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# Effect of Tetrakis (Hydroxymethyl) Phosphonium Sulphate Biocide on Metal Loss in Mild Steel Coupons Buried in a Water-logged Soil

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**Abstract** Mild steel strip coupons were buried in water-logged clay soil sitesin the Niger Delta for 190 days, with one site untreated and the other site treated with a tetrakis (hydroxymethyl) phosphoniumsulphate (THPS)-based biocide. Post-exposure analysis of the coupons showed that there was an increasing trend in metal loss in the coupons as the exposure days increased, for both the untreated and treated soil sites. The trend of metal loss showed an average cumulative increase of 46.7% in the untreated soil site and 34.3% in the treated soil site. Average percentage weight loss (APWL) after the 40, 100 and 190-day observational periods, were 2.3%, 5.5% and 8.5% respectively, in the untreated soil; and 1.2%, 2.0% and 2.8% respectively, in the treated soil. Over the period, there was a cumulative 5.4% metal loss in the coupons from the untreated soil and 2.0% in the treated soil. With biocide treatment of the soil, there was a 59.5% decrease in cumulative APWL, comparing the untreated soil and the treated soil sites during each of the observational periods. Total bacterial counts determined by quantitative polymerase chain reaction (qPCR) showed a 5-log, 2-log and 1-log reduction in total bacterial counts after 40, 100 and 190 days, respectively, representing between 94-100% reduction in the bacterial numbersin the soil treated with 250 ppm of the biocide.

***Keywords:*** *THPS, biocide, mild steel, water-logged, APWL, Niger Delta*

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## 1. Introduction

Corrosion results from physicochemical interactions between metallic materials and the environment. The soil is one such environment that can cause corrosion in metals. Soil can be considered a porous heterogeneous system with colloidal characteristics. The holes between soil particles can be filled with water and/or gas [1]. When soil is compared to another medium, such as the atmosphere or sea water, it is difficult to make a classification of its aggressiveness because of its complexity [2]. Exposure of metals to soil environments accelerates the deterioration of metals due to soil conditions. The corrosiveness of soil can be defined as the capacity of producing and developing the corrosion phenomenon. Soil is defined as an electrolyte and can be studied by electrochemical methods [3].

Several factors affect the corrosivity of soils to metals including moisture content, pH, resistivity, salts, and bacteria, amongst others.Acid soils are aggressive to metals and alkaline soils are benign [4]. Soil corrosion is a geologic hazard that affects buried metals and concrete that is in direct contact with soil or bedrock. Soil corrosion is a complex phenomenon, with a multitude of variables involved. Pitting corrosion and stress-corrosion cracking (SCC) are a result of soil corrosion, which leads to underground oil and gas transmission pipeline failures [5].

The corrosion of metals in soils is enhanced by the presence of a wide range of microbial flora in the soil. Biocorrosion results from interactions – which are often synergistic – between the metal surface, abiotic corrosion products, and bacterial cells and their metabolites. The latter include organic and inorganic acids and volatile compounds such as ammonia and hydrogen sulfide [6].

Microbial colonization of metals and alloys of industrial usage takes place through the formation of biofilms. Simply put, biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface. Basically, biofilms consist of bacteria, extracellular polymeric substances (EPS) and water. Biofilms exist wherever surfaces contact water. More than 99% of all bacteria live in biofilm communities and biofilms can cause problems including corroding pipes [7].

The term, microbiologically influenced corrosion or MIC, is used to designate corrosion resulting from the presence and activities of microorganisms within biofilms on metal surfaces. Microorganisms can accelerate rates of partial reactions in corrosion processes and shift the mechanism for corrosion. Microbiologically influenced corrosion has received increased attention by corrosion

scientists and engineers in recent years with the development of surface analytical and electrochemical techniques that can quantify the impact of microbes on electrochemical phenomena and provide details of corrosion mechanisms. Microbiologically influenced corrosion has been documented for metals exposed to sea water, fresh water, demineralized water, process chemicals, food stuff, soils, aircraft fuels, human plasma, and sewage [8].

The main types of bacteria associated with metals in terrestrial and aquatic habitats are sulfate reducing bacteria (SRB), sulfur-oxidizing bacteria, iron oxidizing/ reducing bacteria, manganese-oxidizing bacteria, and bacteria secreting organic acids and slime [9]. Microbial colonization of metal surfaces drastically changes the classical concept of the electrical interface commonly used in inorganic corrosion: important changes in the type and concentration of ions, pH values, and oxidation–reduction potential are induced by the biofilm, altering the passive or active behavior of the metallic substratum and its corrosion products, as well as the electrochemical variables used for assessing corrosion rates [10]. Therefore, microorganisms influence corrosion by changing the electrochemical conditions at the metal-solution interface. Problems due to biocorrosion and biofouling of industrial systems range from heavy microbiological contamination with consequent energy and efficiency losses, to structural failures owing to corrosion [11].

In carrying out this study, we tried to understand the chances of biocorrosion of mild steel in water-logged soil environment (typical soil-type in the Niger Delta),to establish that microorganismsaffect this process and importantly, to determine the effect of a biocide in limiting the biocorrosion process in the water-logged environment. The significance of this study is that it provides information as guide to the many pipeline-related projects carried out in the vast water-logged soil sites of the Niger Delta, especially by the oil and gas companies.

# 2. Materials and Methods

## 2.1. Site Selection

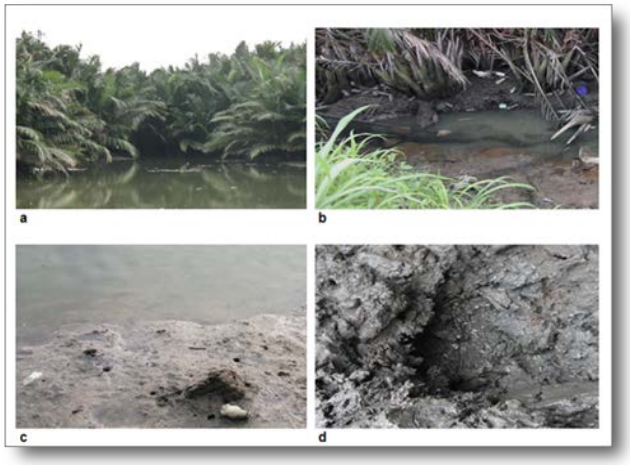


**Figure 1.** Water-logged soil site showing: a – full tide, b – low tide, c – shore-line, d – moist soil

A water-logged swampy clay soil site in the Niger Delta was chosen for this study. Characteristically, soil from this site was wet, smooth, and greyish-black in colour and contained silt and other debris. Pictures of the water-logged site and soil are shown in Figure 1. During the study, mild steel coupons were buried in the soil to a depth of 30cm.

## 2.2. Coupon Specimen and Preparation

Mild steel coupons (MSC) measuring 4”x1” (100mm x 25mm) were used for this study. The pre-weighed coupons were sealed in protective envelopes and were manufactured by Visco Chemicals, Houston.Before use, they were examined for integrity and confirmed to be in good condition by visual examination. During the time of exposure of the coupons, they were carefully taken out of the sealed envelopes and introduced into the untreated and the biocide-treated soil sites.

## 2.3. Pretreatment of Soil with Biocide

To prepare the soil, twenty kilograms (20kg) of the soil was mixed with 5.5 mL of a tetrakis (hydroxymethyl) phosphoniumsulphate (THPS)-based industrial liquid biocide to a concentration of 250 ppm. To ensure proper mixing of the chemical, soil samples were weighed out into four parts of 5kg each. To each of this, 1.1mL of the biocide was added and mixed thoroughly. After this, all of the pre-mixed 5kg samples were then blended together to make the composite 20kg biocide-treated soil for the study. The treated soil was then encapsulated in a cellophane bag (to contain the biocide), the coupons buried in the treated soil and the soil-bag having the coupons was then buried in the soil.

## 2.4. Weight Loss Determination

After 40, 100 and 190 days respectively, the coupons were retrieved from the untreated and treated soil sites and taken to the laboratory for cleaning and analysis to determine the weight loss during the period of exposure. In the laboratory, outer soil debris on the coupons was carefully scrapped-off the surface of the coupon while collecting the debris most proximate to the metal surface for further microbiological analysis. After the surface deposit collection, the coupons were immersed in xylene for 10 minutes and then washed in water. They were then cleaned with 15% HCl and rinsed under running water. Next, the coupons were placed in an oven at 70$^{o}$C for 15 minutes to dry them. After drying, they were placed in a desiccator to cool after which they were weighed using a Mettle Toledo weighing balance (New Classic ML 204, Switzerland). The weight of the retrieved coupons before cleaning was compared to the initial weight, the difference indicating metal loss. The corresponding average percentage weight loss (APWL) was calculated.

## 2.5. Bacterial Quantification and Quantitative Polymerase Chain Reaction Analysis

One gram (1g) of the solid deposit was mixed with 10mL of sterile phosphate buffer saline (PBS) and shaken vigorously. The supernatant was carefully filtered through a 0.22 mm Durapore® membrane Millipore filter. The Millipore filter was air-dried two times by pushing the syringe plunger through the empty barrel twice. The filter

cartridges were packaged in sterile WHIRL-PAK bags and shipped to the laboratory for analysis. Bacterial genomic DNA was isolated from the sample-filtered Millipore filters using the UltraClean Soil DNA Isolation kit (MoBio-Carlsbad, CA) according to the manufacturer's instructions. A DNA fragment, encoding a portion of the bacterial 16S rDNA gene was amplified using 25 mM primers 8F and 338R [12], and 12.5 mL Sybr® Green Real-Time PCR Master Mix (Invitrogen). Amplification reactions were performed using an Applied Biosystems 7500 Real-Time PCR System. The PCR conditions were as follows: 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. A calibration curve was obtained by using a serial dilution of a known concentration of positive control DNA (bacterial 16S rDNA cloned pIDTSMART-AMP vector – IDT). The $C_T$ values that were obtained from each sample were compared with the standard curve to determine the copy number of prokaryotic DNA present in the sample.

## 3. Results and Discussion

### 3.1. Visual Examination

Upon retrieval of the coupons after 40, 100 and 190 days, they were visually examined for any obvious physical changes. This examination of the coupons was done before washing and cleaning them for further analyses. The mild steel coupon before burial in the soil, post-retrieval, and after cleaning, is shown in Figure 2.

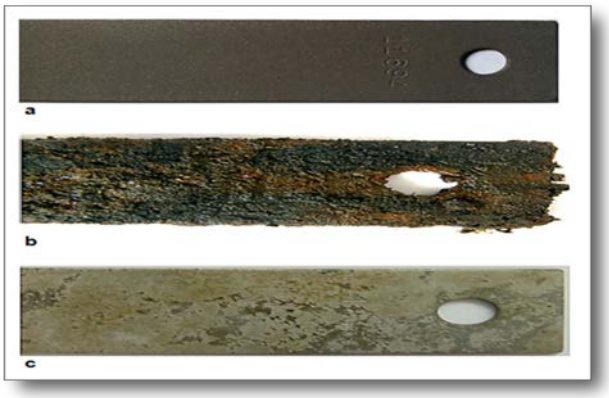


**Figure 2.** Mild steel coupon: a – new, unexposed coupon, b – coupon retrieved from the water-logged soil site, c – coupon after cleaning

### 3.2. Average Percentage Weight Loss (APWL)

Average percentage weight loss in the coupons retrieved from the untreated soil site after 40, 100 and 190 days were 2.3%, 5.5% and 8.5%, respectively, giving an cumulative average of 5.4%; while in the treated site, the APWLs were 1.2%, 2.0% and 2.8%, respectively, for a 2.0% cumulative average (Figure 3).

Comparing the APWLs in the untreated and the treated sitesshowed that there was a 47.8% decrease in APWL in the treated site after 40 days, a 63.6% decrease after 100 days and a 67.1% decrease after 190 days. Overall, this gives acumulative average decrease of 59.5% over the entire observational period.

The results showed that there was more metal loss due to corrosion in the coupons from the untreated site than in those from the treated soil site.With biocide treatment of the soil at a concentration of 250 ppm, there was an apparent effect of the biocide on the bacterial numbers in the soil and consequently on the rate of metal loss in the coupons. The addition of the biocide brought about a reduction in the numbers of bacteria present in the soil and subsequently, a reduction in the biocorrosion activity around and on the coupons.

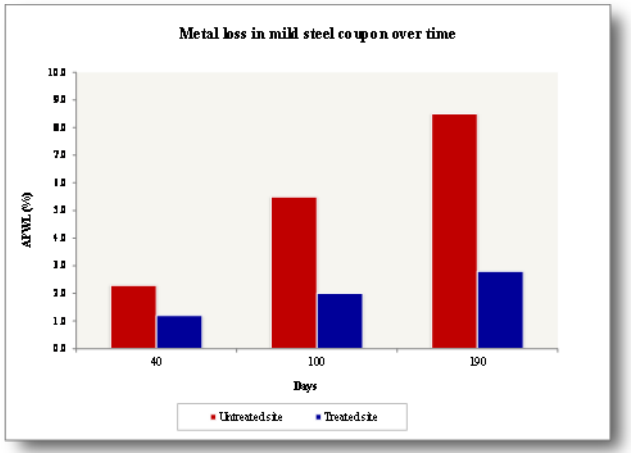


**Figure 3.** Average percentage weight loss (APWL) in mild steel coupons retrieved from untreated and treated water-logged site after 40, 100 and 190 days of soil burial

### 3.3. Total Bacterial Counts

Using the quantitative polymerase chain reaction (qPCR) analysis, total bacterial counts from the untreated soil site after 40 days was $8.56x10^9$ cells/gramand $6.03x10^4$ cells/gram for the treated soil site. This indicates a 100% decrease in bacterial counts. Total bacterial counts for the untreated site after 100 and 190 days were $4.59x10^8$ cells/gram and $2.04x10^9$ cells/gram, respectively (Table 1). Similarly, counts for the treated site were $5.27x10^6$ cells/gram and $1.23x10^8$ cells/gram, respectively after 100 and 190 days. These counts represent decreases of 98.9% and 94% in total bacterial numbers after the 100 and 190-day periods, respectively. Overall, there was a 97.6% decrease in bacterial counts at the treated site during the entire observational period.

**Table 1. Microbial quantification (cells/gram) in untreated and treated soil sites**

| Day | Untreated site | Treated site |
|---|---|---|
| 40 | $8.56 \times 10^9$ | $6.03 \times 10^4$ |
| 100 | $4.59 \times 10^8$ | $5.27 \times 10^6$ |
| 190 | $2.04 \times 10^9$ | $1.23 \times 10^8$ |

There is therefore, evidence of antimicrobial activity in the biocide-treated soil as reflected in the significant reduction in the cell counts in the treated soil site. This may have implied a reduction in microorganism-mediated corrosion of the coupons in the treated soil site.

The results showed that the longer the observational period, the greater the metal loss in the coupons in the water-logged soil, for both the untreated and treated sites. This was evident in the trend of the APWL over the periods of 40, 100 and 190 days. During these periods, APWL in the untreated soil was 2.3%, 5.5% and 8.5% respectively, and 1.2%, 2.0% and 2.8%, respectively, for

the treated soil. The most metal loss occurred after 190 days and the least after 40 days. In their study, [13] found that APWL due to microbiologically influenced corrosion, MIC, caused by *B. cereus-SNB4*, was 0.94%, 3.54% and 32.89% at 50, 100 and 150 days, following exposure of mild steel coupons in wet soils. It is possible that the longer exposure period allowed for increased microbiological activity that may have led to the higher metal losses seen in the 100-day and 190-day samples especially in the untreated site.

Treatment of the soil with the biocide was responsible for the significant reduction in bacterial numbers as no other conditions were altered. In this study, the biocide caused a reduction in total bacterial count in the treated site by as much as 97% over the 190-day observational period. Reference [14] found that viable bacterial counts were reduced 99% by exposure to 0.08 mg of hypochlorous acid (pH 7.0) per liter at 1-2°C for 1 min and [15] found that gluteraldehyde in 2% aqueous alkaline solution is rapidly bactericidal and sporicidal, killing 99.99% of the spores of *Bacillus anthracis* and *Clostridium tetani* in 15 and 30 min, respectively.

In a study to investigate the inactivation of *Staphylococcus aureus* and *Pseudomonas aeruginosa* under different growth conditions (non-agitated tryptone soya broth and tryptone soya agar) by three cationic active chemical disinfectants – benzalkonium chloride, chlorhexidine digluconate and octenidinedihydrochloride – cells of both test organisms were sensitive to all three biocides with *S. aureus* cultures having differences in the reduction factor of up to 5 log steps and *P. aeruginosa*, up to 2.5 log steps [16]. In another study, [17] found that fifty percent of biofilm material of *Burkholderiacepacia* was removed in the first hour of continuous treatment with 24 $mgl^{-1}$ chlorine or 2 $mgl^{-1}$ ozone.These results are consistent with what this study found with regards to the reduction in bacterial counts by treatment of the soil with the THPS biocide.

With the biocide effectively reducing the numbers of bacteria in the treated soil site by as much as 94-100% (considering 40, 100 and 190-day test periods), there was a possible significant reduction in bacterial EPS secretion and a similar reduction in the likelihood of biofilm formation, both of which are important processes for microbiologically influenced corrosion in steel.

## 4. Conclusion

The water-logged soil of the Niger Delta has the potential of initiating and accelerating the corrosion of steel exposed to that environment. Specifically, mild steel has been shown to be susceptible to biocorrosion in the water-logged soil as indicated by metal loss in the coupons. The rate of metal loss in the untreated soil increased as the exposure period increased probably due to a combination of two factors – unaltered microbial numbers and uninterrupted microbial activity. There was evidence that the THPS biocide was effective in reducing the numbers of soil bacteria involved in the biocorrosion process and ultimately, the rate of metal loss in the mild steel coupons.

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## Competing Interests

The authors would like to state that there are no competing interests.

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