Seasonal Variation on the Effect of Heavy Metals (Chromium And Zinc) on the Corrosion Potential of *Desulfovibrio desulfuricans and Desulfovibrio vulgaris* on three Metal Coupons (Stainless Steel, Mild Steel and Carbon Steel) in New Calabar River.

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Abstract
Seasonal variation on the effects of heavy metals (chromium and zinc) on the corrosion potential of Desulfovibrio desulfuricans and Desulfovibrio vulgaris on three metal coupons (stainless steel, mild steel and carbon steel) in New Calabar River was carried out for a period of one year. The River is a brackish water ecosystem which encourages sulphur reducing bacteria proliferation. Desulfovibrio desulfuricans had its highest growth rate in the dry season at 31MPN/100ml and highest growth rate in rainy season at 26MPN/100ml while Desulfovibrio vulgaris had its highest growth rate in the same dry season at 27MPN/100ml and highest growth rate in the same rainy season at 21MPN/100ml.The heavy metals (chromium and zinc) were present at a level tolerated by the organisms in the dry season at (1.12mg/l and 1.87mg/l) respectively while the levels of chromium and zinc at the beginning of rainy season were at 1.32mg/l and 2.41mg/l respectively. The corrosion rates of the three metal coupons stainless steel, mild steel and carbon steel were also highest in the dry seasons namely 0.109g, 3.192g, and 6.037g respectively. The TTEST value for chromium(stainless steel, mild steel and carbon steel) are 0.010126232, 1.13489E-10, 1.02102E-10 respectively while that of zinc(stainless steel, mild steel, and carbon steel) are 9.93492E-07, 1.49608E-07, 5.63435E-08 respectively. The Correlation value between stainless steel and mild steel is 0.484494.The Correlation value between mild steel and carbon steel is 0.69991 while the Correlation value between stainless steel and carbon steel is 0.865626.

Key words: *Corrosion, Stainless steel, Mild steel, Carbon steel, New Calabar River, Chromium, Zinc.*

Introduction
The rapid urbanization and industrialization of the world have given rise to serious degradation of the environment as a whole (Tume *et al*., 2008, Yang *et al*., 2011).
The quality of the spheres namely biosphere, hydrosphere, atmosphere, pedosphere and litosphere are impeded seriously because of pollution from heavy metals any other pollutant (Lourenco *et al*., 2010, Xia *et al*., 2011). The presence of heavy metals in the atmosphere represents a serious threat to the environment and human. Heavy metals as natural components

of the earth's crust are increasingly found in microbial habitats due to several natural and anthropogenic processes.

According to Moosa *et al*., (2002) current and past mining activities as well as various industrial discharges have contributed large quantities of acid waste waters to the environment and this have affected many microbial habitats as well as their activities.

According to Ali and Malik (2011), Alloway (2004) contamination of urban soils with heavy metals has been studied and documented all over the world and in different disciplines due to their implication on human health. Heavy metals can cause surface and ground water pollution and are taken up by plants, released as gases into the atmosphere or bond semi enduringly by soil components such as organic matter and clay particles which can affect human health (Krishna and Gouil, 2007).

According to Mgbemena *et al*., (2012) the main source of pollution particularly by heavy metals is usually linked with areas of intensive industry and high automobile use. We can also say that the natural sources of heavy metals are weathering of parent rocks. Also there could be several point and non-point sources of heavy metals frequently related to the urban land use, economic, industrial and transportation set up (Fong *et al*., 2008).

According to Maas *et al*., (2010) they considered soil a sink a well as a major source of pollution and most significant entity for risk evaluation. Vehicular emission, commercial fertilizers, atmospheric deposition of contaminants via dust and aerosols, industrial and domestic waste, thermal power stations based on coal fired and energy industry based on fossil fuel burning could be said to be the source of heavy metals in urban soils (Odewande and Abimbola, 2008; Cheng *et al*., 2009).

However microbes have evolved mechanisms to tolerate the presence of heavy metals either by efflux, complexation or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration (Gadd, 1992; Nies and Silver, 1995). The first response to toxic metal contamination is a large reduction in microbial activity (Bader *et al*., 2000). This is confirmed by the fact that habitats that have had high levels of metal contamination for years still have microbial population and activities that are smaller than the microbial population in uncontaminated habitats.

Konopka *et al*., (1999) argued that resistance mechanisms do not offer protection at extremely high levels of free ions and with a lethal toxic effect. Resistance of essential heavy metals such as Cu, Zn, Ni, Co, Cr confront the cell with a special problem because of their requirement to accumulate some of these cations at trace levels and at the same time to reduce cytoplasmic concentrations from potential toxic levels. Resistant bacterial strains solve these problems by a careful regulation that results from the interaction between chromosomally determined cation transport systems and metal resistance system that are mostly determined by plasmids (Brown *et al*., 1999).

Many bacterial resistant systems for toxic metals are encoded by plasmids (Silver, 1996). However, bacterial plasmids contain genes that provide extra functions to the cells among which resistance to toxic metal is very important. Also there is the use of different types of microorganisms such as algae, fungi and bacteria that remove metals from solution (Dubey, 2006).

This study is looking at the seasonal variation on the effect of heavy metals on the corrosibility of two sulfur reducing bacteria on three metal coupons in a brackish water habitat.

Materials and Methods

Study Site

New Calabar River is situated in a low energy coastal environment of the Niger Delta of Rivers State, Nigeria. The water is brackish being influenced by the marine influx associated with tidal cycle. The river is subjected to frequent precipitation throughout the long rainy season of March to October. The dry season starts in November and terminates in March. The edges of the river are covered with green vegetation and some mangrove trees.

Activities along the River

Some industrial activities are present in this brackish environment. One of them coded A.S.L is an oil servicing industry; its activities include building and repair of petroleum pipelines. Another company coded I.D specialize in food packaging. Other minor activities along the river where water samples were collected include various lumbering industries, fish ponds and markets.

Metal Coupons

The steel coupons used for this study namely stainless steel ASTM A316L, mild steel ASTM A283 and carbon steel ASTM A36 was obtained from Nigeria Agip oil company (NAOC).

Heavy Metal Samples

The heavy metals used for this study are compounds of chromium and zinc which were purchased from a standard chemical supply store located at East West road Alakahia, Port Harcourt Rivers State, Nigeria.

Chemical Reagents

All chemical reagents employed in this study were produced by Aldrich Chemical Co, Milwaukee, U.S.A, BDG Chemicals, Poole, England and Sigma Chemical Company, St Louis Missouri, U.S.A.

Ecological Quality Parameters

The following parameters were determined namely pH, Salinity, Conductivity, Total Dissolved Solids, Total Organic Carbon and Heavy Metals (Cr and Zn).
The heavy metals were determined using atomic absorption spectrophotometer (Shangai analytical instrument Co. model AA320).
To measure conductivity, WTW electronic conductivity meter model, Germany was used.
Determination of pH of all the samples was carried out using a pH meter (Jenway model 3015).
Parameters such as Total Dissolved Solids, Total Organic Carbon and Salinity were determined employing methods from (APHA, 2000).
Total Dissolved Solids were determined by gravimetric method, Salinity by Argentometric method and Total Organic Carbon by Rapid Oxidation method.

Microbiological Analysis

After weighing the metal coupons, postgate broth was used to enumerate the sulfur reducing bacteria using the conventional five tubes most probable number method (MPN). Postgate broth samples (10ml, 1ml and 0.1ml) were placed in a series of five tubes. After sterilization they were inoculated with dilutions of scrapings from the metal coupons and enumerated after 7days of incubation in an anaerobic gas jar at room temperature.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the heavy metal resistant bacteria isolates were determined by gradually increasing the concentration of the heavy metals by 0.2mg/l each time on the postgate broth medium until the strain failed to give black precipitate indicating that growth had stopped taking place.

Statistical Analysis

Correlation analysis and students T-test from excel 2010 was employed where value of P<5% was considered to be significant and P>5% was considered as not significant for T-test, whereas for correlation analysis +1 (perfect correlation) through 0 (no correlation) to -1 (perfect negative correlation) were taken into consideration.

Results

Table1. Population of microorganisms isolated from the brackish water habitat.

months	Sulphate reducing bacteria MPN index per 100ml	
	Desulfovibrio desulfuricans	*Desulfovibrio vulgaris*
April	11	9
May	17	12
June	26	21
July	9	14
August	5	8
September	4	8
October	<2	3
November	2	4
December	7	7
January	12	14
February	22	20
March	31	27

Table1b: Control Population of Microorganisms Isolated From The Brackish Water Habitat Upstream.

months	Sulphate reducing bacteria MPN index per 100ml	
	Desulfovibrio desulfuricans	*Desulfovibrio vulgaris*
April	16	11
May	20	14
June	28	20
July	25	18
August	19	15
September	12	11
October	10	11
November	9	14
December	15	17
January	23	19
February	29	24
March	36	29

Table 2. Chemical composition of metal coupons.

Element Composition (%)	Carbon steel	Mild steel	Stainless steel
Carbon	0.008-0.20	0.15-0.25	0.03 maximum
Manganese	0.45-0.65	0.45-0.65	2.0 maximum
Phosphorus	-	-	0.04 maximum
Sulphur	-	-	0.03 maximum
Chromium	-	-	16-18
Nickel	-	-	10-14
Silicon	0.25-0.60	0.25-0.60	1.0 maximum
Molybdenum	-	-	2.0-3.0
Copper	0.60	0.60	-

Table 3: Minimum inhibition concentration.

Sulphur Reducing Bacteria	Zinc	Chromium
Desulfovibrio desulfuricans	2.2mg/l	1.2mg/l
Desulfovibrio vulgaris	2.0mg/l	1.0mg/l

Results in table 1 are the bacterial count of anaerobic organisms isolated from the brackish water habitat from April 2017 to March 2018. *Desulfovibrio desulfuricans* increased from 11-26 MPN index per 100ml from April to June, decreased from 9-<2 MPN index per 100ml from July to October, and increased again from 2-31 MPN index per 100ml from November to March.

Results in table 1b served as control for bacterial count of anaerobic organisms isolated from the brackish water habitat from April 2017 to March 2018. *Desulfovibrio desulfuricans* increased from 16-28MPN index per 100ml from April to June, decreased from July to November from 25-9 MPN index per 100ml, increased again from 15-36 MPN index per 100ml from December to March the next year.
Desulfovibrio vulgaris increased from 9-21 MPN index per 100ml from April to June, decreased from 14-3 MPN index per 100ml from July to October, and also increased from 4-27 MPN index per 100ml from November to March.
The chemical composition of the various metal coupons namely stainless steel, mild steel and carbon steel used for this study are shown in table 2.
Table 3 is the result of the minimum inhibitory concentration of the heavy metals used for this study namely Zinc and Chromium on the anaerobic organisms which are *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris*.

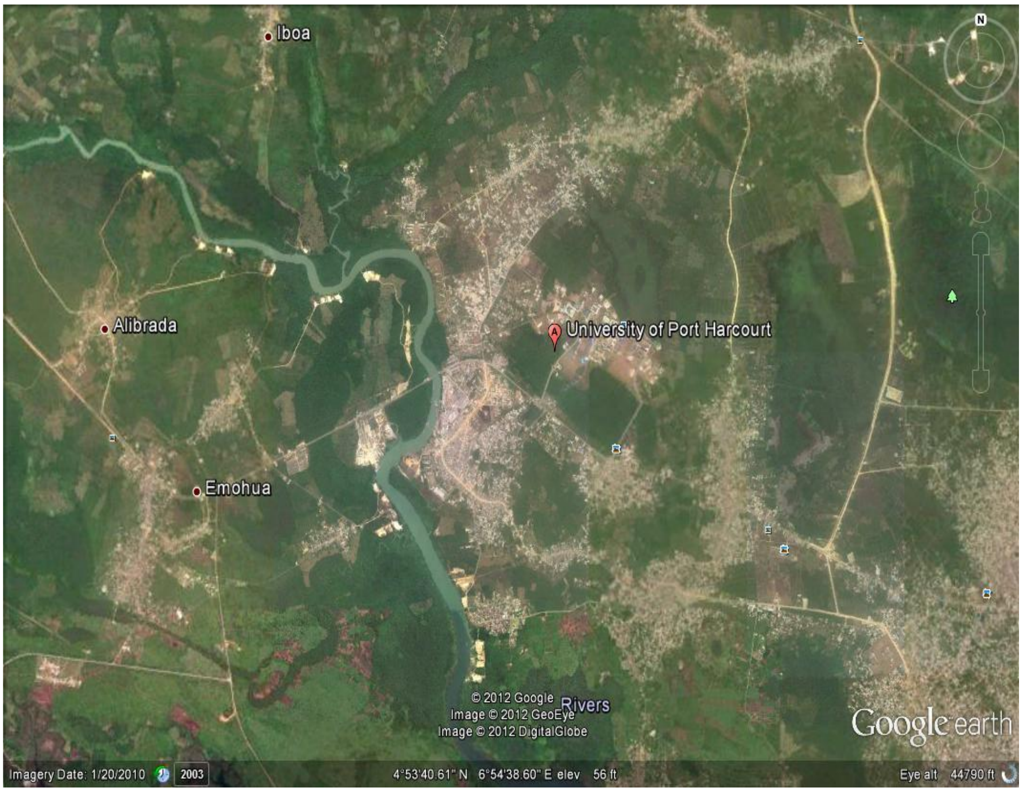

Figure 1. Map of study area New Calabar River.

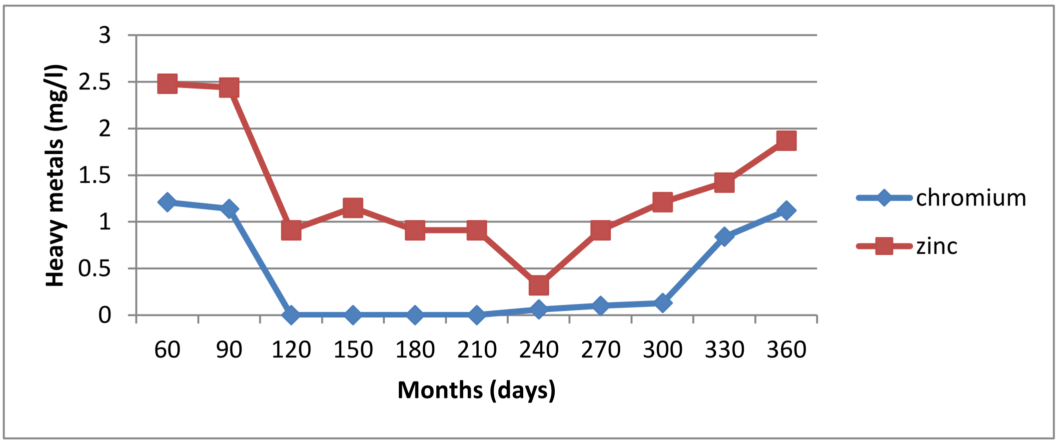

Figure 2. Heavy metals against time.

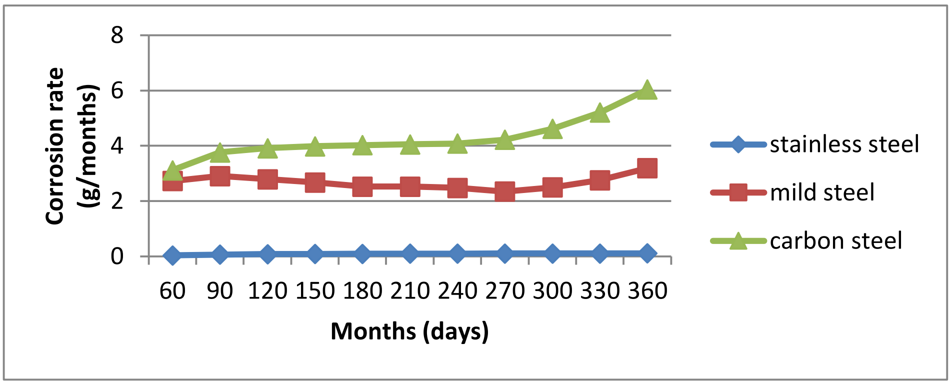

Figure 3. Corrosion rate against time.

Figure1 is the goggle earth map of New Calabar River which is the study area and the river is brackish in nature located in the south-south geopolitical zone of Nigeria.

Figure 2 is concentration of the heavy metals used in this study namely chromium and zinc against time from April 2017 to May 2018. From April to June, we can see that the concentration of chromium and zinc increased from 1.32 to 1.14 and 2.41 to 2.44 respectively. From July to October nothing was recorded for chromium which later started increasing from November to March the next year.
For zinc, there was a decrease in July, little increase in August and another decrease from September to November and increase from December to March the next year.

Figure3 represents the corrosion rates of the three metals used in this study namely stainless steel, mild steel and carbon steel against time from April 2017 to May 2018.
Corrosion rates increased from May to March the next year. For mild steel, corrosion rates increased from April to June and decreased from July to December and increased again from January to March the next year. For carbon steel, corrosion rates followed the same trend like stainless steel which increased from May to March the next year.

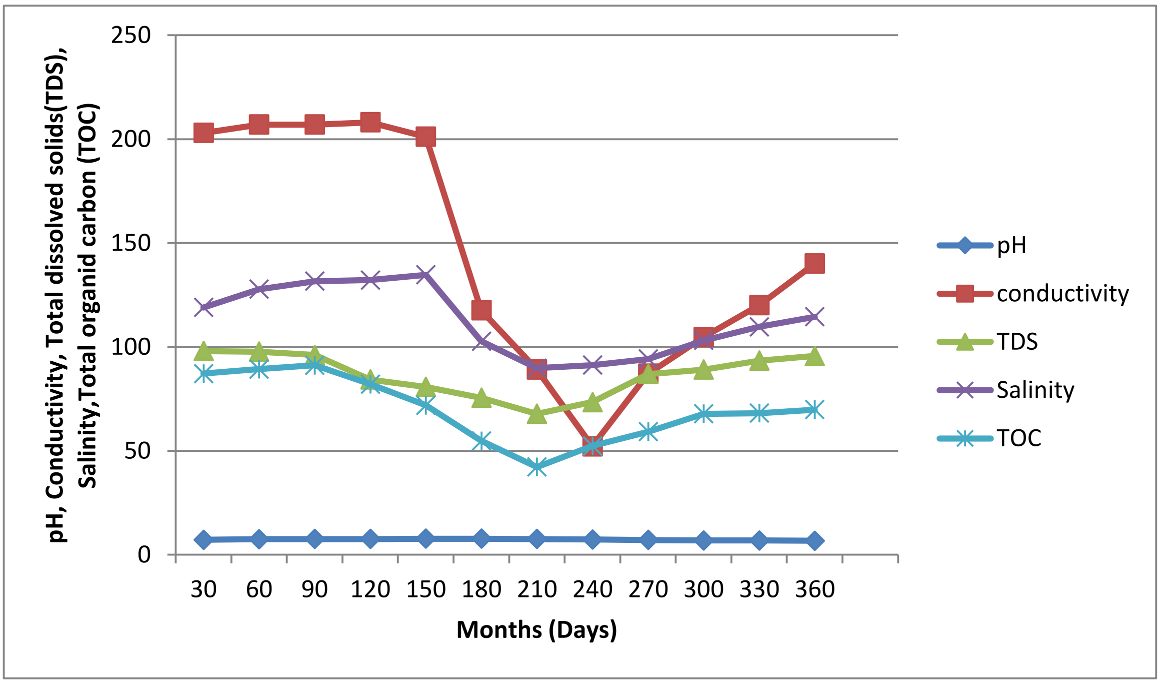

Figure 4. Physico chemical parameters against time.

Figure 4 is a graph of various physico chemical parameters monitored which include the pH, Conductivity, Total dissolved solids (TDS), Salinity, Total organic carbon (TOC).

The pH increased from 7.2 to 7.7 from April to September 2017 and then decreased from 7.6 in October 2017 to 6.7in March the next year.

Conductivity increased from 203µs/cm to 208.11µs/cm from April to July 2017 then decreased from 201.17µs/cm to 52.16µs/cm from August to November 2017 and increased again from 87.12µs/cm in December to 140.09µs/cm in March the next year.

Total Dissolved Solids (TDS) increased from 98.03mg/l to 99.71mg/l from April to May 2017 then decreased from 96.11mg/l to 67.82mg/l from June 2017 to October 2017 then increased from 73.44mg/l in November 2017 to 95.66mg/l in March the next year.

Salinity increased from 119.01mg/l to 134.71mg/l from April to August 2017, decreased from 102.77mg/l in September to 91.24mg/l in November 2017 then increased again from 94.17mg/l in December 2017 to 114.54mg/l in March the next year.

Total Organic Carbon increased from 87.14mg/l to 91.17mg/l from April to June 2017, and then decreased from 71.92mg/l to 42.26mg/l from August to October 2017, increased again from 52.33mg/l in November 2017 to 69.87mg/l in March the next year.

Discussion

The microorganisms isolate for this study in the brackish water environment New Calabar river are Desulfovibrio desulfuricans and Desulfovibrio vulgaris which are both sulphur reducing bacteria as shown in table 1.

From the MPN index per 100ml we can say that Desulfovibrio desulfuricans was a little more abundant in the brackish water New calabar river than Desulfovibrio vulgaris. These organisms play a role in corrosion by inducing oxygen gradient which accelerates corrosion by acting as a depolarizer to form ferrous ions and oxidizing ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}). This latter reaction normally take place in pH values higher than 4 as observed in the pH values found in this study where the highest pH was at a value of 7.7 and the lowest pH was at a value of 6.7.

Microorganisms especially Desulfovibrio desulfricans isolated from this study depolarize surface by directly removing corrosion products such as hydrogen formed at the cathode and this depolarization encourages biocorrosion and this maintains corrosion current (Battersby *et al.*,1985).

Microorganisms are also known to destroy protective coatings of various types and this is attribute to Desulfovibrio desulfuricans which produce hydrogen sulphide which cause hydrogen blistering and embrittlement in metals and structural fittings (Raloff, 1985).

Table 2 showing the chemical composition of metal coupons showed that the alloying elements of stainless steel namely chromium and nickel offered great protection to the metal coupon thereby leading to minimal corrosion recorded in both rainy and dry season unlike the other metal coupons used in this study. This could be said to be attributed to the fact that the alloying elements occur in the lower part of the electrochemical series and are less reactive in nature.

The less corrosion protection witnessed by mild steel coupons and carbon steel coupons used in this study could be due to the fact that they are made of manganese, phosphorous, copper, carbon and silicon which are more reactive in nature hence giving these metal coupons less protection from corrosion.

According to Jaganathan et al., (2011) chromium and nickel are more toxic to microorganisms to higher levels of speciation. They showed that 13% chromium is required for stable passivity of Fe-Cr alloy in acidic and neutral solution not containing inhibitors.

From this study we can see that corrosion rates, heavy metal concentrations and microbial population could be said to have reduced in the rainy season than in the dry season where some significant increase was observed. This could be attributed to runoffs and dilution of the water bodies by heavy rains that increased the water bodies.

This study showed higher proliferation of microorganisms in areas with little or no concentration of heavy metals which was used as the control as shown in table 1b. This is in accordance to the works of Badder *et al*., (2000) which states that the first response to toxic metal contamination is a large reduction in microbial activity. This is confirmed by the fact that habitats which have had high levels of metal contamination for years still have microbial population and activities that are smaller than the microbial population in uncontaminated habitats.

The minimum inhibition concentration of zinc and chromium for *Desulfovibrio desulfuricans* is 2.2mg/l and 1.2mg/l respectively while that of *Desulfovibrio vulgaris* is 2.0mg/l and 1.0mg/l respectively. From the study we can say that these two sulphur reducing bacteria corroded the metal coupons more when the minimum concentration of the heavy metals were not toxic for them. This is in accordance to the argument of Konopka et al., (1999) which states that resistance mechanisms in microorganisms do not offer protection at extremely high levels of free metal ions and with a lethal toxic effect. Resistance of essential heavy metals such as Cu, Zn, Ni, Co, Cr confront the cell with a special problem because of their requirement to accumulate some of these cations at trace levels and at same time to reduce cytoplasmic concentrations from potential toxic levels.

The physico-chemical parameters such as Total Organic Carbon, Total Dissolved Solids, Salinity and Conductivity from this study was also high during the dry season than in the rainy season following same trend as corrosion rates of the metal coupons, minimum inhibitory concentration of heavy metals and proliferation of microorganisms apart from the pH which was between 6.7 and 7.7. This could also be attributed to runoffs and heavy rains especially around June, July and August which may have affected the physico-chemical parameters.

There was a significant difference between stainless steel corrosion and chromium concentration. Same to mild steel corrosion and chromium concentration and carbon steel corrosion and chromium concentration. The significant difference was higher in carbon versus chromium than in mild steel versus chromium and stainless steel versus chromium.
There was also significant difference in stainless steel corrosion and zinc concentration, same to mild steel corrosion and zinc concentration and carbon steel corrosion and zinc concentration.
The significant difference was higher in carbon steel corrosion versus zinc concentration followed by mild steel corrosion versus zinc concentration and stainless steel corrosion versus zinc concentration respectively.

Conclusion

From this study we can say that more construction works should be done with stainless steel than other materials such as carbon steel and mild steel because the sulphur reducing bacteria which are a menace in salty waters corroded the metal coupons (stainless steel) less unlike the other metal materials used in this study at different concentrations of heavy metals.
Also the study was able to show that these sulphur reducing bacteria can corrode metals better at a certain concentration of heavy metal. This could serve as a cleanup threshold concentration for heavy metals when it comes to use of microorganisms in our water bodies.

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