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Microbiologically influenced corrosion of mild steel in some water environments.

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Abstract. Microbiologically influence corrosion of mild steel in effluent, Seawater and fresh water investigated using Weight loss, Culturing technique, Fourier Transforming Inferred Spectroscopic (FTIR), Scanning Electron Microscope (SEM) and X-ray florescence (XRF) methods. Weight loss was used to observed change in weight on metal surface due to accumulations of corrosion products. Culturing technique was used to identify the presences of microorganisms such as bacteria and fungi nutrient agar and potato dextrose agar (PDA) responsible for changes in weights of water environments thrive and produce acidic by products. Effluent was found to contains Escherichia coli (E. coli) bacterial species with average populations of 3.25 x 10^{-3}cfu/ml and 3.28 x 10^{-3}cfu/ml, Sea water was found to contains Streptococcus pneumonia bacterial species with average populations of 1.40 x 10^{-3}cfu/ml and 2.53 x 10^{-3}cfu/ml and Staphylococci aureus bacterial species was found in Fresh water with average populations of 1.07 x 10^{-3}cfu/ml and 2.33 x 10^{-3}cfu/ml before and after immersion of metal coupons respectively. Similarly, same fungal species identified in both effluent and Fresh water, that is Mucormycosis species with average populations of 9.01 x 10^{-3}cfu/ml and 7.22 x 10^{-3}cfu/ml respectively, and Rhizopus species with average populations of 2.98 x10-3cfu/ml was found in Seawater after immersion of carbon coupons. XRF and Atomic Absorption Spectroscopy (AAS) results confirmed the presence of elements in the water environments such asFe, Ni, Zn, Cu, Mn, Pb, Cr, Co, Nb, Mo, Sn and Cs in effluent, Fe, Ni, Zn, Cu, Mn, Pb, Cr, Co, Br, Sn, Nb, Mo, Cd, Ba and La in seawater while Fe, Ni, Zn, Cu, Mn, Pb, Cr, Co, Nb, Mo, Ba and La in fresh water. FTIR results confirmed the presence of functional groups such as amines, amides, Imines, aldehydes which are found in metabolites produced by microorganisms and some of these metabolites produced have a great impact, while some are said to be as waste end products as corrosion products. SEM shows the metal surface microgram. These changes in compositions of elements and weight of mild steel is due to the presences of extensive micro pitting on the surface.

Keywords: Corrosion, Microorganisms, microbiologically influenced corrosion, Bacteria, Fungi, Mild steel, Effluent, Seawater and fresh water.

1. Introduction

The practice of mild steel in industrial and household application is very substantial, Mild steel is found to have low corrosion resistance hence need to be protected against corrosion [4]. It's tremendously difficult to determine economic costs and damages are associated with microbiologically influenced corrosion (MIC), that is, corrosion resulting from the presence and activities of microorganisms [17].

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The most common method for corrosion control is the use of corrosion inhibition. Heterocyclic compounds containing O, N, S and P have attracted many researchers and proved to be good in corrosion protection [5]. However, MIC has been documented for metals exposed to effluents, seawater, freshwater, distilled and demineralized water, process chemicals, foodstuffs, soil, oil, gasoline and aircraft fuels, human plasma, and sewage [18]. Microbiologically influenced corrosion has been documented in chemical, food, pulp and paper processing, conventional and nuclear power generation; exploration, production, transportation, storage, and use of hydrocarbon fuels, marine industries and fire protection systems [11]. Microbiologically influenced corrosion (MIC) is used to label corrosion due to the existence and actions of microorganisms, that is, those organisms that cannot be seen individually with the unaided human eye, which includes bacteria, microalgae, and fungi [19]. Microorganisms accelerate rates of partial reactions of corrosion processes or shift mechanism for corrosion [15]. It occurs in environments where corrosion would not be predicted like low chloride waters, and the rates can be exceptionally high [2]. Microbiologically influenced corrosion reported to account for 50% of total cost of corrosion, industries most affected by MIC are power generation, oil production, transportation, and storage and water distribution [8]. Microbiologically influenced corrosion (MIC), is also acknowledged as a potential problem in long-term nuclear waste storage. In addition, MIC has been expanded to include biodegradation of metal matrix, polymeric composites, polymers, paints, ceramics, glass, concrete and other non-metallic [13]. Microbiologically influenced corrosion (MIC), does not produced a unique type of corrosion, but most MIC are localized corrosion and can take form of pitting, crevice corrosion, under deposit corrosion, and de-alloying, in addition to enhanced galvanic and erosion corrosion [16]. There are numerous mechanisms and causative organisms for MIC that can vary among metals, alloys and operating conditions for same materials [14].
Within the previous decade, there have been several important discoveries as follows:

a) Microbiologically influenced corrosion (MIC) occurs in environment where no corrosion is predicted.
b) Microbiologically influenced corrosion (MIC) rates can be extraordinarily fast.
c) Liquid culture techniques do not provide an accurate assessment of the numbers and types of microorganisms from the natural environment.
d) The chemical composition of the electrolyte influences not only the numbers and type of microorganisms, but also their impact on corrosion.
e) Modification and control strategies have shifted from use of biocides to manipulation of environment, for example, removal of sulfate (SO_4^{2-}) and addition of nitrate (NO_2^-) to control specific microbial populations [1].

Study of MIC has developed into an interdisciplinary science, including electrochemical, microbiological, biotechnological, metallurgical, surface analytical, and biophysical analyses [11].
Corrosion process creates many problems in different environments, many factors can cause corrosion, and the most important ones are microorganisms [9]. These problems of corrosion needs serious interventions, people are drinking and storing water using metallic cups, pots and tanks, as such there is gradual deterioration of a metal container, however, people are not aware of that, inside there is particular product course by deterioration of the container which is corrosion and microorganisms course the corrosion and have harmful or side effects coursing some many diseases such as diarrhea, kidney failure, blood stream infections, meningitis, pneumonia, blindness, skin rashes, GIT issues and cancer [3].These problems demand and encourage us to carry out this research work. The aim of research work is to find out the specific microorganisms and elements responsible for corrosion activities of mild steel in water environments.

2. METHODOLOGY

2.1. Preparation of Mild Steel:

The metal coupons used in the research work were mild steel sheets obtained from Mechanical Engineering Department of Bayero University, Kano (BUK). The mild steel was cut into pieces in a dimension of 2cm x 2cm x 0.2cm and polished to mirror finished with four different grades of silicon carbide emery paper (200, 400, 600 and 800) respectively to remove any existing corrosion effect, washed in distilled water, degrease in acetone, sterilize in 70% ethanol to dried and stored in a desiccator prior to experimental. The polished specimens weighed as initial weight and immersed in 100ml of each sample for a period of (1 to 5) weeks respectively.

2.2. Samples Collection:

Three different samples of water (Effluent, Seawater, and Freshwater) were collected from different environments, Sharada industrial area of Kano state, bar beach of Lagos state and Gwammaja Housing Estate Kano state respectively. Water samples collected in Polyethene bottles according to APHA9060A (2005).

2.3 Preparation of Nutrient Agar Solution:

Preparation of nutrient, agar solutions was performed using microbial grade 96% of nutrient agar powder with distilled water under controlled condition. Dissolve 28.0g of agar was dissolved in 1000ml distilled water, gently heat to dissolve the medium completely, sterilize by autoclaving at 121^0C for 15minutes and dispense the agar medium as desired [12].

2.4. Preparation of Potato Dextrose Agar (PDA) Solution:

Preparation of potato dextrose agar (PDA) solutions was performed using microbial grade 90% of potato dextrose agar powder with distilled water under controlled condition. Dissolve 39.0g in 1000ml distilled water, stir gently and dissolve completely, sterilize by autoclaving at 121^oC for 15minutes and allows to cool to room temperature prior to dispensing for isolation of enumeration of fungi and mold from dairy and other food products [10].

2.5. Determination of Culturable Microbial Population (Bacteria and Fungi):

For each of the three provided samples of water (Effluent, Seawater, and Freshwater), 1.0 ml was weighed into 9.0 ml of sterile distilled water, serial dilutions of the sample were made. Aliquots (0.1 ml) were then taken from $l0^{-3}$ to 10^{-5} dilutions and plated in triplicates on to nutrient agar plates for bacteria while the same quantity was plated in duplicate on potato dextrose agar for fungi. These were incubated at room temperature of 35^0C for 24 hrs for bacteria and 72 hrs for fungi. Colonies on the plates were afterward enumerated in cfu/ml.

2.6. Chemicals Used:

The ethanol (95%), 0.1M HNO_3 (65%) acid and 0.1M H_2SO_4 (99%) used in this research work was of analytical grade, and the solutions were prepared using distilled water in the laboratory.

2.7. Materials characterization and instrumentation:

The characterization of the metal and water samples was determined with the help of spectroscopic and quantitative analysis: Glow discharge spectroscopy (LECO GDS 50A MODEL), at Mechanical Engineering Department of Bayero University, Kano (BUK) performed elemental analysis, Incubator with the model number (UNID 063106), Autoclave machine with the model number (RAU 530D), Pro X: Phenonm world with serial number (MVE015707775) and Model number (800 - 07334) performed scanning electron microscopy (SEM) analysis, A A Agilent 200 Series AA with Model number (240 FS) performed atomic absorption spectroscopy (AAS) analysis, Electronic microscope with the model number (XSZ – 107BN) performed the identification of microorganisms, and Niton XRF Metal Analyzer with the model number (XL2 800) performed the X-ray fluorescence (XRF) analysis.

2.8 Determination of Corrosion Rate:

The best method for assessing a corrosion rate from weight loss is to weigh the corroding sample before and after exposure and divide by the total exposed area and the total exposure time making sure that appropriate conversion constants are used to get the rate in the required units [7]. The method in g/cm^2/week can be represented by equation (1).

$$CR = \frac{\Delta w}{\mathbf{A}} \text{ X T} \quad (1)$$

$Where$: $CR = Corrosion\ rate\ (g/cm^2/week)$
$\Delta w = Weigh\ t\ loss\ in\ gram,$
$A = Exposed\ surface\ area\ of\ Coupon\ (2cm \text{ x } 2cm)$
$T = Time\ of\ exposure\ in\ week.$

3. RESULTS AND DISCUSSION

The result in Table1 shows the elemental composition of metal. It demonstrated that the metal used in this research work is low carbon steel (mild) having percentage by weight of 0.087 ± 0.037 wt % C in the metal steel which is within the standard range of carbon steel (0.008 – 2.1%) and it's considered as mild steel [15].

Table 1. Result of elemental composition analysis of metal.

ELEMENTS	% PRESENT	± δ
Molybdenum (Mo)	0.027	0.005
Carbon (C)	**0.087**	**0.037**
Iron (Fe)	97.80	0.240
Manganese (Mn)	0.420	0.066
Chromium (Cr)	0.145	0.034
Titanium (Ti)	1.410	0.130
Zinc (Zn)	0.015	0.017
Cupper (Cu)	0.087	0.037
Nickel (Ni)	0.092	0.061

Three water samples were analyzed by the AAS technique. The following elements were found: Fe, Mn, Zn, Cu, Pb, Ni, and Co. The variability of the element concentrations in the analyzed water samples were shown in Table 2. AAS is a method that used to analyzed atoms in their ground state, only atoms of elements which are in metallic in nature can be analyzed [21]. The Table 2, revealed

that, seawater has the highest concentrations of heavy metals, followed by effluent then fresh water respectively. The presence of elements make the favorable condition for the survival of microorganisms which allow microbial growth on the surface of metals exposed to water environmental contaminants and can influence its corrosion in most adverse ways. The possibilities of some bacterial and fungal species to grow on metal surfaces are determined by their secreted metabolites which enable them to adapt to new environmental and nourishment conditions, these lead to corrosion products on the metal surface [20].

Table 2. Concentrations of elements in Water Samples**.**

ELEMENTS	EFFLUENT		SEAWATER		FRESH WATER	
	CONC. mg/L	ABS.	CONC. mg/L	ABS.	CONC. mg/L	ABS.
Fe	0.0096	0.0021	0.0197	0.0042	0.0074	0.0016
Zn	0.0642	0.0061	0.1049	0.0100	0.0583	0.0056
Cu	0.0147	0.0009	0.0187	0.0011	0.0160	0.0010
Mn	0.0101	0.0008	0.1380	0.0106	0.0170	0.0013
Pb	0.0047	0.0013	0.0218	0.0061	0.0133	0.0037
Ni	0.0029	0.0012	0.0042	0.0066	0.0049	0.0049
Cr	0.0062	0.0007	0.0044	0.0062	0.0088	0.0068
Co	0.0043	0.0022	0.0065	0.0059	0.0064	0.0054

Table 4, shows the weight loss and corrosion rates of the mild steel in different environments, it indicates strong relationship as the weight loss increased also the corrosion rate increased.

Table 3. Weight loss (ΔW_3) g of Mild Steel at Different Immersion Time.

Time (Weeks)	SAMPLES	INITTIAL WEIGHT (W_1) ± δ	FINAL WEIGHT (W_2) ± δ	CHANGE IN WEIGHT (ΔW_3) ± δ
1	**Effluent**	6.181 ± 0.26	6.148 ± 0.21	0.033 ± 0.24
	Seawater	5.922 ± 0.27	5.921 ± 0.27	0.001 ± 0.27
	Fresh water	6.230 ± 0.18	6.227 ± 0.18	0.003 ± 0.18
2	**Effluent**	6.070 ± 0.21	5.870 ± 0.31	0.200 ± 0.26
	Seawater	5.470 ± 0.42	5.370 ± 0.35	0.100 ± 0.39
	Fresh water	6.000 ± 0.36	5.870 ± 0.40	0.130 ± 0.38
3	**Effluent**	5.970 ± 0.21	5.730 ± 0.12	0.240 ± 0.09
	Seawater	6.070 ± 0.15	5.950 ± 0.10	0.120 ± 0.05
	Fresh water	6.200 ± 0.10	6.070 ± 0.12	0.139 ± 0.02
4	**Effluent**	6.100 ± 0.20	5.849 ± 0.12	0.251 ± 0.08
	Seawater	6.000 ± 0.17	5.870 ± 0.40	0.130 ± 0.23
	Fresh water	6.170 ± 0.12	6.030 ± 0.11	0.140 ± 0.01
5	**Effluent**	6.130 ± 0.15	5.870 ± 0.21	0.260 ± 0.06
	Seawater	6.000 ± 0.17	5.830 ± 0.21	0.170 ± 0.04
	Fresh water	6.033 ± 0.18	5.840 ± 0.12	0.193 ± 0.03

Table 4. Weight Loss and Corrosion Rate of Mild Steel

Time (Weeks)	Weight Loss (g)			Corrosion Rate (g cm^{-2} $week^{-1}$)		
	Effluent	**Seawater**	**Fresh Water**	**Effluent**	**Seawater**	**Fresh Water**
1	0.0330	0.0010	0.0030	0.0083	0.0003	0.0008
2	0.2000	0.1000	0.1300	0.0250	0.0130	0.0163
3	0.2400	0.1200	0.1390	0.0200	0.0100	0.0116
4	0.2510	0.1300	0.1400	0.0160	0.0108	0.0117
5	0.2600	0.1700	0.1930	0.0130	0.0085	0.0097

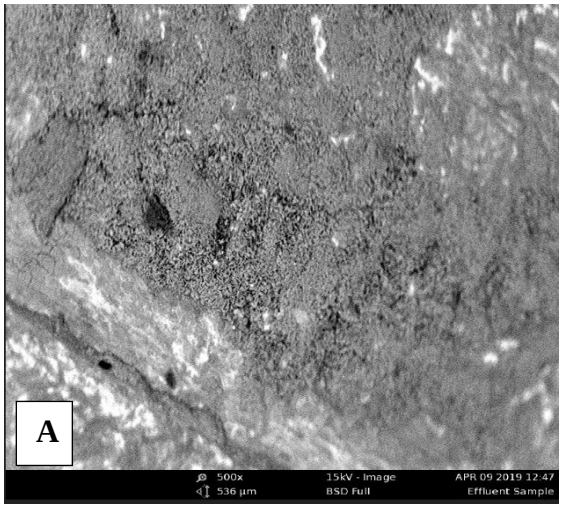
Table 5, shows the microscopic identification of bacteria and fungi in Effluents, Seawater and Fresh water before and after soaking of carbon coupons respectively. It confirmed that same species of bacteria and fungi ware found before and after immersion of mild steels in each samples. i.e Gram Positive *E. Coli* for effluent, *Streptococcus* for Seawater and *Staphylococcus* for Fresh water and Mucor species for both effluent and Fresh water and *Rihzipus* species for Sea water) respectively.

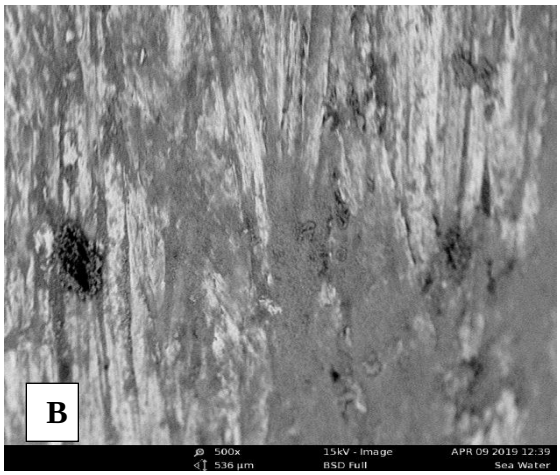
Table5. Microscopic identification of bacterial and fungal species in Effluents, Seawater and Fresh water before and after soaking of mild steel.

SAMPLES	BACTERIAL SPECIES IDENTIFIED	FUNGAL SPECIES IDENTIFIED
Effluent	Gram Positive, *Escherichia coli species*	*Mucormycosis Species*
Seawater	Gram Positive, *Streptococcus pneumonia species*	*Rhizopus Species*
Fresh water	Gram Positive, *Staphylococci aureus species*	*Mucormycosis Species*

The Scanning Electron Microscopy (SEM)micrograph images shown in figure 1, showed that the mild steel sample immersed in effluent, seawater and fresh water samples respectively for two weeks, both the samples has rough smooth surface with full of various corrosion cracks and products due the presence of microorganisms that feeds on the mild steel surface. This clearly provided the information that shows or proof that the effluent has the highest corrosion products follows by fresh water than sea water sample [11].

By comparing the SEM images of the three different samples, we can conclude morphological evolution during oxidation reaction. Effluent has a compact surface structure, while the texture Sea and Fresh Water shows cracks and breaks, which can be attributed to the release of a large amount of carbon dioxide by bacteria and fungi during oxidation process [23]. Microorganisms such as bacteria and fungi produce waste products like CO_2, H_2S, and organic acids that corrode the metals by increasing the toxicity of the metal surfaces [22].





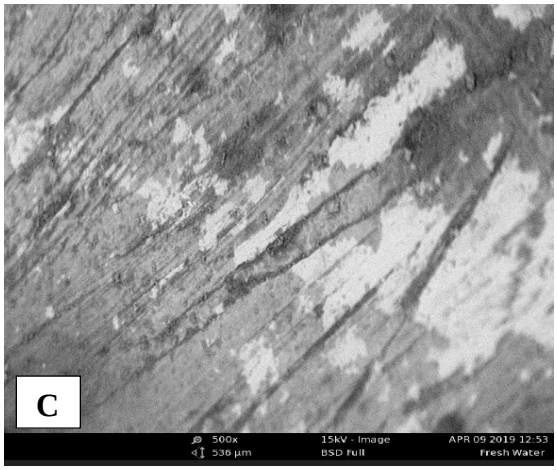
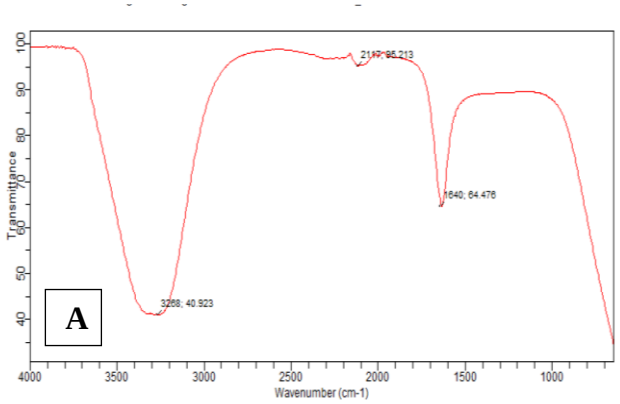
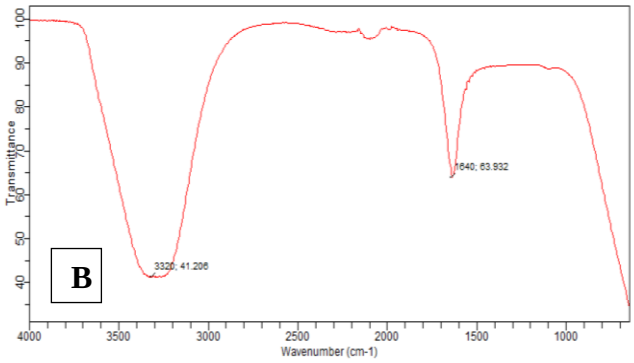


Figure 1. SEM Micrograph of mild steels immersed. in different water environments





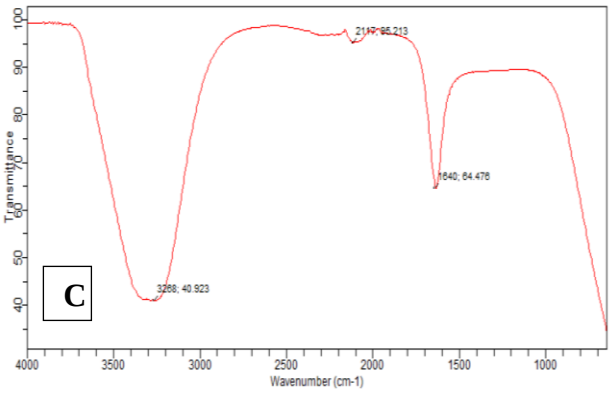


Figure 2. FTIR Results of corrosion products

(A) = Effluent, (B) = Seawater and (C) = Fresh water

Fig 2A. Shows the FTIR spectra of effluent sample which give the characteristic of the classes of compounds present with the broad peak at 3260 cm^{-1} which corresponding to the N – H and O – H stretch vibration frequencies of water, amides, amines and imines. The broad peak at 2110 cm^{-1} corresponding to the either C≡N or O = C = O stretch vibration of cyano or acid, the broad peak at 1640 cm^{-1} corresponding to the C = O stretch vibration frequency of aldehyde. Thus the results of

FTIR Spectra of effluent sample obtained shows that effluent sample contains organic compounds that are rich in carbon, hydrogen, oxygen and nitrogen in form of aromatic rings [6]. Fig 2B. Shows the FTIR spectra of sea water sample which gave two characteristic of the classes of compounds present which are similar to that of effluent, the broad peak at 3260 cm^{-1} which corresponding to the N – H and O – H stretch vibration frequencies of water, amides, amines and imines. The broad peak at 1640 cm^{-1} corresponding to the C = O stretch vibration frequency of aldehyde. Thus the results of FTIR spectra of sea water sample obtained shows that, the sea water sample contains organic compounds that are rich in carbon, hydrogen, oxygen and nitrogen in form of aromatic rings. Fig 2C. Shows the FTIR spectra of fresh water sample which gave the characteristic of the classes of compounds present with the broad peak at 3260 cm^{-1} which corresponding to the N – H and O – H stretch vibration frequencies of water, amides, amines and imines. The broad peak at 2117 cm^{-1} corresponding to the either C≡N or O = C = O stretch vibration of cyano or acid, the broad peak at 1640 cm^{-1} corresponding to the C = O stretch vibration frequency of aldehyde. Thus the results of FTIR Spectra of fresh water sample obtained shows that, the fresh water sample contains organic compounds that are rich in carbon, hydrogen, oxygen and nitrogen in form of aromatic rings. This is to implies that both the three samples shared some commons characteristics of functional groups which allow the facilitations and growing of microorganisms such as bacteria and fungi that caused the microbiologically influence corrosion at the surface of mild steel in each samples.

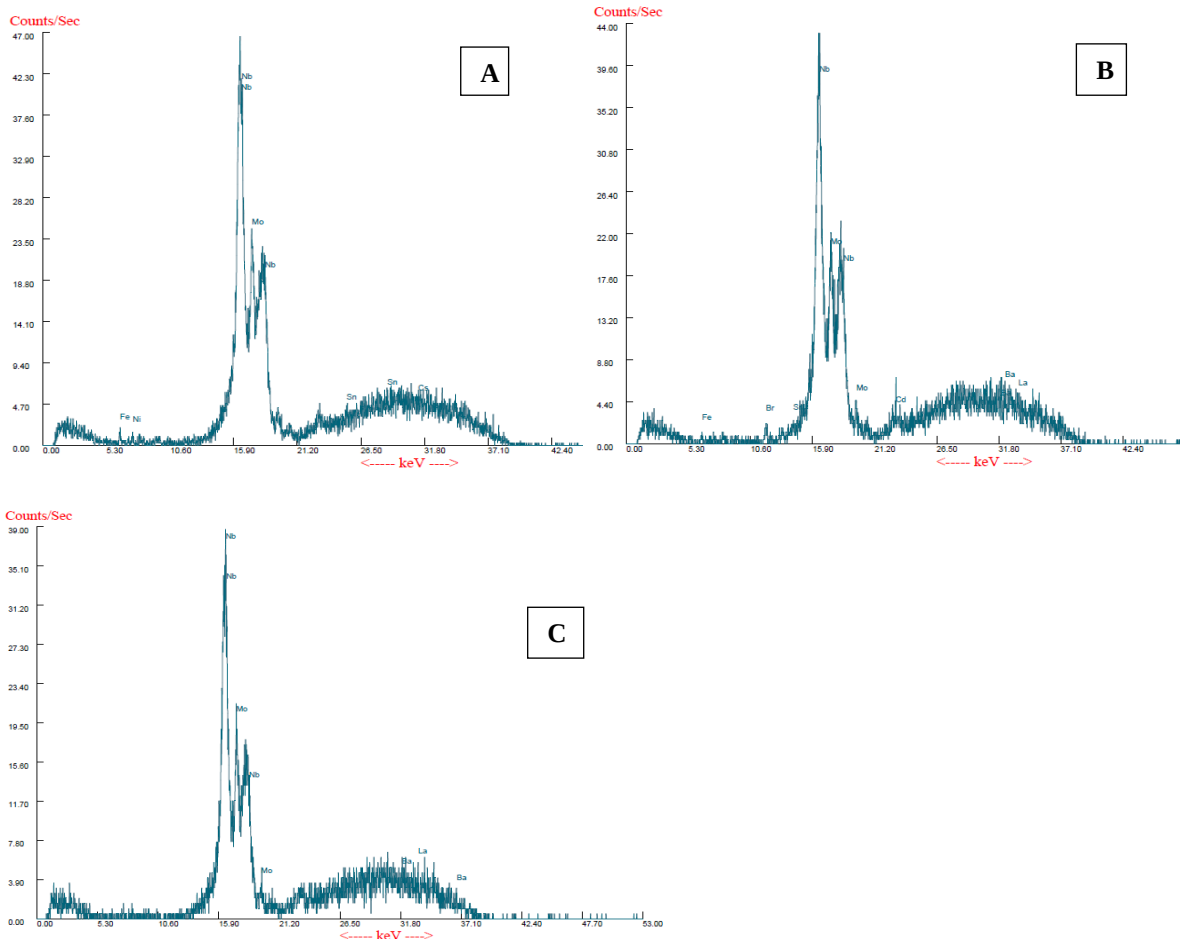


Figure 3: XRF Results Pattern after immersion of mild steel, A= Effluent, B= Seawater and C= Fresh water

X-Ray Fluorescence (XRF) Spectrometry is a versatile tool to many analytical problems which analysed major, minor and trace elements in different or various kinds of samples to perform qualitatively as well as quantitatively. It worked based on the excitation of the sample atoms by high-energy x-rays, followed by the emission of characteristic photons with some energy. One of the features of method besides the accurate, rapid, multielement capacity is that the analysis can be performed non-destructively. There is almost no sample that cannot be analyzed by technique, as long as elemental analysis is required. Three water samples were analyzed by the technique. The following elements were determined: Mo, Fe, Zn, Br, Sn, Nb, Ba, Ni, Cd, La and Cs. The variability of the element concentrations in the analyzed water samples is shown in figure 3A, 3B and 3C, which indicate the Pattern of Effluent, Seawater and Fresh water samples after immersion of mild steel for 168 hours. The results of effluent pattern give the characteristic of the classes of elements present within the sample, these elements include Fe, Ni, Nb, Mo, Sn and Cs. Seawater pattern gives the characteristic of the classes of elements present within the sample, these elements include Fe, Br, Sn, Nb, Mo, Cd, Ba and La. While fresh water pattern gives the characteristic of the classes of elements present within the sample, these elements include Nb, Mo, Ba and La. Both the samples show high concentrations of Niobium (Nb) and Molybdenum (Mo) and Iron (Fe) is commonly found in effluent and seawater.

4. CONCLUSIONS

From the results of the study, microorganisms and their populations ware found in effluent, Seawater and Fresh water respectively. The microorganisms are Gram Positive *E. Coli* with average populations of 3.25 x 10^{-3}, Gram Positive *Streptococcus* with average populations of 2.88 x 10^{-3} and Gram Positive *Staphylococci* with average populations of 1.07 x 10^{-3} for bacterial species respectively. While for fungal species, *Mucor* species were found in both Effluent and Fresh water with average populations of 9.01 x 10^{-3} and 7.00 x10^{-3} respectively. *Rhizopus* species were found in Seawater with average population of 1.40 x 10^{-3}. The results also revealed that, many bacteria and fungi adhere to mild steel surface and form mat of biofilms on surfaces, with interactions governed strongly by the mild steel surface properties and adhesion mechanisms. The possibilities of some bacterial and fungal species to grow on mild steel surfaces are determined by their secreted metabolites which enable them to adapt to new environmental and nourishment conditions, these lead to corrosion products on the mild steel surface. These metabolites include amides, amines and imines and aldehyde. These confirmed or justify the results of FTIR that shows the characteristics of the classes of compounds. This shows that the corrosion of metals in the water environments is influenced by microorganisms. The corrosion rate increases with increase in the population of microorganisms, weight loss and immersion time in all water environments. Currently different methods are used to prevent MIC. Understanding of microbial corrosion mechanism is one of primary tools to get better result in corrosion prevention. Liable on the environmental conditions, one mechanism or a combination of several mechanisms can happen. Further studies must be done to find out how microorganisms such as bacteria and fungi grow and protect themselves from biocides and other prevention methods.

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