course of the deterioration study on day 1 and day 48 (table 4).

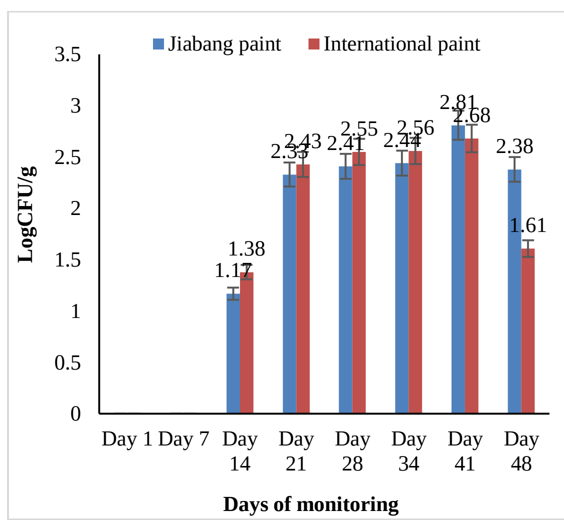

Fig. 3: Fungal count of the paint during the biodeterioration study

Conductivity reduced from 0.174 ±0.00 on day 1 to 0.081±0.00 on day 48 for the marine paint, International, while the decrease from 0.180±0.01of day 1 to 0.113±0.028 on the day 48 was recorded in Jianbang marine paint. During the biodeterioration investigation, there was no significant difference ($p \leq 0.05$) in the conductivity between the two paintings. During the biodeterioration investigation, the viscosity of Jianbang marine paint and International marine paint dropped from 23.1±0.01±0.00 to 16.7±0.028 and from 28.4±0.02 to 19.8±0.014, respectively, with a significant difference ($p \leq 0.05$) between the two paints. The pH of the international marine decreased from 7.1±0.00 to 5.94±0.00 (increase in acidity) from the day 1 to the day 48 while the decrease in pH was also recorded in the deterioration study of marine paint, Jianbang from 6.2±0.02 to 6.11±0.014 from day 1 to day 48 with significant difference ($p \leq 0.05$) throughout the biodeterioration investigation between the two paints. This decrease is attributed to different chemical composition of the paints studied. The decrease in pH, conductivity and viscosity can also be attributed to the microbial growth and their products of metabolism as the compounds are degraded into water, CO_2, and some other inorganic end product (Pandey and Kiran, 2020).

Table 4: Change in physicochemical parameters during the biodeterioration study

Parameters	International Paint		Jianbang Paint		
	Day 1	Day 48	Day 1	Day 48	p-value
Conductivity	0.174±0.00	0.081±0.00	0.180±0.01	0.113±0.028	0.251
Viscosity	28.4±0.02	19.8±0.014	23.1±0.00	16.7±0.028	0.00
Ph	7.1±0.00	5.94±0.042	6.2±0.02	6.11±0.014	0.33

Conclusion: The study demonstrated that marine paints could biodeteriorate over time, due to microorganisms common in aquatic environments and this could affect the longevity of the paints, by modifying the physical and chemical properties, thus resulting in spoiling. The high counts of bacteria found in the paint indicate that they are rich in organic nutrients that may support microbial growth.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data are available upon request from the corresponding author.

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