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Microbiological Pollution of Marine Environment of the Coastal of Agadir. Impact on the Corrosion of Mild Steel

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The chemical and biological specificities of seawater make it an aggressive environment against the corrosion of many materials including steel low or moderate allies. These materials are widely used in the manufacturing of marine port infrastructure. These structures are exposed to two kind of corrosion: general corrosion and localized corrosion. The immersion of a material in seawater provokes the formation of a biofilm containing bacteria and microalgae, where are fixed the macro-organisms, which can also play a decisive role in the corrosion of metallic materials. This work addresses this issue related to the bacterial contamination by sulfite-reducing bacteria of seawater of the Agadir coastal and its potential impact on the resistance of the corrosion of the mild steel. For carrying out this work, we used the microbiological, electrochemical and the surface analysis techniques. We observed that the seawater of Agadir coastal is microbiologically polluted and it has a considerable effect on the corrosion of steel.

Keywords: Biocorrosion; mild steel; seawater; sulfite-reducing bacteria; biofilm.

1. INTRODUCTION

The water is a very abundant compound in the nature with covers more of 70% of the earth surface[1]. However, the most of this water is as brine in the sea and oceans. Considering its stable composition and huge volume, the seawater is an original electrolytic solution that deserves more attention. It contains many mineral salts, dissolved gases, like oxygen, bacteria, suspended solids and sediment that sometimes give him a high turbidity. So, seawater is not just a solution of sodium chloride. Indeed, the sulphate ions and the iron are involved in the mechanisms associated to marine corrosion of steel [2]. Corrosion problems originating from the presence of microorganisms affect various areas of activities, and the industrial installation witch can be damaged by the biocorrosion are several: transportation systems of raw water, heat exchangers, fire circuits, condensers and deionizers. In this type of installation, bacterial corrosion usually occurs simultaneously with the appearance of a gelatinous biofilm. Aerobic organisms, through respiration inside the biofilm, create an oxygen gradient. The metal completely anoxic zones, strictly anaerobic species can develop and participate, alongside aerobic bacteria, on the development of microbiologically influenced corrosion. When the metal is exposed to seawater witch contained toxic metals and chemicals, the SRB in the biofilm, produces the EPS. These extapolymers substances, which contained proteins and polysaccharides, were responsible to increase the corrosion of the mild steel, because they contain the acidic and iron binding nature of the EPS. But, we don't have a relationship between the degree of the increase of corrosion and the toxicity of metals to SRB, witch reduce the sulfate, or the increased of production of the EPS [3]. In other hand, SRB biofilms is able to obtain the electrons from carbon steel in order to accelerate the microbial corrosion rate of the steel [4]. It is demonstrated that in presence of SRBs, the potential E_{corr} decreased towards the positive values which are nobler compared to that in sterile. Also, a high amount of elemental sulphur was detected as corrosion products in the presence of SRBs [5]. The contamination of seawater by the anaerobic bacterium sulfate-reducing provokes the pollution of water by the sulphides ions which decrease E_{corr} into the active values after a certain immersion duration [6]. In this section, the Desulfovibrio bacteria contributed on the corrosion increases because of the high concentration of these bacterial metabolites [7]. In other work, the influence of the adhesion and the enzymatic activity of biofilm on the electrochemical behavior of stainless steels immersed in river water were studied [8]. But, it is demonstrates that the biofilm bacteria Desulfovibrio.sp inhibits the accelerated damage of the steel for a limit time in synthetic seawater [9]. The impact of the sulfate-reducing bacteria Desulfovibrio vulgaris on the inhibitory efficacy of imidazoline in marine environment against the corrosion of steel AISI 1018 was studied. This work has revealed two forms of corrosion: localized corrosion and mixed corrosion due to the interaction between bacteria and imidazoline [10]. So, it appears that the issue related to the biocorrosion needs more studies dealing more parameters. In our study, we are interesting to examine four marine zones seawater as an electrolyte solution for the mild steel by investigating the microbiological, electrochemical, gravimetric and microscopic measurements.

2. MATERIALS AND METHODS

2.1. Materials

The metal tested in this work is mild steel with the chemical composition (wt %): C (0.14), Si (0.05), Mn (1.30), P (0.11), S (0.33) and Fe (remainder). For the electrochemical tests, the working electrode was prepared at cylindrical mild steel rod. The surface area of this rod was 0.50 cm^2. Before each measurement, the surface of mild steel was abraded by different grades of sandpaper, which ended up with the 1200 grade. Then, this electrode was cleaned by distilled water, acetone, distilled water, respectively, and immersed in the solution quickly. In other hand, the gravimetric tests are mounted by the samples that were cut into plates with defined surface and follow the same polishing procedure.

2.2. Medium preparation

The seawater solution tested in this work is the natural seawater collected from four zones of Agadir coastal which differs by the type of industrial activity and the wastes. To characterize the seawater collected, we determine some physic-chemical properties in situ such as: temperature, pH, conductivity, salinity and dissolved oxygen. Additionally, the enumeration of microbiological species is done for the total flora (agar PCA medium), the fecal coliforms (middle Lactose TTC Tergitol7 medium) and the sulfate-reducing bacteria (agar meat-liver medium). The bacterial analysis is described in the following constructions:

2.2.1. Enumeration of the total flora

To quantify the bacterial pollution of Agadir seawater, we count the total flora. For every marine zone, we realize many dilutions until 10^{-7} M to better visualize the development of bacteria in the culture medium. Then 1ml of each solution is seeded in a petri box containing the PCA medium (Plate Count Agar).

2.2.2. Enumeration of sulfite-reducing bacteria

As part of the study of the microbiological influenced corrosion, and exceptionally by the bacteria reducing the sulfate, it is primordial to highlighting the bacterial genus of sulfite-reducing bacteria. In this section, we make different dilutions until 10^{-5}M. Then 1ml of each solution is introduced into a tube containing agar-liver meat medium (meat-Liver Glucose Agar) in the liquid state by a helical movement of the bottom to the top. Several passages were performed to confirm the presence of this group of bacteria and to its isolation.

2.2.3. Enumeration of fecal coliforms

To reveal pollution by pathogens bacteria in the study areas, we must characterize and highlight the most dangerous species. In this part, we chose the species *Escherichia coli* as fecal coliform, given its contribution to anomalies and serious diseases. In this work, we make the identification and characterization of this bacterial species. The sea water samples are collected under the conditions of temperature. Then the volume (1 ml, 10 ml or 100 ml) of this water is filtered through a diameter of 0.45 µm by a membrane under aseptic conditions. This membrane is placed on a box containing Tergitol medium with TTC solution (triphenyl) at 1 ml to 250 ml of medium. After incubating the test boxes, were isolated colonies under sterile conditions. These colonies are seeded in boxes that contain the EMB medium (Medium eosin methylene blue). In this study, we used the taxonomy through a gallery named API 20 E, by biochemical tests, instead of conventional microbiological tests. This gallery is made up of 20 micro tubes containing hydrated substrates. Firstly, a slurry solution is prepared by introducing the culture carried out before in the physiological water. From this suspension, drops are added to each micro tubes of the gallery which is humidified with physiological water. Then the initial color of each tube is noted. After a period of 24 hours, were read the results.

2.3. Gravimetric testing

The measurements of mass loss are the first approach to use for studying the microbial corrosion of mild steel in the seawater of a four marine areas of Agadir coastal (Z1, Z2, Z3 and Z4).The samples are prepared and then immersed in the electrolyte solutions for a period of 2 days, 10 days, 20 days and 30 days. After the immersion time, the samples were removed. They are rinsed with distilled water and scraped to remove the inorganic and organic matter deposited on the surface, with nitric acid(2M).After drying the samples, their final masses are measured in order to calculate the loss of mass and the speed of corrosion using the following role:

$$V_{corr} = \frac{\Delta m}{S.t}$$

2.4. Electrochemical measurements

The electrochemical measurements were realized using Voltalab PGZ 301 Electrochemical Analyzer attached to a computer control. For these tests, an electrochemical cell remounted by three electrodes was used. A platinum electrode and a saturated calomel electrode (SCE) were employed as the auxiliary and the reference electrode, respectively. Before electrochemical measurements, the working electrode is maintained at open circuit potential for half an hour, and its surface is polished before each measurement. The current-potential curves are made in potentiostatic mode with a potential scan rate of 1 mV/s. These curves are stored in a potential range between -300 and -1200 mV/SCE. Then, EIS and potentiodynamic polarization measurements were carried out subsequently. The EIS experiments were realized in the frequency domain from 100 kHz to 10 mHz at E_{ocp}.

2.5. Scanning electron microscopy and EDS analysis

Was examined the surface of mild steel after its exposure to the different seawater after 33 days of immersion, by a scanning electron microscopy (SEM) and EDS analysis.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analyzes

Biocorrosion occurs in marine environments where the nutrient content, the temperature, the pressure and the pH are different **[11]**. The physicochemical analyzes are measured in situ in order to determine the quality of seawater collected. The values obtained for the date of June 2013 are summarized in the table 1.We find that the physicochemical parameters studied are not much affected by the sampling zone of seawater.

Table 1. Physicochemical properties of the seawater of the four zones of Agadir coastal

	Beach zone (Z1)	Port zone 3m (Z2)	Port zone 6m (Z2)	Anza zone (Z3)	Aghroud zone (Z4)
pH	8.63	8.62	8.62	8.39	8.63
T (°C)	25	25	25	24	25
Conductivity (mS/cm)	55	54.9	54.5	53.9	54.7
Salinity (g/l)	36.3	36.3	35.9	35.5	36.1
O_2 dissolved	7.2	7.2	7.2	7.2	7.2

3.2. Microbiological tests

The results obtained from microbiological tests about reality of bacterial pollution in the studied areas. The enumeration of the total flora allows to characterize these areas with a high bacterial load zones: Z2 (6m) and Z3 zones contains a relatively high bacterial load compared to others (Table. 2).While the enumeration of sulfite-reducing bacteria allows us to classify areas as follows: Z3 > Z2(6m) > Z2(3m) > Z1 > Z4 [12]. In another aspect, in the Table 3, it is mentioned that the four zones are microbiologically contaminated by pathogenic bacteria through germ contamination indicators searching *EscherichiaColi*.

Table 2. Bacterial counts in the seawater of the four zones of Agadir coastal

	Total flora (UFC/ml)	Sulfite-reducing bacteria (UFC/ml)
Z1	$13.0\ 10^{1}$	$1.0\ 10^{0}$
Z2 (3m)	$35.6\ 10^{4}$	$2.0\ 10^{0}$
Z2 (6m)	$16.1\ 10^{5}$	$8.0\ 10^{0}$
Z3	$13.1\ 10^{5}$	$47.5\ 10^{1}$
Z4	$25.3\ 10^{4}$	$0.0\ 10^{0}$

Table 3. Enumeration of *Escherichia Coli*. in the seawater of the four zones of Agadir coastal

	1 ml	10 ml	100 ml
	Incubation à 44°C		
Z1	34	7	2
Z2 (3m)	Claire	10	38
Z2 (6m)	2	14	70
Z3	10	8	19
Z4	Claire	1	3

After incubating the boxes containing the suspension into the EMB medium, it was observed the presence of yellow colonies surrounded by orange rings. It was the colony of *Escherichia Coli.*

The gallery obtained is illustrated in the following figure:

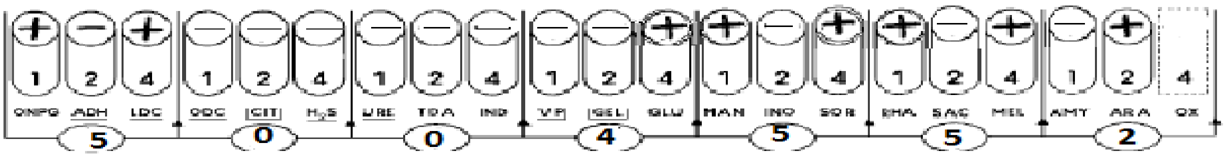


Figure 1. AP 20 E Gallery used for identification of Escherichia coli.

The first observation that we can make is the revelation of the change of color in the tube of glucose. In our experience, it means that this species are capable to degrading glucose. Then other reagents are added to some tubes (ADD, IND, VP and GLU). After a calculation, we removing the code of the species studied (5,004,552). Moreover, this is why this test is called a numerical taxonomy. This code is verified in a percentages table. The result was that it is Escherichia coli. with id = 56.2% and T = 0.79 (IND 89%).

3.3. Lost Weight

The Fig.1 shows that the rate of corrosion has the same appearance for all the sampling zones. This velocity increases to attain a maximal value at 10 days. It decreases for the next periods to be established at 0,3mg/cm².days. This allure can be explained by the blockage of the active sites by the products deposits into the surface that are the results of the oxidation of the steel in seawater. Consequently, the rate stabilizes in a long immersion. Additionally, we find that the rate of corrosion in the zones Z1 and Z2 (depth 6m) is very important than others. However, it is low in the zone Z3 because a natural biofilm is formed in the surface. This biofilm blocks the active sites of corrosion. We can also justify this result by the capacity of biofilm to inhibit the corrosion of the steel for some time [13].

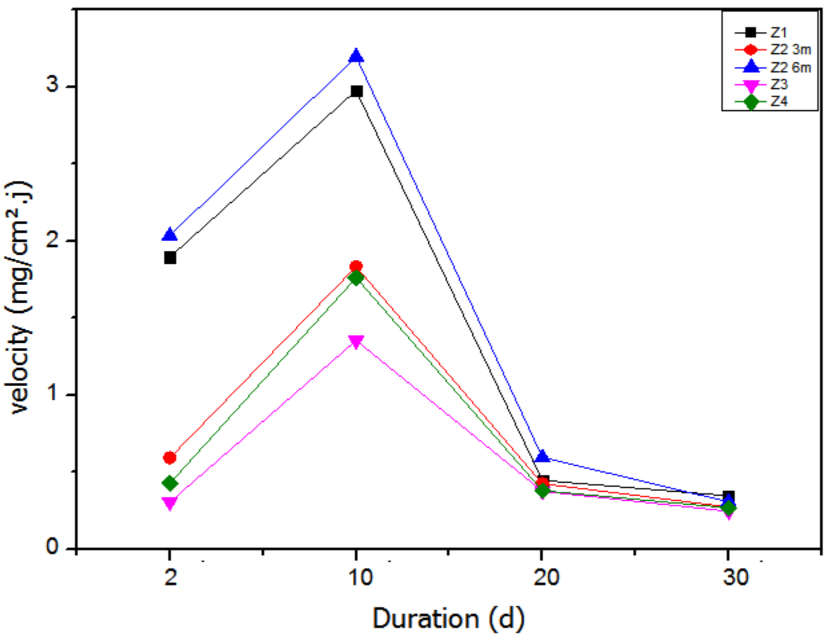


Figure1. The evolution of the velocity of corrosion for mild steel in seawater of the four zones of Agadir coastal

3.4. Potentiodynamic polarization

The microbial activity of biofilms formed on the surfaces of metallic materials can affect the kinetics of cathodic and/or anodic reactions [13] and can also considerably modify the chemistry of any protective layers, leading to either acceleration or inhibition of corrosion [14,15].

The representative potentiodynamic polarization curves of the mild steel electrode, which were obtained in seawater solution, are given in Fig. 2. In order to extract more information about the kinetics of the corrosion, some electrochemical parameters such as corrosion potential (E_{corr}), corrosion current density (i_{corr}) and anodic pent (β_a) values were calculated from the corresponding polarization curves and the obtained data are given in Table 4.

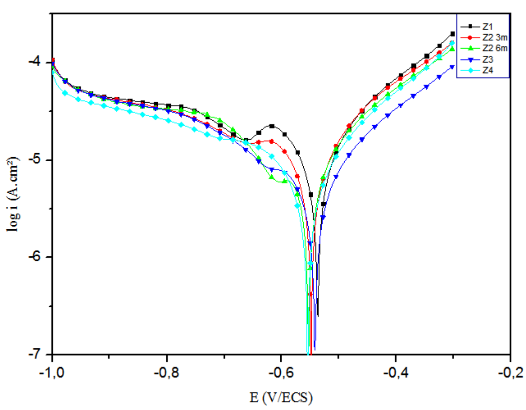


Figure 2. Polarization curves for mild steel in the seawater of the four zones of Agadir coastal

Table 4. Electrochemical data for mild steel in seawater of the four zones of Agadir coastal

Medium	E_{corr} (mV/ECS)	I_{corr} (µA/cm²)	$β_a$ (mV/Dec.)
Z1	-540	7.3	117
Z2 (3m)	-549	5.9	114
Z2 (6m)	-554	5.6	127
Z3	-542	3.7	121
Z4	-551	4.4	122

We find that the zone Z1 presents a cathodic pic with is attributed to reduction of an electro active substance in solution. In fact, we have always the same value of corrosion potential and $β_a$. The values of current density in different zones are compared and classified like Z1 > Z2 (3m) > Z2 (6m) > Z4 > Z3. The same result has obtained in the study of the effect of the SBR biofilm on the accereration of the corrosion [4].

3.5. Electrochemical impedance spectroscopy

The representative Nyquist diagrams of the mild steel electrode that were obtained in seawater solution are shown in Fig. 3. We find that in all cases, diagrams of Nyquist have the shape of a circle at high frequency. We also note that the diameter of the circle depends on the study area. This diameter allows us to calculate the transfer resistance and the double layer capacity. Also, the electrolyte resistance is negligible compared with that of transfer (Table 5).

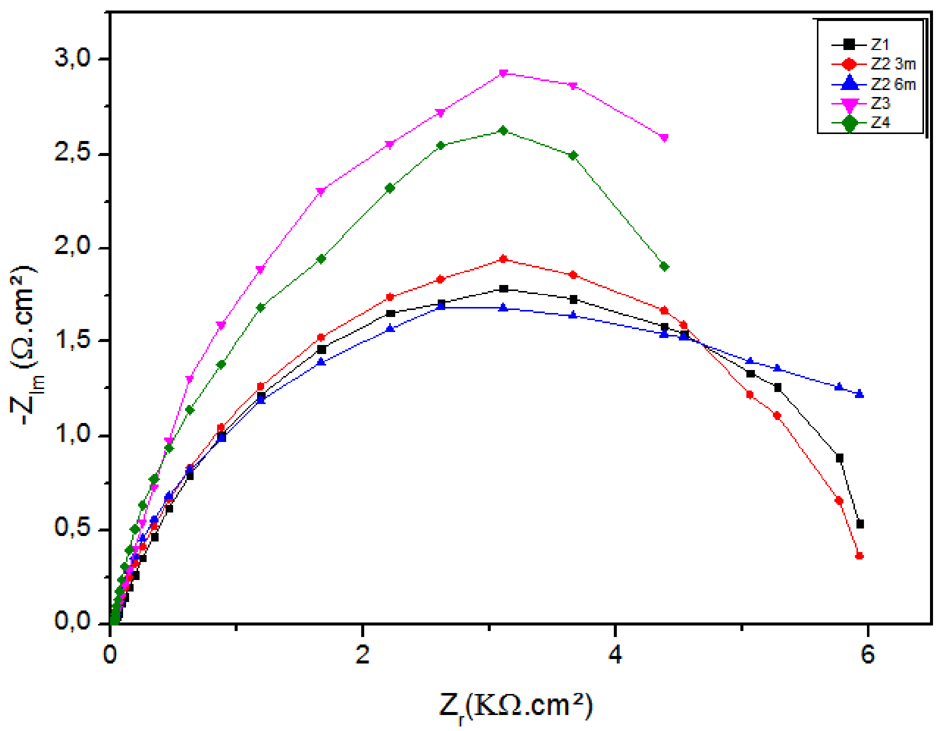


Figure 3. Nyquist diagrams for the mild steel in the seawater of the four zones of Agadir coastal

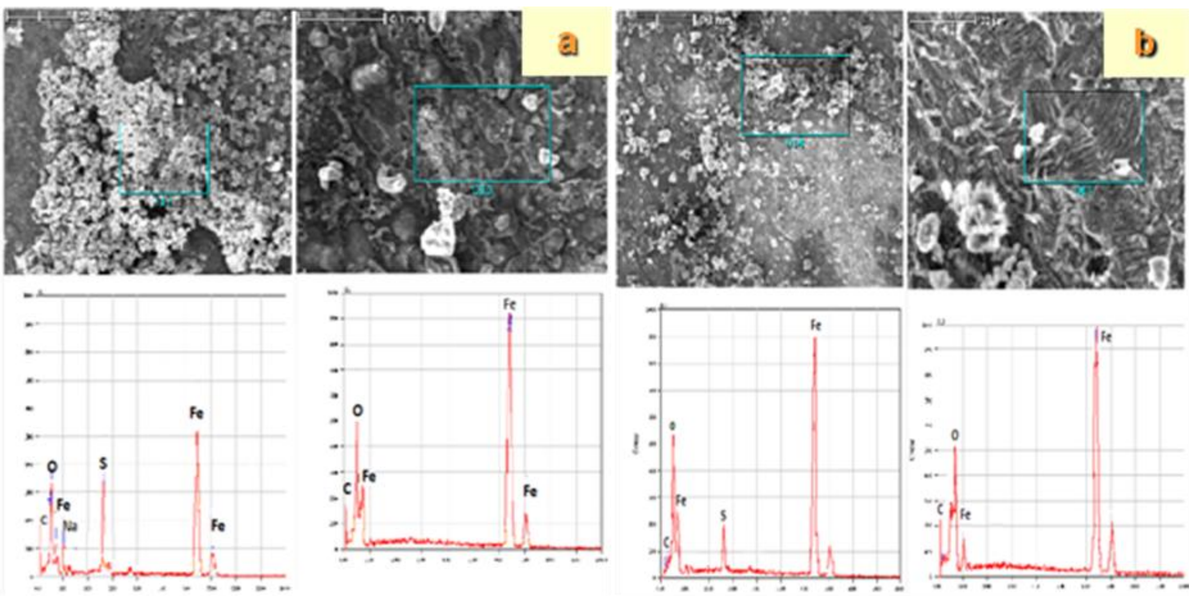
Table 5. Impedancemetric parameters for the mild steel in the seawater of the four zones of Agadir coastal.

Zones	R_e ($\Omega.cm^2$)	C_{dl} ($\mu F.cm^2$)	R_t ($\Omega.cm^2$)	$1/R_t$ ($10^{-4}\ \Omega^{-1}\ cm^{-2}$)
Z1	29	99	6446	1.55
Z2 (3m)	75	103	6176	1.62
Z2 (6m)	81	189	8426	1.19
Z3	21	65	9733	1.03
Z4	65	69	9183	1.09

By analyzing the ability of the double layer, it is noted that it depends on the nature of the studied area: it is very important in the zone Z2 (6m) compared to other areas. Comparing the corrosion current density of different areas that show the zones Z1, Z2 (3m) and Z2 (6m) have a significant corrosion rates compared to the zones Z3 and Z4.

3.6. Scanning electron microscopy and EDX Analysis

Fig.4 shows a comparison between coupon surfaces in different solutions of seawater.



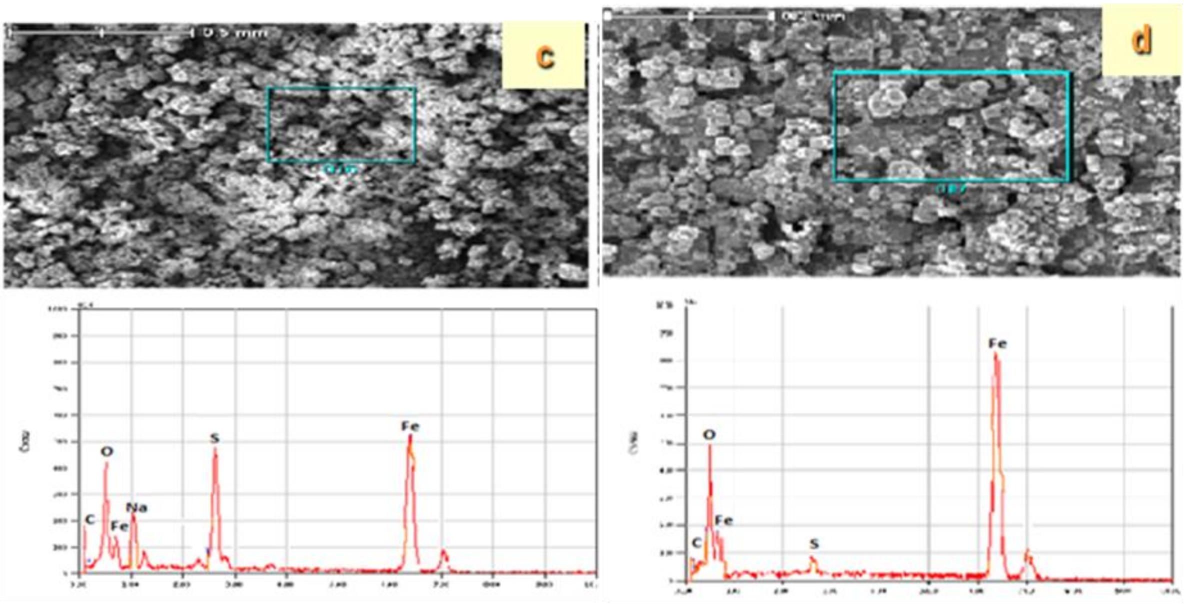


Figure 4. SEM images for mild steel immersed in natural seawater solutions of Z1 (a), Z2 (b), Z3 (c) and Z4 (d) for 33 days

The micrographs show the existence of two layers for both zones (Z1 and Z2 (3m)): internal and external layer. In the outer layer, the EDS analysis shows the presence of iron and oxygen. These two elements are probably from the deposition of the rust (Fe_2O_3, nH_2O) on the surface. Additionally, we observe the presence of sulfur witch comes from FeS. This result confirms the microbiological results showing the presence of sulfite-reducing bacteria. The carbon is also present as an element of steel. In the inner layer, we find the Iron, Oxygen and Carbon. In this layer, there is probably the natural formation of rust. In zone Z3, there is the formation of a single layer to the metal surface. The EDS analysis shows the existence of Iron, Oxygen (elements of rust), sulfur and sodium. There is probably precipitation of sodium sulfide Na_2S. The last area studied in this work is the Z4 area. The analysis showed that the deposition of corrosion products to the inner layer of the metal surface is made of iron, oxygen, carbon with a low amount of sulfur. This result agrees well with the microbiological tests confirming the absence of sulfite-reducing bacteria in the zone Z4. This result is confirmed by the study concerning the increasing of the heterogeneity of the steel surface when it is exposed to seawater polluted by SBR bacteria [16].

4. CONCLUSION

Based on the results and discussion along this study, these conclusions were drawn:

- The gravimetric and electrochemical tests show that the aggressiveness of this marine environment of Agadir, trough the corrosion of a mild steel varied depending of the zones.
- It depends really on many factors specially the bacterial pollution.

- The seawater of Agadir coastal is polluted by the sulphite-reducing bacteria. The quantity of these germs varies from one zone to another, but the zone Z3 is the most polluted area.

- The group of sulfite-reducing bacteria has an important contribution on the corrosion of the mild steel in natural seawater.

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