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Comparative Eco-toxicological Effect of Corrosion Inhibitors on Aquatic Nitrogen Fixing Bacterium in Fresh and Brackish Water Ecosystem

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aquatic nitrogen-fixing bacteria play a crucial role in maintaining the nitrogen cycle and supporting primary productivity in freshwater and brackish water ecosystems. The activities of oil and gas industries operating within the coastal region of PortHarcourt releases effluents that contain various chemicals including corrosion inhibitors into the aquatic environment which can significantly affect the aquatic organisms including the nitrogen-fixing bacteria. Hence, this study was aimed to determine the comparative eco-toxicological effect of corrosion inhibitors on aquatic nitrogen fixing bacterium (*Nitrosomonas* sp.) in Fresh and Brackish water ecosystem. Fresh water and brackish water samples were collected from Chokocho stream in Etche Local Government Area and Eagle Island Port Harcourt, all in Rivers State, Nigeria. Standard toxicity procedure was applied on rectangular cut-out crude oil pipeline metal (ME) coated with corrosion inhibitors: Ambercil (AMB) and X-PRO 99 inhibitor (XPRO) immersed in freshwater (FW) prepared at concentrations of 25%, 50%, 75% and 100% for *Nitrosomons sp*. These inhibitors were tested with *Nitrosomonas sp.* at 0,

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4, 8, 12 and 24hrs separately for each toxicant. The median lethal concentration (LC_{50}) was employed to compare the toxicities of the different toxicants on the test organisms. The result for percentage survival of *Nitrosomonas* sp decreased when exposed to various concentrations listed above for 24. The LC_{50} (%) result (noting, that the lower the $LC_{50,}$ the more toxic the toxicant): of *Nitrosomonas* sp decreased in the following order: FW + ME (85.5) > BW + ME (86.7) > Both FW + AMB & FW + XPRO (86.8) > BW + XPRO (87.9) > BW + ME + XPRO (89.1) > BW + AMB (89.7) > FW + ME + XPRO (94.9). The above results revealed that *Nitrosomonas* sp decreased in percentage survival with an increase in concentration. The result showed that the effluents of metals coated with corrosion inhibitors (ME + AMB & ME + XPRO) were more toxic to *Nitrosomonas* sp in brackish water than freshwater while the effluents of XPRO & AMB not coated on metals were more toxic in freshwater than in brackish water. In conclusion, corrosion inhibitors when coated on metals, AMB corrosion inhibitor was more toxic than XPRO corrosion inhibitors in brackish and freshwater. Based on the findings, the use of XPRO corrosion inhibitor should be a best option for coating crude oil pipeline due to its relative low toxicity in upstream sector of the Nigeria petroleum industry.

Keywords: Eco-toxicology; corrosion inhibitors; comparative; aquatic ecosystem.

1. INTRODUCTION

Corrosion inhibitors are chemical compounds that are added to different systems to reduce the corrosion rate of metals. They function by forming a protective layer on the metal surface, preventing the corrosive agents from reaching the metal. Commonly used corrosion inhibitors include organic compounds such as amines, imidazolines, and phosphates [1]. After use, these chemical compounds do not undergo any chemical changes. With concentrations below inhibitory limits, CIs like QUATs have become commonplace and can be found in a variety of settings, including surface water, wastewater, soil and aquatic sediments. Corrosion inhibitors are widely used in various industries to protect metal surfaces from corrosion Udotong et al., [2]. However, their potential ecotoxicological effects on aquatic ecosystems, particularly on nitrogen-fixing bacteria, remain largely unexplored. Nitrogen-fixing bacteria are microorganisms capable of converting atmospheric nitrogen (N2) into a biologically useful form, such as ammonia (NH3) or nitrate (NO3-), through the process of nitrogen fixation, these nitrogen-fixing bacteria especially *Nitrosomonas* species play a crucial role in maintaining the nitrogen cycle and supporting primary productivity in freshwater and brackish water ecosystems. However, various anthropogenic stressors, such as pollution and habitat degradation, pose significant threats to these bacteria [3]. The impacts that have been discussed are potential effects that can be avoided, diminished, or managed with prudence. The industry has been successful in creating management systems, business practices, and engineering technologies aimed at lowering environmental effects, and as a result, the number of environmental mishaps has significantly decreased [4]. The main environmental changes that occur during the production of crude oil and natural gas within the oil and gas industry are production practices as well as long-term ecosystem changes (including plant part repairs or replacements, waste disposal (e.g., processed water), noise (e.g., from good operations, compressor or pump stations, flare stack, vehicle, and equipment), worker involvement, and potential leaks are all factors to consider) decreased [4].

Most common among corrosion inhibitors are compounds with a long aliphatic chain attached. It can be seen that within one functional group, the increase in the side chain, which means an increase in the hydrophobicity of organic compounds, increases the toxicity. The number in mg/L are LC50 (96 hr) values. One has to remember that the smaller the LC50 value, the larger the toxicity, and vice-versa. Examples are cited below in which the LC50 values are compared. a. Aliphatic amines: The toxicity of n-butyl amine is 267 mg/L. It increases as we increase the length of the side chain and reaches 1.0146 mg/L for n-decyl amine. b. Acetylenic alcohols: Increase from 10.09 mg/L for 2-butyn-3-ol to 0.412 mg/L for I-octyn- ~ c. Fatty acids: The toxicity increases from 4688.7 mg/L for propionic acid to 103.8 mg/L as one reaches nonoic acid. d. Aliphatic Nitriles: The toxicity of acetonitrile is 1640 mg/L. It increases to 0.429 mg/L for dodecanitrile (Cl~). A theoretical explanation for non-reactive toxicity was first developed by Overton and Meyer in the late 19th century [5]. Their theory relates the toxicity of a

non-reactive chemical to its volubility in lipids. Ferguson extended this theory in 1939 by applying the concept of thermodynamic activity. In an aqueous environment, the physiological effect of a chemical can be related to its activity in the aqueous phase. Thus, as aqueous solubility increases, the concentration of toxicant required to produce a given biological effect increases. Since hydrophobicity k s related to the non-polar nature and hence to its volubility, an increase in hydrophobicity (e.g. a long alkyl chain) makes the organic compound more and more soluble into the lipid layers of the cell membranes and hence exerts damage upon them. Hence one can make a general statement: hydrophobicity is a killer. These chemicals also make hydrophobic binding in pockets of specific enzymes in the central nervous system. This is the reason why empirical methods like molecular connectivity indices have been able to estimate the toxicity of non-polar organic compounds so well. This study was therefore aimed to comparatively assess the ecotoxicological effects of corrosion inhibitors on aquatic nitrogen-fixing bacteria in both the fresh and brackish water ecosystems [5].

2. MATERIALS AND METHODS

2.1 Sample Collection/Study Area

Freshwater was obtained from Chokocho stream in Etche Local Government Area, while brackish water sample was collected from Eagle Island Port Harcourt, all in Rivers State, Nigeria, these samples were collected in sterile plastic containers and transported with ice pack to the Laboratory in the Department of Microbiology, Rivers State University, Port Harcourt, Nigeria, for analyses within 24 hours. Two products of corrosion inhibitors designated as product AMB and XPRO were purchased from Garrison junction, Aba road, Port Harcourt.

2.2 Isolation *Nitrosomonas* Species

The total *Nitrosomonas* sp*.* in fresh and brackish water was isolated and enumerated using microbiological standard methods [6]. Winogradsky Agar medium composition as modified by Odokuma and Nrior, (2015) was used: Agar agar 15.0g/l, $FeSO_4.7H_2O$ 0.4g/l, NaCl 2.0g/l, K_2HPO_4 1.0g/l, $MgSO_4$.$7H_2O$ 0.5g/l, and $(NH_4)_2SO_4$ 2.0g/l were dissolved in 1000ml of distilled water and autoclaved at 121ºC (psi) for 15 minutes after which it was allowed to cool to about 40^0C and the medium was poured on the Petri-dishes. Then, the medium was allowed to solidify before progress to the hot air oven to dry the moisture. An aliquot from 10^{-2} dilution of serially diluted freshwater and brackish water respectively were inoculated unto the prepared and oven dried Winogradsky agar and incubated for 48hours at room temperature (30± 2ºc), after which the colonies were counted and the average of the count recorded accordingly. Pure isolate from the respective media was characterized and identified based on its morphological, biochemical and physiological features [6-8]. Grayish, mucoid, flat colonies revealed pear-shaped, and Gram negative of *Nitrosomonas.*

2.3 Sample Processing

2.3.1 Effluent preparation

Two products of corrosion inhibitors were aseptically forced open and 20 ml of each product was measured and carefully applied to a pipeline metal then allow to dried for one week. The metal with the respective corrosion inhibitor was stock in 1500ml of the water samples: freshwater, and brackish respectively allowed for another one month which served as effluent toxicant with metal for the toxicity testing [5]. Also 20 ml of the respective corrosion inhibitor was measured and transferred to 1500ml of the water samples and allowed from one month for effluent without metals.

2.3.2 Toxicity test procedure

The test was carried out in five (5) separate test tubes containing appropriately habitat water: fresh and brackish water habitat of the test organism separately in each of the test tubes, the toxicant concentrations used were (%); 25, 50, 75 and 100 each effluent toxicant [9,10]. The control contains the water sample (habitat water) and the organism without toxicant. Test procedure for the test organisms from freshwater, and brackish water; One milliliter (1 ml) of the test organism was added to each toxicant concentration in test tubes. Aliquot (0.1 ml) from each of the concentrations respectively set-up was then plated out using spread plate technique on media Winogradsky, agar immediately after inoculation as zero (0) hour, inoculation and spreading continues after 4, 8, 12 and 24hours respectively and was incubated for 24 hours at 37± 2ºC. After incubation the total viable count on the plate were taken and converted to $\log_{10}$ [11,3].

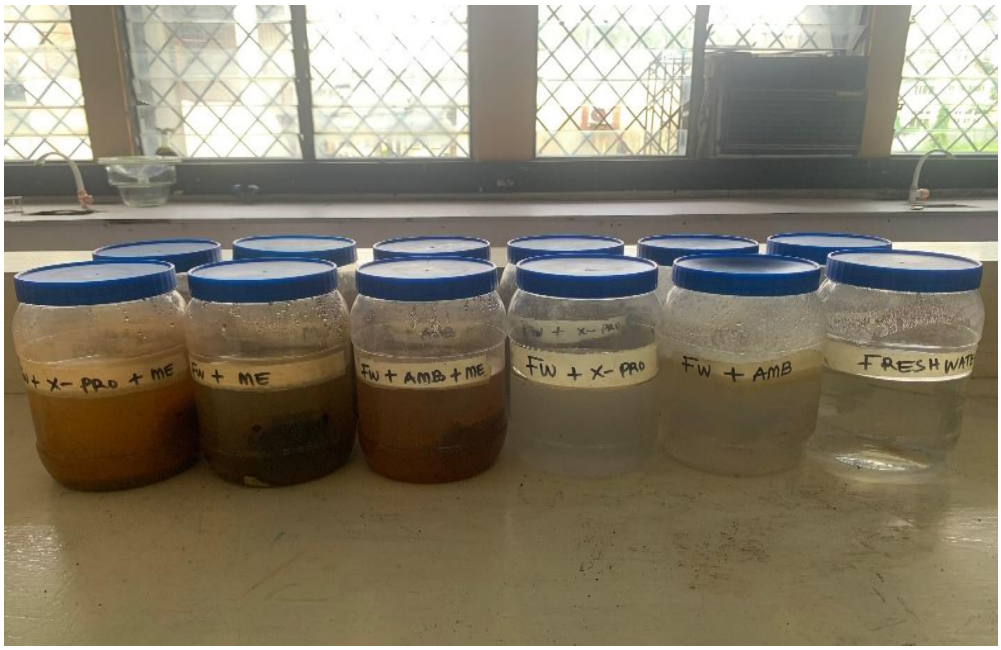

Plate 1. Effluent preparation of toxicants (metals with corrosion inhibitors soaked in fresh and brackish water for 30 days before toxicity setup)

2.3.3 Determination of percentage log survival of the microorganism

The percentage log survival of the test organisms was determined according to the method adopted by Nrior and Kpormon [5] dividing logarithmic count of the toxicant concentration, by the logarithmic count of the control and multiplying by 100.

$$\text{Percentage (\%) logarithmic survival} = \frac{\text{Log C}}{\text{Log c}} \times 100$$

Where;

Log **C** = logarithmic count of the toxicant,
Log c = logarithmic count of the control

2.4 Percentage Log Mortality

The Percentage (%) log mortality of the test organisms exposed to the toxicant were determined by subtracting the one hundred from the value of the percentage log survival
Percentage (%) = 100 - % log survivals

2.5 Median Lethal Concentration (LC_{50})

The median lethal concentration of the corrosion inhibitor on the test organisms in both aquatic ecosystems were determined by subtracting the value of the highest concentration used (75%) from the sum of concentration difference, multiply by mean percentage mortality and divide by the control (100).

$$LC_{50} = LC_{100} - \frac{(\sum \text{conc. Diff.} \times \text{mean \% mortality})}{\text{\% control}}$$

2.6 Statistical Analysis

The data obtained during the study was analyzed statistically using a computer-based program, SPSS version 22 for analysis of variance (ANOVA) of the data in the respective ecosystems.

3. RESULTS AND DISCUSSION

The result of the log survival count showed the sensitivity of the *Nitrosomonas Sp* to the toxicity of corrosion inhibitors (XPRO and AMB) in du-aquatic water with effect to their salinity. The log survival and mortality shown in Table 1 to Table 2 indicates that the test organism showed a decrease in log survival and an increase in log mortality as concentration increased from 25% to 100% at 0 hours, 4 hours, 8 hours, 12 hours and 24 hours in both the fresh and brackish water respectively. Fig. 1 showed the summary of median lethal concentration of the toxicant on the test organism (*Nitrosomonas sp*) in the di aquatic ecosystem.

Table 1. Percentage survival of *Nitrosomonas sp* exposed to corrosion inhibitors in freshwater

Toxicant Conc. (%)	Treatment				
	ME	AMB	ME + XPRO	ME + AMB	XPRO
0	100±0.0 ^{d}	100±0.0 ^{d}	100±0.0 ^{c}	100±0.0 ^{d}	100±0.0 ^{d}
25	91.0±2.2^{c}	93.1±3.0 ^{c}	97.3±0.8 ^{b}	92.4±2.9 ^{c}	93.1±3.0 ^{c}
50	88.6±3.1^{c}	89.6±2.9 ^{c}	95.1±0.6 ^{ab}	89.2±2.7 ^{bc}	89.6±2.9 ^{c}
75	83.4±2.5^{b}	85.3±1.3 ^{b}	93.7±3.2 ^{a}	86.8±1.8 ^{b}	85.4±1.3 ^{b}
100	78.9±3.8^{a}	78.9±4.2 ^{a}	93.0±3.4 ^{a}	82.1±2.6 ^{a}	78.9±4.2 ^{a}

Key: ME= metal, AMB= Ambersil corrosion inhibitor, XPRO= X-pro corrosion inhibitor

Table 2. Percentage survival of *Nitrosomonas* spp. exposed to corrosion inhibitors in brackish water

Toxicant Conc. (%)	Treatment				
	ME	AMB	ME + XPRO	ME + AMB	XPRO
0	100±0.0^{d}	100±0.0 ^{c}	100±0.0 ^{c}	100±0.0 ^{d}	100±0.0 ^{e}
25	92.6±4.6^{c}	91.8±4.9 ^{b}	93.5±3.0 ^{b}	96.1±2.7 ^{c}	95.4±2.4 ^{d}
50	91.3±2.7 ^{bc}	89.9±5.6 ^{b}	90.9±2.6 ^{c}	93.2±4.4^{bc}	90.9±2.6 ^{c}
75	85.9±3.9^{b}	880.8±6.3^{a}	85.5±3.4 ^{b}	88.3±6.5 ^{b}	85.5±3.4 ^{b}
100	76.7±3.9^{a}	4.8±6.2 ^{ab}	84.1±2.6 ^{a}	81.0±6.3 ^{a}	79.8±3.7 ^{a}

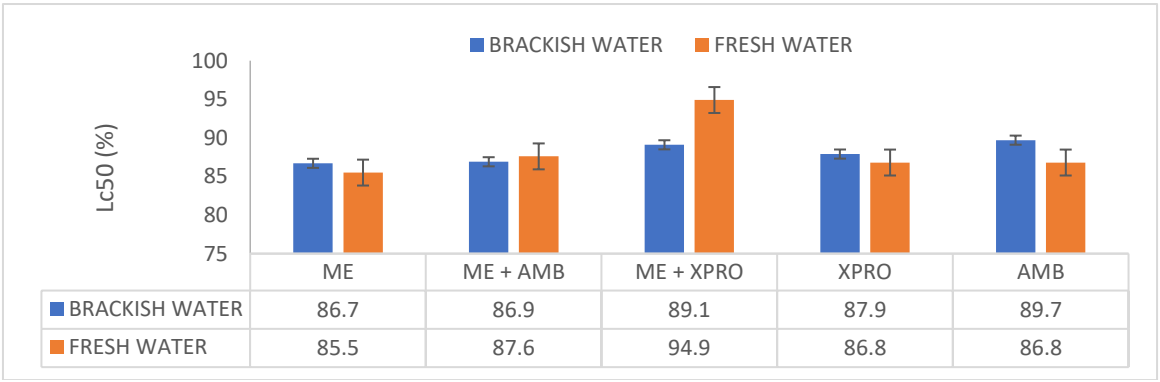

Fig. 1. Summary of median lethal concentration of the toxicant on the test organism (*Nitrosomonas sp*) in the di aquatic ecosystem

From the study, the increase in the mortality of the test organisms in the aquatic ecosystems increased with increase in the exposure time. This is in line with the study of Nrior and Kpormon [5] in the study to evaluate and compare the effect of used phone batteries on *Nitrosomonas* spp. in tri aquatic bodies in which there was reduction in the percentage survival of the test organisms as the time of exposure increased. The toxicity of the corrosion inhibitor and the metal effluent (ME) produced range of mortality result on the test organisms in the di-aquatic ecosystems as shown in this study. The effluents from rusted metals (ME) were observed to be more lethal to the survival of the *Nitrosomonas* in both the fresh and brackish water as least mean lethal concentration (LC_{50}) was recorded compared to the both inhibitors (AMB and XPRO) and their combinations however the LC_{50} recorded by these toxicants to the *Nitrosomonas* in this study were high (with the range of 86.7% to 89.9 in brackish water and 85.5% to 94.9%) compared to those recorded in many other studies [12]. The mean lethal concentration (LC_{50}) which is used to determine the amount or concentration of material or toxicant that is expected to kill 50% of the test organism when there is exposure to the environment [2,3]. The lower the LC_{50} of a substance, the more toxic the substance [3]. The higher toxicity recorded by the metal effluent can be attributed to the presence of rust (Fe_2O_3) in this effluent formed through electrochemical reaction [13]. The mean lethal concentration of the different toxicants (of the corrosion inhibitors and the combination to the metal effluent) in the brackish water to *Nitrosomonas* was recorded as 86.7% for ME < 86.9% for ME+AMB < 87.9 for

XPRO < 89.1% for ME+XPRO < 89.9% for AMB while the LC_{50} of the toxicants to *Nitrosomonas* in fresh water was recorded as 85.5% for ME < 86.8% for XPRO and AMB < 87.65 for ME+AMB < 94.9% for ME+XPRO. The effect of metal effluent from corrosion can result in bioaccumulation of heavy metals which results in the reduction of the population of some important aquatic microorganisms including Ammonia oxidizing bacteria (AOB) such as *Nitrosomonas* [14,15]. The reduction of *Nitrosomonas* in the environment by most chemicals results in the distortion of an important phase in nitrification process and this has been reported by other studies [16,17].

4. CONCLUSION AND RECOMMENDA-TIONS

Understanding the comparative ecotoxicological effects of corrosion inhibitors on nitrogen-fixing bacterium in fresh and brackish water ecosystems is crucial for sustainable industrial practices and the preservation of aquatic biodiversity. The findings of this study provide valuable insights into the ecotoxicological effects of corrosion inhibitors on nitrogen-fixing bacterium in aquatic ecosystems. The results can contribute to the development of guidelines and recommendations for the safe use of corrosion inhibitors in industries, aiming to minimize their potential negative impacts on aquatic ecosystems and the overall nitrogen cycle. Therefore it is essential to recommend the e further education oil and gas industry operators on the usage of eco-friendly products to reduce the hazardous load on the environment and to enforce stricter regulations for the disposal of effluents containing corrosion inhibitors into the surrounding aquatic environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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