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ABIOTIC VARIABLES INFLUENCING *PSEUDOMONAS AERUGINOSA*'S SURVIVAL IN TWO MOROCCAN HUMID ZONES (SEAWATER AND WELL WATER)

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ABSTRACT

***Pseudomonas aeruginosa* is a pathogenic bacterium that grows in moist conditions. It is the source of several high-risk disease (affecting the lungs, bloodstream, or heart valves...). The purpose of this study is to investigate at the survival of *Pseudomonas aeruginosa* in two samples from Mehdia saltwater (S1) and Mnasra well water (S2) in Morocco from 0 to 72 days. The impact of the physico-chemical parameters of the two zones on *Pseudomonas aeruginosa* growth shows that temperature (19.5 °C in S1, and 26°C in S2), pH (7.4°C in S1, and 6.3 in S2), electrical conductivity (7.7S/cm in S1, and 1015 S/cm in S2), and bicarbonates (161.62 mg/l) have positive effects on the bacterium's abundance (1.15 10^9 UFC/ml). Temperature (19.5°C in S1, and 26°C in S2), pH (7.4°C in S1, and 6.3 in S2), electrical conductivity (7.7S/cm in S1, and 1015 S/cm in S2), and bicarbonates (161.62 mg/l) have positive effects on the bacterium's abundance (1.15 10^9 UFC/ml). However, the amounts of sodium (89 mg/l at S2) and potassium (3.5 mg/l at S2) restrict the growth of *Pseudomonas aeruginosa*. High-suspended solids (813.5 mg/l), chemical oxygen demand (410 mg/l), and biochemical oxygen demand (200 mg/l) in both zones, promoted its development.**

KEY WORDS : Survival, *Pseudomonas aeruginosa*, Seawater, Well water, Morocco.

INTRODUCTION

Pseudomonas aeruginosa is a common soil bacterium and a versatile opportunistic pathogen that may cause life-threatening infections in a wide range of situations and patient groups.

Each year, 440,000 individuals in the United States contract a Healthcare-Associated Illness (HAI), resulting in increased patient mortality and projected healthcare expenditures of $10 billion. While progress has been achieved in preventing HCAI-causing germs from spreading from patient to patient, much remains unknown about how these infections are transferred between patients and spread from one person to another (Bachta *et al.*, 2020).

Due to its nutritional requirements, this bacterium can maintain an exponential multiplication in different wetlands (soil, river, lake etc.). Thus, its ability to form biofilms inside the pipes gives it a resistance to disinfectants (El blidi *et al.*, 2003).

The present study deals with the evaluation of the impact of physico-chemical parameters of water taken from two wetlands (seawater and well water) on the survival time of *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

1 Origin of the bacterial strain

The strain of *Pseudomonas aeruginosa* was isolated and identified at the level of the wells of Strainer of the laboratory "Animal, Vegetal and Agro-Industries Productions", Ibn Tofail University-Kenitra, Morocco.

The present work took place during the period August 2019 to July 2020.

Sites for water sampling

Characteristics of the sampling site of the sea water from the Mehdia beach

Mehdia is a city in Morocco, located in the region of Rabat-Salé-Kénitra (RSK). It is a picturesque, small coastal town located near the city of Kenitra 30 km northeast of the capital Rabat. (El blidi *et al.*, 2003). The loop of Oued Sebou in the North, the Merja of Fouarat in the East and the Merjas of El Alwi and Sfassef in the North-East and A forest of Maâmora in the South and South-West (Snoussi, 2019) (Figure 1).

Characteristics of the Mnasra well water sampling site

The second study area corresponds to the rural commune of Mnasra, in the coastal zone of the Gharb plain (Morocco). This area is generally characterized by unevenly distributed rainfall. Only the southern part, close to Kenitra, was distinguished by market gardening. Intensive agriculture of high technicality makes a generalized use of groundwater. The climate is Mediterranean with an average temperature of 18°C (Bricha *et al.*, 2007) (Figure 2).

Sampling

In sterile glass bottles with a capacity of 250 ml, samples were collected at both locations. These were brought to the lab in a portable cooler (4°C) (ISO 5667/3 -1994). The samples were kept in a refrigerator at 2 to 5°C until they were analyzed.

Study of physico-chemical parameters of the two-sample collection locations

The evaluation of the physico-chemical parameters

Fig. 2. Geographic location of the Mnasra well water-sampling site (S2).

was carried out according to the techniques of Rodier (Rodier *et al.*, 2016). Indeed, Temperature, pH, and conductivity are measured in situ using a portable multi-parameter (Consort, type C 835). While, sodium (Na^+), potassium (K^+), and chloride (Cl^-) are determined by a flame atomic emission photometer. In addition, calcium (Ca^+) and magnesium (Mg^+) are determined by the EDTA volumetric method. Bicarbonates (HCO_3^-) are analyzed by volumetric determination with HCl at 0.1 N, nitrites (NO^{2-}) are determined by colorimetric determination with a spectrophotometer (UV/visible Lamba 2).

Suspended solids (SS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) are measured by methods described in the ISO 5667/3 standards (Hamdani *et al.*, 2004).

Fig. 1. Geographical location of the study site (S1) of Mehdia-Kenitra beach.

Water sample preparation

Wet sterilization at 121° for 20 minutes is applied to samples taken from both locations, followed by filtering through a 0.45m diameter cellulose filter.

***Pseudomonas aeruginosa* strains revification**

Bacterial strains that had been kept in glycerol at -4 °C are revived by seeding them in nutrient broth repeatedly until they are viable and cultivable.

The tubes that demonstrate turbidity after 24 hours of incubation at 37°C are judged positive. These are *P. aeruginosa* colonies with the typical shape and coloration.

Revivification of *Pseudomonas aeruginosa* strains

Bacterial strains previously preserved in glycerol at -4°C are revived by successive inoculation in nutrient broth, in order to obtain viable and cultivable bacteria.

After incubation for 24 h at 37 °C, the tubes showing a turbidity are considered positive. These are colonies with the characteristic morphology and pigmentation of *P. aeruginosa.*

***Pseudomonas aeruginosa* Survival**

The Most Probable Number (MPN) technique is used to calculate the survival dynamics of *Pseudomonas aeruginosa*.

After adjusting the bacterial concentration to 7.88 10^8 UCF/ml (using a wavelength of 600 nm to measure the optical density), subsequent dilutions were produced. The tubes were then incubated for 72 days at 35 °C.

We may calculate a Characteristic Number based on the number of positive tubes gathered (CN). A three-digit number is the NC. The first value refers to the greatest number of positive tubes obtained at the highest dilution. For the computations, this dilution will be taken into account. The other two numbers represent the number of positive tubes in the two dilutions that follow.

According to Mac Grady's table, the volume sown of the dilution evaluated corresponds to a more probable number of germs for each characteristic number.

Expression of results

The outcomes of determining the amount of *Pseudomonas aeruginosa* per milliliter of water are given as MPN= most likely number obtained by reading the Mac Grady table (Rodier, 2016).

$$N = \frac{MPN}{Vcultivation} \times F_{dilution}$$

Statistical Analyses

In order to obtain an assumption of a normal distribution, the numbers were transformed to decimal logarithmic values. To explore the influence of specific variables, the data were submitted to an analysis of variance using the TUKEY test (strains and environment).

RESULTS AND DISCUSSION

Physico-chemical parameters of the two sampling sites

Table 1 reports the values of the parameters considered in this study, which include temperature, pH, electrical conductivity, Sodium, Potassium, Magnesium, Nitrite, Chlorine and Bicarbonates (HCO^{3-}) and the content of BOD_5, SM, COD.

Table 1. Average values of physico-chemical parameters measured in the study sites.

Sites	Mehdia seawater	Mnasra Well Water	OMS Standard
T°C	19,5	26	–
pH	7,4	6.3	–
Electric conductivity (µS/cm)	15 952	1650	–
Sodium Na^+mg/l	120	89	150
Potassium K^+mg/l	2.73	3.5	12
Calcium Ca^+mg/l	174	100	70
Magnesium mg/l	93.08	17.32	50
Bicarbonate (HCO^{3-}) mg/l	113.52	162,61	200
Chloride (mg/l)	155.57	173	200
Nitrite NO^{2-} (mg/l)	0.001	96.84	0.1
Biochemical oxygen demand (BOD_5) (mg/l)	200	132	–
Suspended matter (SM) (mg/l)	813,5	48	–
Chemical Oxygen Demand (COD) (mg/l)	410	112	–

We noticed that the average temperature measured in both media was 19.5 °C for Mehdia water and 26 °C for Mnasra water during the test period.

The electrical conductivity is only 1650 µs/cm for the water of Mnasra, while that of seawater, reaches 15 952 µs/cm. This translates into levels of Sodium, Potassium, Magnesium, Calcium, Bicarbonates (HCO^{3-}), Nitrite and Chlorine, that are respectively as follow 89 mg/l, 3.5 mg/l, 100 mg/l, 17.32 mg/l, 162.61 mg/l, 96.84 mg/l and 173 mg/l. For the sample of Mnasra, the concentrations were higher in the second sample (120 mg/l, 2. 73 mg/l, 2.73 mg/l, 174 mg/l, 93.08 mg/l, 0.001 mg/l, 155.57 mg/l and 200 mg/l respectively). This corresponds to a strong mineralization of these waters.

The Organic Suspended Matter (OSM) present in the Mehdia seawater (813.5 mg/l) is higher than in the Mnasra well water (48 mg/l). These organic matters present in these waters could be of natural origin (biological activity of the hydrous environments: decomposition of plants or microorganisms, etc...), or be related to domestic pollution. The presence of organic matter in these waters is an indirect indicator of microbiological risk and can pose two types of problems (Timoléon and Fulbert, 2013):

- It can react with water disinfection products (especially chlorine) and form undesirable by-products such as Tri-HaloMethanes (THMs) or cause a bad taste in water.
- It can initiate bacterial growth problems.

The concentrations of TSS in both types of water partly explain the high values recorded for Biochemical Oxygen Demand (BOD5) (200 mg/l and 132 mg/l respectively for seawater and well water) and Chemical Oxygen Demand (COD) (410 mg/l and 112 mg/l, respectively for seawater and well water). For example, high oxygen demand (COD or BOD_5) presumes the abundance of organic matter in the media.

In vitro survival kinetics of *Pseudomonas aeruginosa* in aqueous solutions from seawater and well water

Monitoring of the survival of *Pseudomonas aeruginosa* in seawater and well water is performed at 35 °C for 72 days. Figure 3 reports the results of the survival of the bacteria for 72 days in seawater and well water. We noticed that after two days of culture at 35 °C, *Pseudomonas aeruginosa* showed exponential growth, which was expressed by an increase in concentration of 8.81 10^{10} UFC/ml. In the following two days, we observed a decrease in microbial activity until 2.15 10^{10} UFC/ml. Then, once it reached its stationary phase where there is a

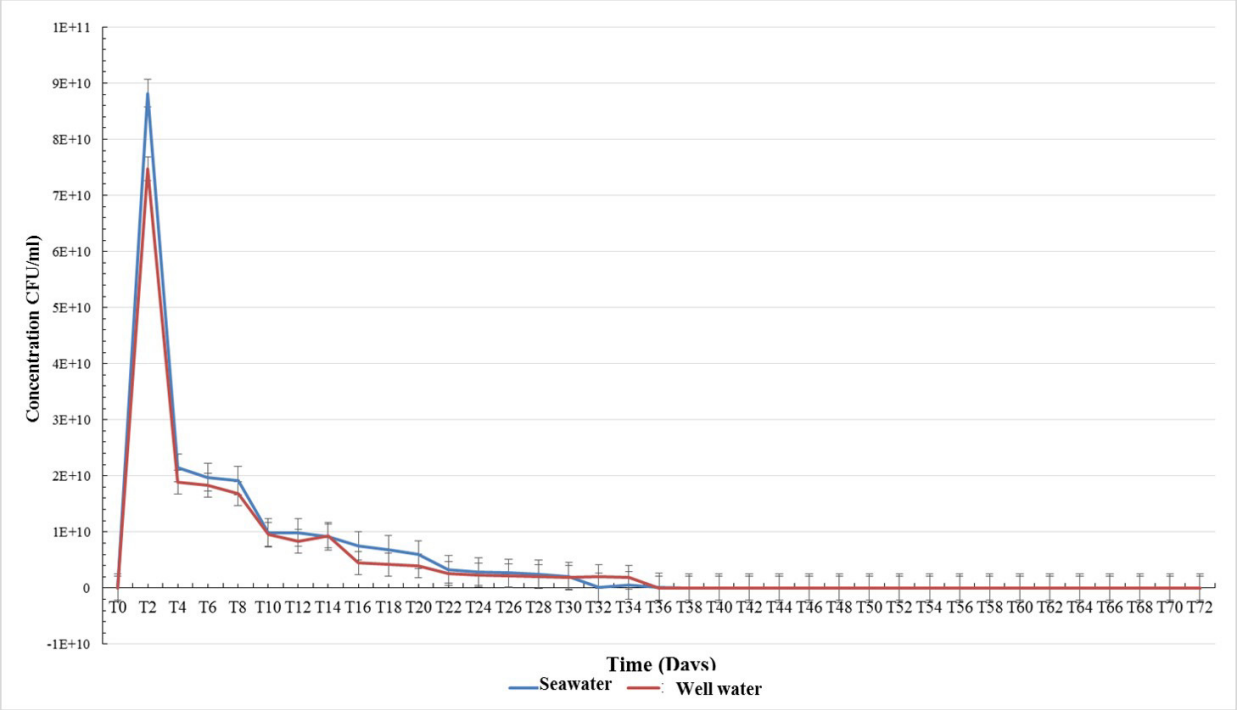

Fig. 3. Kinetics of *Pseudomonas aeruginosa* survival in seawater (S1) and well water (S2).

compensation between the cells that die and those that multiply, the bacteria persisted for 4 days before reaching the decline phase, which lasted 26 days (from T10 to T36).

However, inoculation of Mnasra well water with the Pseudomonas aeruginosa strain allowed it to persist for 72 days at 35 °C. Its exponential phase started on the second day (T2), which translates into an increase in the bacterial concentration of 7.5 10^{10} CFU/ml. The latter is maintained for two more days, before it begins its decrease to 1.9 10^{10} CFU/ml (T4). Then, the bacterial population continued to survive at the same concentration for four more days (T8). Subsequently, in its decline phase, the abundance of *Pseudomonas aeruginosa* in the medium decreased significantly to reach 72 days of total disappearance (T72 = 0 CFU/ml).

Environmental factors have been shown to impact bacterial physiology in recent research (Wilson *et al.,* 2008; Crabbé *et al.,* 2011). Phosphate and oxygen availability can also affect how bacteria grow and respond in the environment. For example, oxygen influences several aspects of "facultative anaerobic" *Pseudomonas aeruginosa* development and physiology, including growth, motility, and biofilm formation (Hong *et al.,* 2004).

The hypothesis regarding the influence of low bicarbonate concentration on the number of bacteria in the water environment was validated in a research on spring and well water from Yaoundé (Cameroon) (Nola *et al.,* 2001). Maximum monthly quantities of *Pseudomonas aeruginosa* (1. 10^3 to 22. 10^3 CFU/100 ml) were found in both kinds of medium (spring and well water). This research backs up our findings on bacteria resistance (162.61 mg/l) in Mnasra well water (S2), which has a low bicarbonate content.

The kind of anion in the growth medium has a significant impact on microorganism growth rates. In the case of *P. aeruginosa*, all ions have a detrimental impact on the bacteria's ability to survive. The activities of DNA restriction enzyme, horseradish peroxidase, and lipase A are all affected (Nostro *et al.,* 2005). In contrast to sulfate and nitrate, chloride has been demonstrated to protect microorganisms against heat stress (Hurst *et al.,* 1980). Cl^- is also necessary for some halophilic and halotolerant organisms in general (Muller et Oren, 2003).

Furthermore, studies have demonstrated the relevance of the membrane in the survival of bacteria in a variety of settings, including those that are especially harsh (Mykytcz *et al.,* 2007). *P. aeruginosa* biofilm development is inhibited by high salt and iron concentrations (Musk *et al.,* 2005), which explains the bacteria's disappearance after 32 days of seeding in salt-rich saltwater.

Declaration of interest statement

The authors declare no conflict of interest.

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REFERENCES

Bachta, K. E., Allen, J. P., Cheung, B. H., Chiu, C. H. and Hauser, A. R. 2020. Systemic infection facilitates transmission of *Pseudomonas aeruginosa* in mice. *Nature Communications.* 11(1) : 1-13.

Bricha, S., Ounine, K., Oulkheir, S., El haloui, N. and Attarassi, B. 2007. Etude de la qualité physicochimique et bactériologique de la nappe phréatique M'nasra (Maroc). *Afrique Sciences.* 03(3) : 391-404.

Crabbé, A., Schurr, M.J., Monsieurs, P., Morici, L., Schurr, J., Wilson, J.W., Ott, C.M., Tsaprailis, G., Pierson, D.L., Stefanyshyn-Piper, H. and Chery, A. Nickerson, 2011. Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. *Appl Environ Microbiol.* 77: 1221-1230.

El blidi, S. and Fekhaoui, M. 2003. Hydrologie et dynamique marégraphique de l'estuaire du Sebou (Gharb, Maroc), Bulletin de l'Institut Scientifique, Rabat. *section Sciences de la Vie*. N°25, 57-65;

Hamdani, A., Chennaoui, M., Assobhei, O. and Mountadar, M. 2004. Caractérisation et traitement par coagulation-décantation d'un effluent de laiterie. *Le Lait.* 84 (3) : 317-328.

Hong, C.S., Shitashiro, M., Kuroda, A., Ikeda, T., Takiguchi, N., Ohtake, H. and Kato, J. 2004. Chemotaxis proteins and transducers for aerotaxis in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett.* 231 : 247-252.

Hurst, A., Hughes, A. and Pontefract, R. 1980. Mechanism of the temperature protective effect of salts on *Staphylococcus aureus J. Microbiol.* 26 511-7

Markou, P. and Apidianakis, Y. 2014. Pathogenesis of intestinal *Pseudomonas aeruginosa* infection in patients with cancer. *Front. Cell. Infect. Microbiol.* 3: 115.

Muller, V. and Oren, A. 2003. Metabolism of chloride. Halophilic prokaryotes. *Extromophiles.* 7: 261-6

Musk, D. J., Banko, D. A. and Hergenrother, P. J. 2005. Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. *Chem Biol.* 12 : 789-796.

Mykytczuk, N. C. S., Trevors, J. T., Leduc, L. G. and Ferroni, G. D. 2007. Fluorescence polarization in studies of bacterial cytoplasmic membrane fluidity under environmental stress. *Progress in Biophysics and Molecular Biology*. 95(1-3): 60-82. Albelo S.T., C.E. Domenech, Carbons from choline present in the phospholipids of *Pseudomonas aeruginosa. FEMS Microbiol. Lett.* 156 : 271-274.

Nola, M., Njine, T., Sekati, V. and Euphrasie, D. 2001. Distribution of *Pseudomonas aeruginosa* and Aeromonashydrophila in ground water in equatorial zone in Cameroon and relationships with some environmental chemical factors, Revue des sciences d'eau. *Journal of Water Science*. 14(1) : 35-53;

Nostro, P. L., Ninham, B. W., Nostro, A. L., Pesavento, G., Fratoni, L. and Baglioni, P. 2005. Specific ion effects on the growth rates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Physical Biology*. 2(1) : 1.

Rodier J. et Legube B. 2016. *L'analyse de l'eau*, Dunod 10 édition. Dunod, Paris.p.1824.

Snoussi, M. 2019. Contribution à l'élaboration d'un Plan degestion intégrée pour les zones côtières de la Région de Rabat-Salé-Kenitra Tâche 1 : Diagnostic des zones côtières de la Région de Rabat-Salé-Kenitra, p.70.

Timoléon and zi Barhéa et Fulbert Bouakab 2013. Caractérisation Physicochimique et Chloration des Eaux de Puits Consommées dans la Ville de Brazzaville-Congo (Physicochemical Characterization and Chlorination of Well Water Consumed in Brazzaville Congo). *J. Mater. Environ. Sci.* 4 (5) : 605-612.

Wilson, J.W., Ott, C.M., Quick, L., Davis, R., Hönerzu Bentrup, K., Crabbé, A., Richter, E., Sarker, S., Barrila, J. and Porwollik, S. 2008. Media ion composition controls regulatory and virulence response of *Salmonella* in spaceflight. *PLoS One.* 3:e3923.