Research Article

Evaluation of Bacteriological Contamination of Agadir Seawater (Southern Morocco): Impact on Copper Corrosion Resistance

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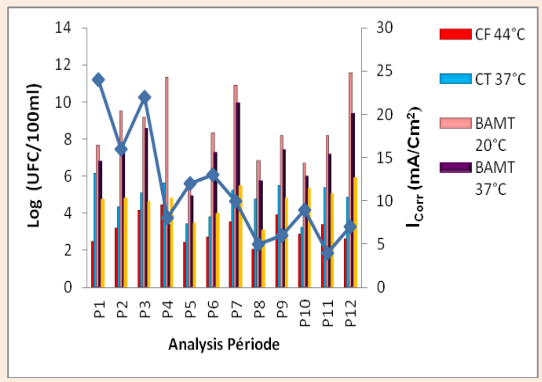
Abstract
In this work, we study the bacterial contamination of marine environment of Agadir city in southern Morocco and evaluation of its impact on copper corrosion resistance. This study was carried out by coupling of microbiological analysis techniques with that of corrosion measurement. Monthly periodic samplings (between October 2012 and September 2013) of sea water were carried out in Anza industrial zone (Z3). Thus, after each sampling, a study of the corrosion behaviour of copper was carried out. The electrochemical parameters such as corrosion potential (Ecor), corrosion current density (Icor) and charge transfer resistance (Rt) were evaluated. The results obtained show that seawater analyzed has a bacteriological pollution. The total mesophilic aerobic bacteria (TMAB), total coliform (TC), fecal coliform (FC) and sulfite-reducing bacteria (SRB) have been identified. The electrochemical parameters values were discussed. Correlation between corrosion rate of copper and the bacterial load was proposed.



Keywords: Bacterial, pollution, sea water, corrosion, copper

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Introduction

In Morocco, the marine environment plays an important role in socio-economic development. The opening of his ribs on two fronts (Atlantic and Mediterranean) extending over 3500 km of coastline, offers a strategic position. Indeed, the Moroccan coast has the advantage of being among the richest in the world with a wide variety of ecosystem. Moreover, much of this area is an ideal place for the installation of many towns and industrial units. Thus, about 60% population and more than 70 % industries are concentrated in urban centres located along the coast. What made these ribs are exposed to chronic contamination caused by direct discharges, mainly from industrial and domestic evacuated (60% wastewater) without any treatment. [1] Agadir Bay (southwestern Morocco) presents several sources of pollution. It has in recent years an important industrial activity; leading to a high microbial pollution in the seawater and sediment of the Bay. In addition to waste from tourism and industrial activities, the Bay receives a considerable contribution of wastewater from the Agadir city and its suburbs. Previous studies conducted in 1997 and 1998 showed that some sites in the bay of Agadir are heavily contaminated with microorganisms. [2] Since the work of Mimouni R. and al [1-2], no studies have been performed, to our knowledge, on the bacteriological contamination of sea water of the bay of Agadir. Corrosion of metals is a fundamental industrial issue which has received considerable attention. The marine environment is a favourable environment deterioration of metallic materials. The aggressiveness of the medium results in particular from its physicochemical properties: high conductivity due to high salinity, high chlorinity, dissolved oxygen ... This is also reinforced by the action of living micro-organisms contained in the medium. [3]. Due to its high electrical and thermal conductivity and good mechanical workability, copper is a material widely used in pipelines for domestic and industrial water utilities, including sea water, heat conductor, and heat exchangers. [4] In spite of the relatively noble potential of copper, its corrosion takes place at a significant rate in sea water and chloride environments. [4-6] However, despite their stainless character, copper are not fully immunized and may be susceptible to some forms of corrosion in specific environments. [7] Sea water polluted by certain types of bacteria constitutes an aggressive environment from corrosion of these materials. [8] The role played by microbiological contaminants on copper corrosion in natural seawater from the Bay of Agadir has not been the subject of previous work. This has led us to undertake our research to determine the microbiological status of industrial Agadir seawater (Z3) and the effects of population density of bacteria studied on copper corrosion resistance.

Experimental

Samples were collected every month. They are then placed in sterile vials (500 m1) for microbiological analyzes or in sterile polyethylene bottles (1.5 L) for the electrochemical study. The sea water samples were transported to the laboratory under isothermal conditions between 4 and 6°C in the dark. Microbiological analyses are performed in the first three hours of sampling. The physico-chemical parameters (pH, temperature, conductivity, salinity and dissolved O2) were measured in situ. Study of bacteriological analysis of seawater and copper corrosion were carried out in the laboratory. In this work, we focused on the identification of the following bacteria: Total Mesophilic Aerobic Bacteria at 37 °C (TMAB at 37 °C); Total Mesophilic Aerobic Bacteria at 20 °C (TMAB at 20 °C). - Total Coliform (TC); Fecal Coliform (FC); Sulfite-Reducing Bacteria (SRB). The culture media used are: PCA (Plate Count Agar): It is a medium for the enumeration of total viable bacterial flora, whether saprophytic environmental germs or germs that may cause illness. These bacteria were counted using the counting technique by incorporation into the PCA agar. [9]; Agar medium in Tergitol and Triphenyl Tetrazolium Chloride (TTC - Tergitol 7): It is a selective medium for the isolation and enumeration of CF (medium recommended by AFNOR). Enumeration of TC and FC was performed by membrane filtration. Three volumes of 100 ml each were filtered through a millipore membrane (pore diameter 0,45μm). These are deposited on three agar plates tergitol and triphenyl tetrazolium chloride (TTC) before being incubated at 37 °C for CT and 44.5 °C for the CF for 24 hours. From the values supplied by the three counts for a sample of sea water, we calculate the average density and CF CT per 100 ml. [9] The enumeration of the spores of sulfite-reducing bacteria was performed by inoculation in the agar medium meat-liver (test tube 220 x 22 mm) and covered with paraffin oil to create the anaerobic conditions necessary for the development of these spores before being incubated at 37 ° C for 24 hours. [9] The polarization curves of copper in sea water are recorded with a potentiostat PGP 201, controlled by a computer. The scan rate is 30 mV/min and the potential is ranged from catholic to anodic potentials. Before recording each curve, the working electrode is maintained with its free potential of corrosion Ecorr for 30 min. We used for all electrochemical tests a cell with three electrodes and double wall thermostats (Tacussel Standard CEC/TH). Saturated calomel (SCE) and platinum electrodes are used as reference and auxiliary electrodes, respectively. The working electrode is in the form of a disc from pure copper of the surface 1 cm2. The electrochemical impedance spectroscopy (EIS) measurements are realised with the electrochemical system (Tacussel), which included a digital potentiostat model Voltalab PGZ100 computer at Ecorr after 30 min immersion in solution. After the determination of steady-state current at a corrosion potential, sine wave voltage (10 mV) peak to peak, at frequencies between 100 kHz and 10mHz are superimposed on the rest potential. Computer programs automatically controlled the measurements performed at rest potentials after 30 min of exposure. The impedance diagrams are given in the Nyquist representation. Experiments are repeated three times to ensure the reproducibility.

Results and Discussion

Microbiological analysis

Microbiological analysis results of sea water (Z3) for the period: October 2012 - September 2013 are given in Table 1. Evolution of the concentration of the five pathogens studied according to the period of analysis is shown in Figure 1. We conclude that sea water of Z3 is polluted during all the period of year. The concentration of the five types of bacteria studied varies with the period of analysis. We find that the concentration of BAMT is significantly higher than that of other bacteria for all period of analysis. The highest values were recorded during the months of January, April and September. However, the concentration of CF undergoes a slight change with time. It represents the lowest concentration versus five pathogens studied. CT bacteria and BSR have also little variation during the sampling periods. Their concentrations are however higher than that of CF. The distribution of microbial pollution at Z3 could be linked with discharges of urban and industrial wastewater at this level. The average concentrations of CF and BSR are well above the standard Moroccan guide (2000 CF / 100ml) value; indicating that sea water of Anza beach is poor quality (class D). [2] The existence of a high quantity of BSR in seawater of Z3 can be explained by the presence of a recent pollution which is probably due to intense industrial activity.

Table 1 Average values of the concentration of pathogens studied in Sea water (Z3) as sampling periods

	Group of germs					
	Period of prelevement	**BAMT at 37 °C (UFC/100ml)**	**BAMT at 20 °C (UFC/100ml)**	**CT at 37°C UFC/100ml**	**C F at 44 °C UFC/100ml**	**BSR (UFC/100ml)**
Sea water of Anza Beach Z3	**05/10/2012 (P_1)**	$6.0*10^{6}$	$4.2*10^{7}$	$1.6*10^{3}$	$3*10^{2}$	$5.5*10^{4}$
	05/11/2012 (P_2)	$2.5*10^{7}$	$3.0*10^{9}$	$2*10^{4}$	$1.5*10^{3}$	$6.5*10^{4}$
	05/12/2012 (P_3)	$3.5*10^{8}$	$1.5*10^{9}$	$1.1*10^{5}$	$1.4*10^{4}$	$4*10^{4}$
	05/01/2013 (P_4)	$2.6*10^{10}$	$2.0*10^{11}$	$4*10^{5}$	$2.5*10^{4}$	$6.5*10^{4}$
	05/01/2013 (P_5)	$8.2*10^{4}$	$6.1*10^{5}$	$2.5*10^{3}$	$2.6*10^{2}$	$3*10^{3}$
	05/01/2013 (P_6)	$1.8*10^{7}$	$2.0*10^{8}$	6.10^{3}	$5*10^{2}$	$1.1*10^{4}$
	05/01/2013 (P_7)	$8.6*10^{9}$	$7.1*10^{10}$	$1.7*10^{5}$	$3.3*10^{3}$	$2.7*10^{5}$
	05/01/2013 (P_8)	$5.3*10^{5}$	$6.5*10^{6}$	$5.5*10^{4}$	$1*10^{3}$	$1.2*10^{3}$
	05/01/2013 (P_9)	$2.6*10^{7}$	$1.5*10^{8}$	$3.0*10^{5}$	$8*10^{3}$	$6.5*10^{4}$
	05/01/2013 (P_{10})	$9.0*10^{5}$	5*106	$1.6*10^{3}$	$7*10^{2}$	$2.1*10^{5}$
	05/01/2013 (P_{11})	$1.4*10^{7}$	$1.4*10^{8}$	$2.2*10^{5}$	$2.4*10^{3}$	$1.1*10^{5}$
	05/01/2013 (P_{12})	$2.3*10^{9}$	$3.3*10^{11}$	$7.1*10^{4}$	$4*10^{2}$	$7.8*10^{5}$

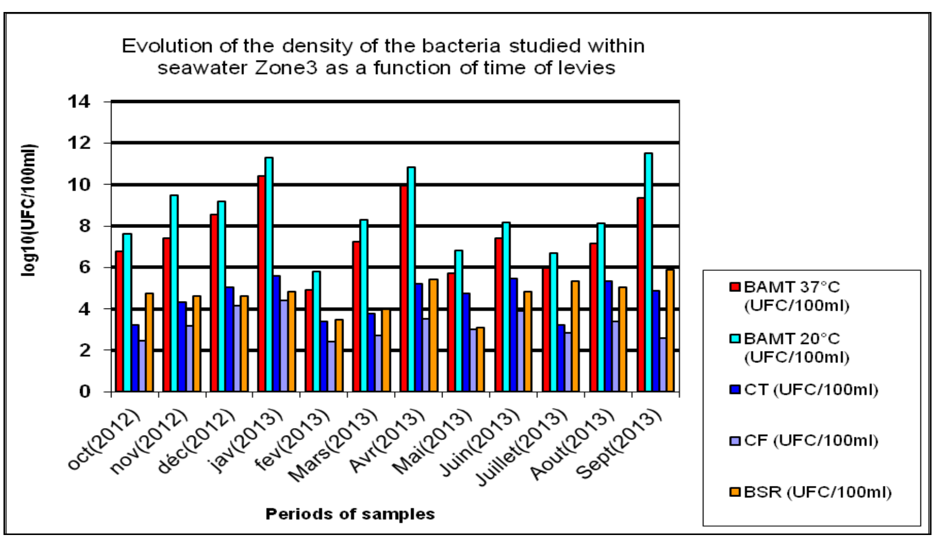

Figure 1 Evolution of concentration with analysis period of five germs in sea water of Z3

Electrochemical measurements

Polarization curves

Figure 2 shows polarization curves of copper obtained in sea water of Z3 with some various period of analysis after 30 min of immersion. All cathodic and anodic polarization curves are similar to one plotted in Synthetic sea water. [10]**.** It is well known that the cathodic reaction of copper in NaCl solution is simple, which can be described by reaction of oxygen reduction. [11-12]

$$O_2 + 4e^- + 2H_2O \rightarrow 4OH^- \quad (1)$$

The cathodic current (Figure 2) follows the Tafel law, and then the corrosion current density can be determined readily after diffusion correction. Anodic dissolution of copper occurs through oxidation of Cu(0) to Cu^+ [13]

$$Cu \rightarrow Cu^+ + 1e^- \quad (2)$$

This Cu^+ reacts with chloride ion from the solution and forms CuCl,

$$Cu^+ + Cl^- \rightarrow CuCl \quad (3)$$

The formed CuCl has poor adhesion, is unable to protect the copper surface, and transforms to the soluble cuprous chloride complex, $CuCl_2^-$ [14-15]

$$CuCl + Cl^- \rightarrow CuCl_2^- \quad (4)$$

Here, three distinct regions can be identified for Cu in sea water of Z3: a Tafel region at lower over-potentials extending to the peak current density due to the dissolution of copper into Cu^+, Eq. (2); a region of decreasing currents until a minimum is reached due to formation of CuCl, Eq.(3); a region of sudden increase in current density as a result of $CuCl_2^-$ formation, Eq.(4), which is the responsible on the dissolution of Cu. [16] The electrochemical parameters deduced from Figure 2 are summarized in Table 2.

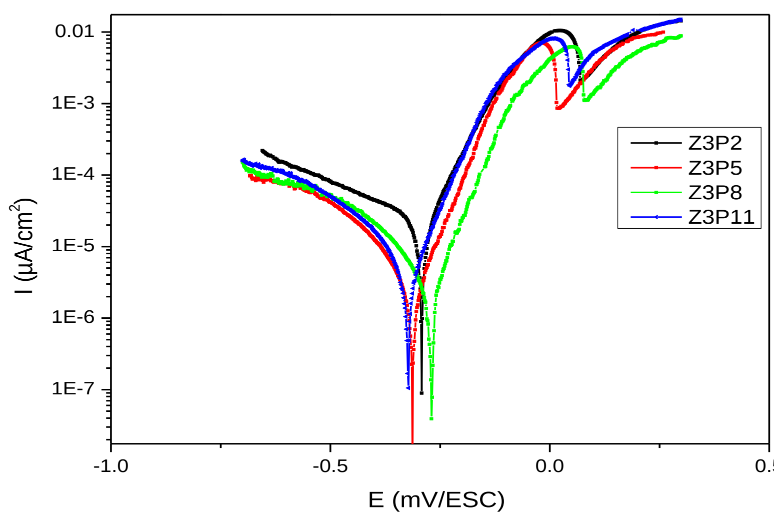

Figure 2 Cathodic and Anodic polarisation curves of copper in sea water of Z3 at different periods of analysis

We observe that for all periods considered, the values of E_{cor} and β_a are sensibly modified. However, the corrosion current density I_{cor} depends on the time of seawater retrieval. Highest values are recorded in the months of October, November and December 2012. While the lowest values in the months May, June and August 2013.

Table 2 Electrochemical parameter for copper corrosion in sea water at different period of analysis

Period of analysis	**Electrochemical parameter**				
	E_{cor} (mV/ECS)	**I_{cor} (µA/cm^2)**	**β_a (mV/decade)**	**R_t (Ω cm^2)**	**R_t^{-1} (Ω^{-1} cm^{-2}).10^3**
05/10/ 2012 (P_1)	-280	24	67	4167	0,240
05/11/2012 (P_2)	-290	16	72	6250	0,160
05/12/2012 (P_3)	-290	22	69	4545	0,220
05/01/2013 (P_4)	-300	8	63	12500	0,080
05/02/2013 (P_5)	-302	12	71	8333	0,120
05/03/2013 (P_6)	-270	13	67	7692	0,130
05/04/2013 (P_7)	-280	10	67	10000	0,100
05/05/2013 (P_8)	-270	5	67	20000	0,050
05/06/2013 (P_9)	-295	6	65	16667	0,060
05/07/2013 (P_{10})	-290	9	77	11112	0,090
05/08/2013 (P_{11})	-303	4	72	25000	0,040
05/09/2013 (P_{12})	-280	7	64	14286	0,070

Electrochemical impedance spectroscopy measurements EIS

EIS is a powerful, non destructive electrochemical technique for the characterization of electrochemical reactions at the metal/interface and the formation of corrosion products and biofilms in MIC. [17] The impedance spectra of copper specimens in the sea water Z3 after different period of analysis are illustrated in **Figure 3**. In the Nyquist plots, the diameters of impedance loops depend the time of seawater retrieval implying a variation in the corrosion rates of the copper. The Nyquist plots present a capacitive semicircle in the high frequency region. This capacitive semicircle is attributed to the combination of charge transfer resistance and the double layer capacitance, and its diameter represents the charge transfer resistance (Rt). [15] The values of R_t^{-1} as function of time analysis of sea water are also regrouped in table 2. We note that R_t value depends on the time of sea water analysis. Value of Rt^{-1} is directly proportional to the corrosion current density and then to the corrosion rate. [18] The higher value of R_t^{-1} is obtained for the month of October, November and December 2012. While the lowest values in the months May, June and August 2013.

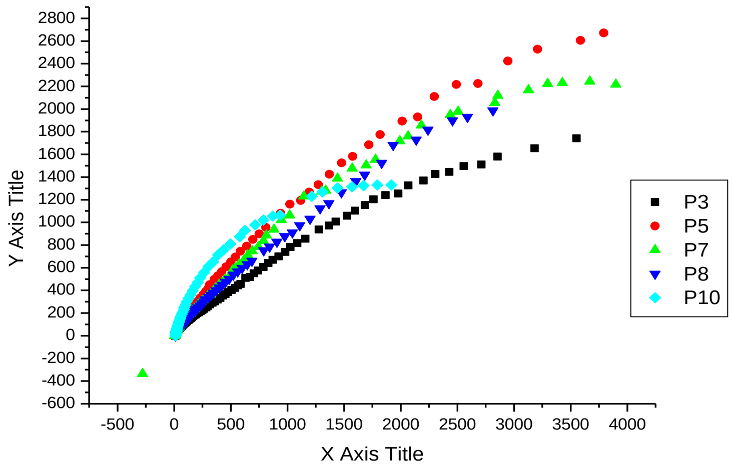

Figure 3 Nyquist plots for the copper electrode in seawater of Z3 at different analysis period

Correlation between corrosion rate and bacterial pollution

To illustrate the impact of pollution of sea water by the germs studied on copper corrosion, we plotted the curves of the simultaneous variation of corrosion rate and the concentration of the studied bacteria with sampling period of seawater. Figure 4 shows evolution of corrosion rate (I_{cor}) and bacterial germ concentrations with the sea water analysis period.

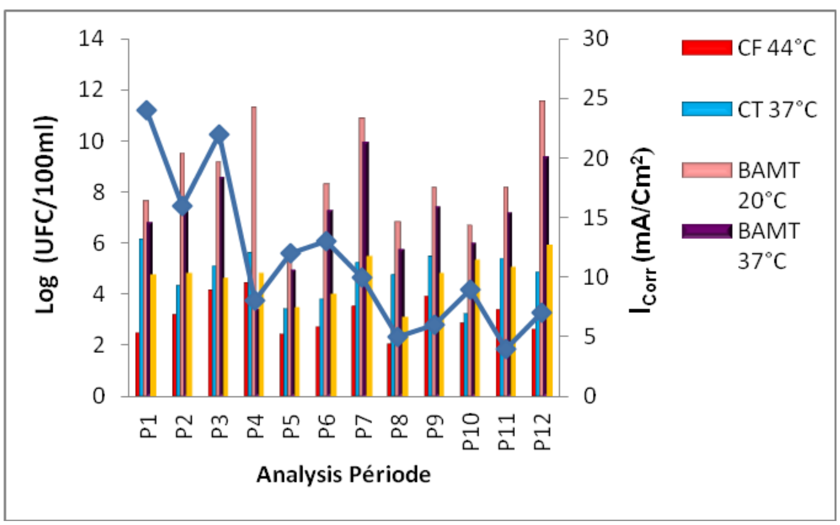

Figure 3 Evolution of concentration of five germs in sea water of Z3 and corrosion current density (I_{cor}) with analysis period

We find that the degree of correlation depends on the period of analysis of Z3 seawater. It is satisfactory for the periods P1, P2, P3, P5 and P6. During the analysis periods, the corrosion rate is even higher than the overall concentration of bacteria is high. For the other periods of sea water analysis, the corrosion rate does not seem affected by the variation of the concentration of these pathogens. This could be attributed to other parameters that influence copper corrosion in seawater. It could also come from the complex mechanism of action of these germs on the copper surface. Although the electrochemical nature of corrosion remains valid for microbiologically influenced corrosion

(MIC), the participation of microorganisms in the process nonetheless induces several unique features, the most significant being the modification of the metal–solution interface by biofilm formation [19]. Biofilms affect interactions between metal surfaces and the environment [20]. Biofilms formation on metals is the result of an accumulation process -not necessarily uniform in time or space [21] - that starts immediately after metal immersion in the aqueous environment. A thin film (approximately 20–80 nm thick), due to the deposition of inorganic ions and organic compounds of high relative molecular mass, is formed in a first stage. This initial film can alter the electrostatic charges and wettability of the metal surface, facilitating its further colonization by bacteria. In a short time (minutes or hours depending on the aqueous environment in which the metal is immersed), microbial growth and EPS production result in the development of a biofilm. This biofilm is a dynamic system, and the different transport processes and chemical reactions occurring at the biofouled interface will thus take place through the biofilm thickness. [22] Microorganisms influence corrosion acts by changing the electrochemical conditions at the metal–solution interface. These changes may have different effects, ranging from the induction of localized corrosion, through a change in the rate of general corrosion, to corrosion inhibition. [23] The stability of such layers depends on their chemistry and morphology and determines the overall susceptibility of the metal to corrosion. Microbial activity within biofilms formed on surfaces of metallic materials can affect the kinetics of cathodic and/or anodic reactions [24] and can also considerably modify the chemistry of any protective layers, leading to either acceleration or inhibition of corrosion. [25-26].

Conclusions

The main conclusions of the present study can be summarized as follows:

- The Agadir Bay sea water (Z3) is strongly polluted by microorganisms. We identified and analysed periodically five pathogen germs.
- The average concentrations of CF and BSR are well above the standard Moroccan guide value; indicating that sea water of Anza beach is poor quality.
- The presence of sulphite-reducing bacteria indicates the possibility of the microbiologically influenced corrosion.
- The instantaneous corrosion rate of copper is function of the analysis period.
- The degree of correlation between corrosion rate of copper and pathogen concentration depends on the type of this pathogen and period of analysis of Z3 seawater.

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