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Ecotoxicity of Selected Marine Paints on *Tilapia guineensis, Palaemonetes africanus, Tympanotonus fuscatus, Aspergillus flavus and Pseudomonas aeruginosa*

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Toxicity, Mortality, Heavy metals, Marine paint, Total Petroleum Hydrocarbon (TPH), Poly Aromatic Hydrocarbon (PAH).

ABSTRACT

Physiochemical analyses of two marine paints; Jotun and International paints, revealed 437.87mg/l and 382.13mg/l for TPH, 11.47 and 14.96mg/l for PAH. Heavy metals present included Nickel (6.904 and 9.208mg/L), iron (601.36 and 1,620.48mg/l), lead (16.47 and 174.40mg/l), Copper (5.848 and 22.732mg/l), Zinc (25.152 and 52.56m/l), Cadmium (0.41 and 1.43mg/l) and Chromium (2.632 and 3.348 mg/l). Mortality was used as an index for the 96hr acute toxicity test for fish, mollusc and crustacean while 24hr and 48hr were used for Bacteria and Fungi. Median lethal concentration (LC50) was calculated using the Probit method. The 96hr LC50 for *Tilapia guineensis*, *Palaemonetes africanus*, and *Tympanotonus fuscatus* for International paint were 5.01, 9.14, and 7.15ppm while that of Jotun paint were 7.62, 5.76 and 7.97ppm. The 24hr LC50 for *Tilapia guineensis*, *Palaemonetes africanus*, and *Tympanotonus fuscatus* for both toxicants were 0.21 and 0.26ppm respectively. The 24hr LC50 for *Pseudomonas aeruginosa* for both toxicants were 0.26 and 0.35ppm respectively. The 48hr LC50 for *Aspergillus flavus* for both toxicants are 0.21 and 0.26ppm. There was no significant difference between the LC50 of both paints to the various test organisms. Iron was found to be more predominant in the *Tilapia guineensis* than other metals. These findings further portray that the use of marine paint should be continued but manufacturers should develop environment-friendly nonstick coatings to prevent the adhesion of fouling organism by providing an extremely smooth surfaces on which these organisms have great difficulty in settling.

1. INTRODUCTION

Almost 70% of the earth's surface comprises the marine ecosystem, making it one of the planet's major aquatic systems [1]. It ranges from the productive near-shore regions to the ocean floor. These ecosystems encompass diverse habitats ranging from estuaries and salt marshes to coral reefs and mangrove forests, supporting a wide array of marine life. It faces significant challenges from the effects of marine paints used to coat marine vessels and structures [2,3]. Marine coatings serve as protection [4] against saline water and limit the increase in frictional drag due to surface deterioration and biofouling [5]. Marine paints contain antifouling agents [6]; tributyltin (TBT) [2], Irgarol 1051[7], Diuron, Sea-Nine 211 [8], Oxides of copper, Chlorothalonil, Zinc pyrithione, and Dichlofluanid. Over the years, pitch, tar, and copper sheathing have been used to

protect vessels from biofouling [9]. These antifouling agents were being added to the marine paints because of their ability to repel, prevent and stop marine organisms such as algae, bannacles, mussels and other invertebrates from growing, living, and attaching themselves on various surfaces of the marine vessels [10]. Some of the biocides were used to preserve the marine paint on the marine vessels and to prolong their operations in the sea for a very long period. These paints ensured fuel efficiency, reduced cost in ship repairs, rusting and leakage [2]. According [11], vessel bottoms gather up to 150 kg of fouling per square meter in six months, increasing the fuel consumption by up to 50% compared to when no antifouling paint is applied. In 1981, the US Navy consumed 18 million barrels of fuel, with 3.3 million barrels attributed to biofouling losses [12]. According to [13], [6,3] and [15], antifouling agents cause impairments of growth, death, deformities, and reproductive anomalies in various species persisting in the ecosystem for up to 30 years. In the case of Oryzias latipes, slowed developmental rate and tail abnormalities were reported [16]. Studies have shown that TBT is detrimental to the immune system and can lead to immunosuppression in mammals such as sea otters and dolphins. [10] emphasized that most of these marine paints were found to leach out from the walls of the vessels making them accumulate in sediments where they persist for a very long period of time, posing dangerous effects on the environment and persisting in the ecosystem for up to 30 years. The persistence of these marine paints and their components in the marine ecosystem pose a great threat to survival, continuity, and existence of some species in the marine ecosystem. The International Maritime Organization (IMO) called for a global treaty that bans the application of TBT-based paints starting from 1st January 2003, and total prohibition by 1st January 2008 [17]. IMO is a specialized agency under the United Nations with the purpose of developing international conventions and guidelines to regulate shipping between nations and the use of substances in antifouling paints globally. One of their objectives is to prevent pollution from ships. However, present, and future restrictions will unfortunately not immediately remove TBT and its degradation products from the marine environment, since these compounds are retained in the sediments where they persist [18]. Additionally, while the use of antifouling paints containing TBT has been banned in countries that join the IMO, it is likely that organotin compounds will continue to be produced and used as effective biocides, especially in developing countries and those countries that do not join the IMO [18]. Bio corrosion has been a major challenge in marine structures asset owners. To prevent this, paint manufacturers has developed marine paints that will help deter the colonization of microorganisms and macro-organisms on metal surfaces in the marine environment. The paint manufacturers hope to develop marine.

2. MATERIALS AND METHODS

2.1. Sample Collection of Water, sediment, and Marine Paint

Two marine paints namely: International paint and Jotun paint were obtained from mile 3, Rivers State, Nigeria

2.2 Source of Test Organisms

***Higher organism*:** *Palaemonetes africanus, Tilapia guineensis Pseudomonas aeruginosa, Aspergillus flavus, Tympantonus fuscatus* were collected from the Nigerian Institute for Oceanography and Marine Research (NIOMR), Rivers State.

Microorganism: *Pseudomonas aeruginosa, Aspergillus flavus* was isolated from same Habitat.

2.3 Collection of higher organisms (Tilapia guineensis)

Juvenile fish of equal size were randomly caught with a hand net of mesh 0.5mm and transferred into the test vessel. The fish were not touched with hand during the selection to avoid stress due to handling. Only active and healthy fish were selected.

2.4 *Isolation of microorganism*

The habitat water was serially diluted and plated onto sterile centrimide plates and nutrient agar plates. The plates were incubated at 37^{O}C for 48 hours. The resultant isolates were isolated and purified by subculturing a discrete colonus unto another freshly prepared Centrimide agar, Nutrient agar and Patato dextrose agar. The resultant growth was then Gram stained and subjected to further biochemical tests. The cultures were stored in Bijou bottles for use in toxicity testing.

2.5. Identification of characterization of isolates

Pure isolates from the corresponding agar slants were characterized and identified using morphological (colonial morphology, motility, and gram reaction), biochemical and physiological attributes [19].

2.6. Chemical composition and water samples

The seawater sample was analyzed for its physiochemical properties using pH, total dissolved solids, electric conductivity, salinity, dissolve oxygen, total suspended solid, total organic carbon, biochemical oxygen demand, chemical oxygen demand, nitrate, phosphate, and sulphate.

2.7. Chemical Composition of Marine Paint

The chemical composition of each marine paint was carried out using parameters such as pH, Dissolved oxygen (DO), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total hydrocarbon, total petroleum hydrocarbon, nitrate, sulfate, phosphate, and chloride.

2.8 Ecotoxicity procedure of marine paint on <u>Pseudomonas sp</u>

2.8.1. Preparation of test medium

The effluent was prepared following the procedure outlined in APHA, (1998), 10ppm, 100ppm, 1000ppm, 10000ppm and 100000ppm concentrations of the toxicants were prepared using 0.5dilution factor respectively.

2.8.2. Preparation of test organism (<u>Pseudomonas sp</u>.)

A loopful of the test organism was transferred into 10ml sterile appropriate broth. This was incubated for 2-4days at room temperature (28±2 C) and stored in refrigerator at 4 C. Aliquot (1ml) of the 24h culture was transferred into fresh sterile broth (10ml), incubated for 24h (to ensure that actively growing organisms were used for toxicity test) and preliminary standard Inoculum determined [20].

2.8.3. Preparation of standard bacterial inoculum

Tenfold serial dilution of the organism was made and aliquot (0.1ml) was inoculated onto cetrimide agar in triplicates using spread plate technique. The plates were incubated for 48hours for pseudomonas sp. After the incubation periods, the plates were examined for discrete colonies. The dilution that gave between 200 and 300 colonies was noted and used as reference dilution to obtain the standard Inoculum for the toxicity bioassay.

2.9 Ecotoxicity procedure of marine paint on <u>Tilapia guineensis</u>

2.9.1. Acclimatization

The test organisms were acclimatized separately in glass tanks shortly after sampling at room temperature for ten days. The water in the acclimatization units was replaced with water from the organism's habitat water. A maximum of fifty organisms were kept in each tank. This number was kept like this to prevent crowding. The dimensions of holding tanks were 2 x 6 x 6m.

2.9.2. Toxicity texting

The test vessels had the following dimensions, 1m x 1m x 1m. The vessels were wrapped with dark polyethylene. The vessels contained brackish water

from Buguma River. Six logarithmic concentrations of the test chemicals; 0, 10, 100, 1000, 10000 and 100000 were prepared using water from the habitat of the test organism, as diluents. A preliminary range finding test was first performed before these concentrations were arrived at. The 96h acute toxicity bioassay was carried out on *Palaemonetes africanus*, *Tilapia guineensis* and *Tympanotonus fuscatus* using the procedure of APHA1998. Seven different toxicant concentrations 0, 10, 100, 1000, 10000 and 100000ppm were prepared for the experiment with controls of filtered clean water from the habitat of the test organisms (dilution water). Ten shrimps of equal sizes were randomly caught with hand net and carefully transferred into each test vessel. The organisms were not touched with hand during the selection to avoid stress due to handling. Only healthy and active organism was selected. Mortality was recorded after 4, 8, 12, 24, 48, 72 and 96 hours. Dead shrimps were removed at each observation. Mortality was plotted against the concentration on a log graph. Regression analysis was used to obtain the line of best fit. The one-way analysis of variance and the least significance difference test (LSD) were employed for analysis of data [21].

2.9.3. *Percentage log survival of Pseudomonas sp.*

The percentage log survival of the bacterial isolates in the toxicant used in the study was calculated using the formular adopted from [22]. The percentage log survival of bacterial isolates in the toxicant was calculated by obtaining the log of the count in each toxicant concentration, dividing by the count in the zero toxicant concentration and multiplying by 100.

Thus; % log survival $\frac{Log\ C\ x\ 100}{Log\ c}$

Where Log C = log of the count in each toxicant concentration, Log c = log count in the zero-toxicant concentration

2.9.4. *Percentage mortality of freshwater juvenile test specie*

Palaemonetes africanus (brackish water crustacean) and freshwater fish (Tilapia guineensis) were used in the study as a specimen of higher organism to assess the probable toxic effect drilling fluid, oil spill dispersant, degreaser and industrial detergents could have on fishes and other higher organisms in the aquatic environment. The formular for the percentage mortality was adopted from [20]. The percentage mortality was done by dividing the number of organisms that died at each exposure hour by the total test organism and multiplying by 100.

$$\%\ Mortality = \frac{No.\ of\ dead\ organisms}{Total\ No.of\ Organisms} \times \frac{100}{1}$$

2.9.5. *Statistical analysis and Median Lethal Concentration (LC_{50})*

Data representing % mortality and concentration from semi-static bioassay were analysed using the probit analysis software to determine the LC values. The results obtained from toxicity screening were subjected to statistical analysis using Analysis of Variance (ANOVA) and student t-test at 0.05 confidence limit [22] to determine the significant difference between the susceptibility of the *Pseudomonas sp*. (test bacteria) and freshwater fish *Tilapia guineensis* to the test toxicants (marine paint).

3. RESULTS AND DISCUSSION

The physiochemical characterization of the Habitat water of the test organisms are represented in table 1. Copper, Zinc, Lead, Cadmium, Iron, and Nickel were very low with values of <0.01, <0.01,0.04, <0.01 and <0.01 respectively. The Habitat water contained high levels of total dissolved solids and Sulphate. In Table 1, Cd and

Pb were not detected while Zn and Cu occurred in low concentration. PAH was also low indicating that the habitat sediment had not been exposed to organic carbon of petroleum origin. The conductivity, Magnesium, Chloride, and salinity of Jotun paint was higher than International paint in table 3. This indicated that Jotun paint was more suitable for the Marine environment than International paint. Both paints displayed similar levels of THC indicating that hydrocarbon solvents were part of their composition. The concentration of lead in both paints was high with values of 16.70- 174.40mg/l, it is far higher than the recommended 0.005mg/L by Environmental guidelines and standard for the petroleum industry in Nigeria [23] and the established threshold of 0.05mg/L. This can cause the accumulation of Pb in the aquatic environment and can also be cancerous to marine lives. Nickel found in the marine paint ranged from 6.904-9.208mg/kg. According to [24], concentrations of Nickel above 0.10mg/L in drinking water could result to liver and heart damages as well as skin irritation.

Table 1. Physiochemical Parameter for Habitat water sample

S/N	Parameter (s)	Habitat water
1	TSS (mg/l)	2
2	Conductivity (µs/cm)	40,700
3	TDS (mg/l)	28,550
4	BOD (mg/l)	4.38
5	COD (mg/l) (mg/l)	2.06
6	PAH (mg/l)	0.258
7	Cd (mg/l)	0.040
8	Fe (mg/l)	<0.001
9	Cu (mg/l)	<0.001
10	Nitrate NO_3 (mg/l)	2.1
11	Sulphate SO_4 (mg/kg)	560
12	Pb (mg/l)	<0.01
13	Salinity (mg/l)	21.340
14	pH	6.98
15	Temperature	27.8
16	Ni	<0.001
17	Cr	<0.001

Table 2. Physiochemical Parameter for Habitat sediment sample

S/N	Parameter (s)	Habitat Sediment
1	TOC (%)	0.663
2	PAH (mg/kg)	0.001
3	Cd (mg/kg)	<0.001
4	Pb (mg/kg)	<0.001
5	Zn (mg/kg)	3.000
6	Cu (mg/kg)	1.500

Table 3: Characterization of the poly aromatic hydrocarbon of Marine paint samples (International and Jotun)

Name	International (ppm)	Jotun paint (ppm)
Naphthalene	4.51862	0.0007
2- methyl Naphthalene	1.73	0.00012
Acenaphthylene	4.82	0.003
Fluorene	0.0311	0.0009
Acenaphthene	0.0034	0.003
Phenanthrene	0.0025	0.001
Anthracene	0.0010	5.47054
Fluoranthene	0.0052	0.031
Pyrene	0.0012	0.012
Benzo (a) anthracene	0.0004	0.001
Chrysene	0.0003	3.82138
Benzo (b) fluoranthene	0.0001	1.34231
Benzo (k) fluoranthene	0.0012	0.016
Benzo (a) pyrene	0.00009	0.0027
Dibenz (a, h) anthracene	0.00024	0.0044
Indeno (1,2,3-cd) pyrene	0.00071	2.35750
Benzo (g, h,i) perylene	0.00006	1.21414
Totals	**11.47508**	**14.96066**

Table 4: Characterization of the Total Petroleum Hydrocarbon of Marine paint samples (International and Jotun)

Group name	Compound name	Jotun paint	International paint
C_8	n-Octane	0.0012	99.5009
C_9	n-Nonane	0.0032	12.2479
C_{10}	n-Decane	0.0023	14.3436
C_{11}	n-Undecane	0.0615	95.8418
C_{12}	n-Dodecane	5.0801	1.3029
C_{13}	n-Tridecane	15.6436	0.9598
C_{14}	n-Tetradecane	5.0548	3.9033
C_{15}	n-Pentadene	6.3532	3.3165
C_{16}	n-Hexadecane	4.1346	3.1312
C_{17}	n-Heptadecane	7.2359	2.5808
PR	Pristane	9.6696	6.0975
C_{18}	n-Octadecane	6.05	3.168
PH	Phytane	4.8848	3.896
C_{19}	n-Nonadecane	11.6417	2.3025
C_{20}	n-Icosane	7.895	4.9912
C_{21}	n-Heneicosane	4.1596	1.6946
C_{22}	n-Doicosane	76.8002	0.757
C_{23}	n-Tricosane	6.7919	6.0077
C_{24}	n-Tetracosane	8.3701	11.0466
C_{25}	n-Pentacosane	51.9527	13.7384
C_{26}	n-Hexacosane	40.4797	4.0238
C_{27}	n-Heptacosane	3.2713	1.9643
C_{28}	n-Octacosane	6.2733	18.1606
C_{29}	n-Nanocosane	32.8292	6.883
C_{30}	n-Triacontane	24.3058	6.8532
C_{31}	n-Hentriacontane	2.8125	1.6046
C_{32}	n-Dotriacontane	19.7011	25.8912
C_{33}	n-Tritriacontane	8.8302	14.6377
C_{34}	n-Tetratriacontane	2.3674	1.277
C_{35}	n-Pentatriacontane	3.5678	3.1107
C_{36}	n-Hexatriacontane	2.4733	3.0916
C_{37}	n-Heptatriacontane	4.3149	0.9273
C_{38}	n-Octatriacontane	5.3492	0.4904
C_{39}	n-Nonatriacontane	0.7815	1.5928
C_{40}	n-Tetracontane	117.7314	0.7979
	TOTALS	**437.8742**	**382.1343**

Mortality was used to determine the lethal effects of the marine paint on the test organisms which include *Tilapia guineensis*, *Palaemonetes africanus*, *Tympanotonus fuscatus, Pseudomonas aeruginosa,* and *Aspergillus flavus.* This index involves the mortality of the test organisms with response to increasing concentration and exposure time. This index has been used [21], [26], [27], [28]. *Tilapia guineensis*, *Palaemonetes africanus*, *Tympanotonus fuscatus* was exposed to varying concentration (0.01ppm to 1000ppm) for both marine paints (International and Jotun paint) for 96hrs and the mortality was recorded at 24hrs intervals for 96hrs. LC_{50} was calculated using Probit method. The 96hr LC_{50} for *Tilapia guineensis, Paleamonetes africanus, and Tympanotonus fuscatus* for International paint are as follows*:* 5.01, 9.14, and 7.15ppm while that of Jotun paint were 7.62, 5.76 and 7.97ppm respectively. The 24hr LC_{50} for *Pseudomonas aeruginosa* for both toxicants were 0.26 and 0.35ppm respectively. The 48hr LC_{50} for *Aspergillus flavus* for both toxicants are 0.21 and 0.28ppm. At all concentration of the toxicant no mortality was recorded for both toxicants at 0hr, but at 96hrs exposure time, the highest concentration of 1000ppm recorded the highest mortality for both toxicant as represented in the tables below.

Table 5 Acute Response of *Tilapia guineensis* to the Marine paint at 96 hours

Parameter	International	Jotun
LC_{50}	5.01	7.67
LOEC	1.25	1.79
NOEC	0.63	0.9
TU_a	19.97	13.12
TU_c	80	55.87

Table 6: Acute toxicity response of *Palaemonete africanus* to the marine paint at 96 hours

Parameter	International	Jotun
LC_{50}	9.14	7.97
LOEC	2.39	1.91
NOEC	1.20	1.0
TU_a	10.94	12.55

Table 7: Acute toxicity response of *Tympatonus africanus* to the marine paint at 96 hours

Parameter	International	Jotun
LC_{50}	7.15	5.76
LOEC	1.76	1.41
NOEC	0.88	0.71
TU_a	13.99	17.35

Table 7: Acute toxicity response of *Pseudomonas aeruginosa* to the marine paint at 24 hours

Parameter	International	Jotun
LC_{50}	0.26	0.35
LOEC	0.12	0.32
NOEC	0.06	0.16
TU_a	390.60	310.40

Table 8: Acute toxicity response of *Aspergillus flavus* to the marine paint at 48 hours

Parameter	International	Jotun
LC_{50}	0.21	0.28
LOEC	0.11	0.15
NOEC	0.055	0.075
TU_a	473.19	363.35

LC_{50}-median lethal concentration, LOEC-Lowest effective concentration, NOEC-no effective concentration- TUa-Acute toxicity unit, TUc-Chronic toxicity unit, ppt-parts per thousand-parts per thousand

Figure 1, 2 3, 4, 5 shows the tissue concentration of heavy metals in *Tilapia guineensis* exposed to NOEC and LOEC concentration of International and Jotun paint. Iron was found to be more predominant in the *Tilapia guineensis* than other metals.

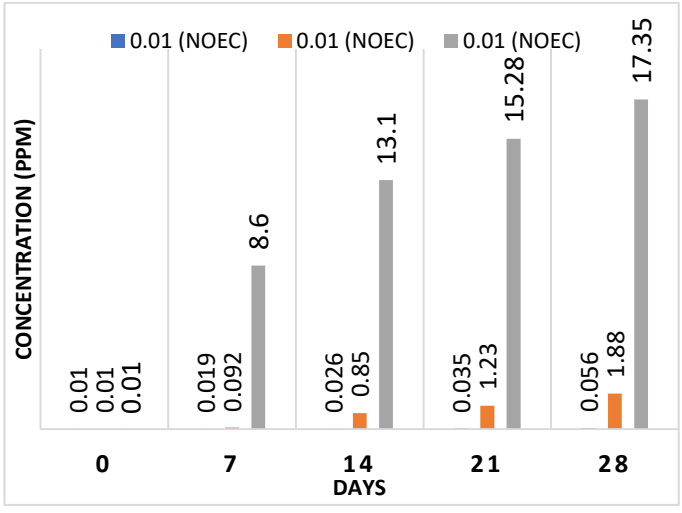

Figure 1: Tissue concentration of metal components in International paint for NOEC on *Tilapia guineensis*

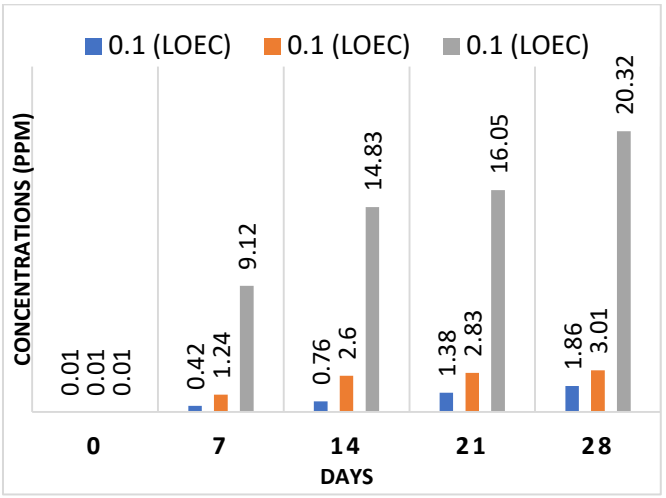

Figure 2: Tissue concentration of metal components in International and Jotun paint for LOEC on *Tilapia guineensis*

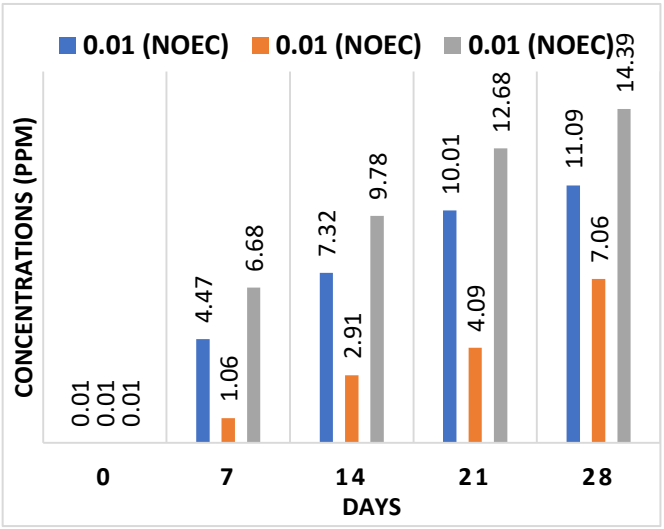

Fig 3: Tissue concentration of metal components in Jotun paint for NOEC on *Tilapia guineensis*

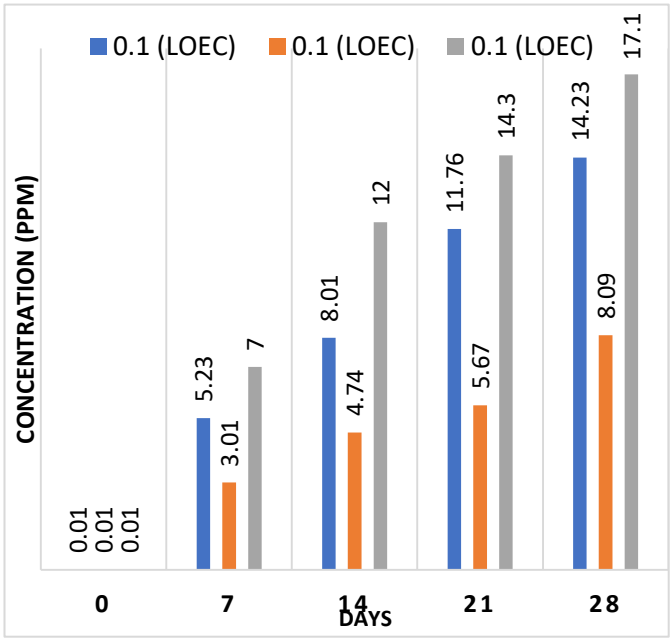

Figure 4: Tissue concentration of metal components in Jotun paint for LOEC on *Tilapia guineensis*

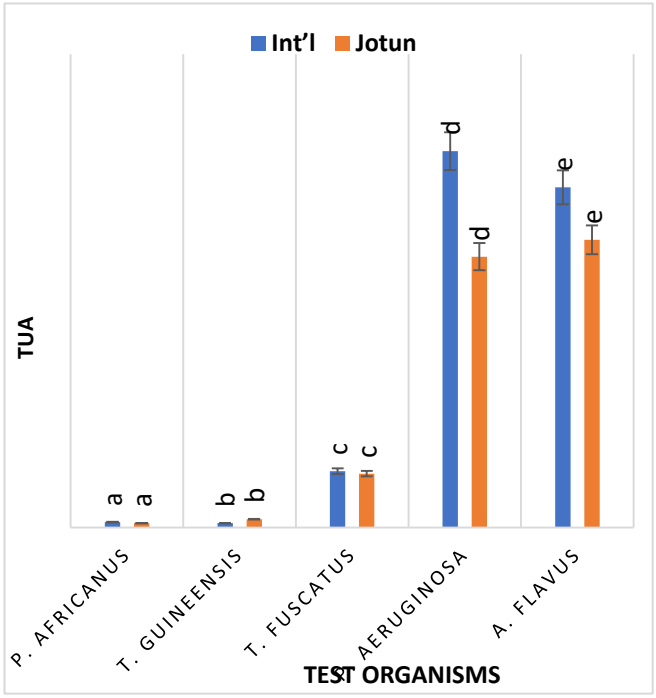

Figure 5: Average Acute Toxicity unit (TUa) for Int'l paint and Jotun Paint on the different test organisms

Note: Bars for a given test organism with the same alphabet are not statistically significant

5. CONCLUSION

In conclusion, the ecotoxicity assessment of selected marine paints on *Tilapia guineensis*, *Palaemonetes africanus*, *Tympanotonus fuscatus*, *Aspergillus flavus*, and *Pseudomonas aeruginosa* reveals varying degrees of toxicity across species. The findings indicate that these paints pose significant environmental risks to aquatic organisms, potentially disrupting ecological balance. *Tilapia guineensis* and *Palaemonetes africanus* showed considerable sensitivity, suggesting the need for stringent regulation of marine paint usage to minimize ecological harm. Additionally, the study highlights the importance of developing less toxic alternatives to protect marine biodiversity and ecosystem health.

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Conflict of Interest Declaration

The author(s) declare that for this article, they have no actual, potential, or perceived conflict of interest.

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