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(RESEARCH ARTICLE)

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Use of bacterial bioluminescence (Microtox test) as a tool for assessing the toxicity of pure substances and complex effluents (Moroccan Atlantic coast)

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Abstract

The Microtox test is a rapid, simple, and widely used test in the context of screening and comparative studies. The aim of this study is to use the *Vibrio fischeri* bacteria luminescence inhibition test to assess the toxicity of metals and those of industrial unit discharges.

The assessment of the effects of discharges on *V. fischeri* luminescence reveals the great sensitivity of this bioindicator to discharges and highlights a high risk of deterioration in the quality of the marine environment, particularly in the areas where discharges, with EC50s which can reach 0.25% discharge into the test medium for petrochemical effluents after 5 minutes of exposure. The analysis of these results made it possible to classify the discharges according to their toxicity towards the bacterium *V. fischeri* and to draw up a profile of the impact of these discharges according to the EC50 values. The results of tests carried out on metals show that mercury is the most toxic metal (EC50 = 0.63 mg Hg L-1), followed by lead (EC50 = 1.08 mg Pb L-1) and cadmium (EC50 = 1.87 mg Cd L-1) and finally copper (EC50 = 2.57 mg Cu L-1). The results obtained during this study show that the Microtox test represents a good model for evaluating the toxicity of complex discharges and pure substances and that bacterial luminescence constitutes an excellent bioindicator for screening the medium quality.

Keywords: Microtox; Ecotoxicology; Global toxicity; *Vibrio fisheri*; Effluents; Metals

1. Introduction

The development of anthropogenic activities has generated the production and diffusion of several chemical contaminants in aquatic environments. The Moroccan Atlantic coast concentrates on the country's main industrial activities: agro-food, textiles, chemistry and para-chemistry, and mechanical and electrical industries. The consequences of this intense industrial activity on the environment are numerous, including the pollution of marine waters by spills and industrial discharges (eutrophication, odors, reduction of biodiversity, regression of seagrass beds, etc.), which causes the degradation of fauna and flora.

The physicochemical approach to water quality makes it possible to individually characterize specific pollutants, but it assumes that the contaminants are identifiable and relatively limited in number, which is rarely the case. Thus, the use of biological tools, which make it possible to study the global effects of pollution on the environment, provide information on the exposure of organisms to polluting substances, and biological tests make it possible to assess the impact of discharges on organisms (Boucheseiche et al., 2002).

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Among the most widely used tests, the Microtox test has shown its effectiveness in several ecotoxicological studies (Beckman, 1982). Indeed, it is recommended for routine monitoring and offers good sensitivity while being easy to implement and perfectly suited to large series of samples (TOUSSAINT et al., 1995).

The purpose of this study is to assess the toxicity of metals (mercury, copper, lead, and cadmium) and that of discharges dumped in the Atlantic coastal fringe from Mohammedia to Safi on the luminescence of the bacterium *Vibrio fischeri* after an exposure of 5, 15 and 30 minutes.

2. Material and methods

The Microtox toxicity test uses the marine bioluminescent bacteria *Vibrio fisheri* as the test organism. The Microtox Model 500 toxicity analyzer was used. The ‘‘Basic Test’’ protocols were utilized based on the level of toxicity. The bacterial luminescence, the endpoint of this assay, was measured for 10 effluent samples and 4 metals after 5, 15, and 30 min of exposure at 15 °C. The bioassay protocol used in this study is inspired by the NM ISO 11348-3 (2014) standard using freeze-dried bacteria.

The toxicity of ten industrial effluents (Figure 1) was assessed, namely:

2.1. Mohammedia area

- The electrochemical effluent (EL) emanates from an electrochemical company whose main activity is the production of polyvinyl chloride (PVC), chlorine, and soda, which involves mercury and lead in the production processes.
- The effluent from an oil refinery (RP). This refinery specializes in refining petroleum products.
- The Oued Mellah (OM) effluent, which flows into the coastal area of Mohammedia, but its estuarine section has been transformed into an open-air outfall, draining wastewater from several industrial units and leachate from the Mohammedia city solid waste landfill.

2.2. Casablanca area

- Lesieur effluent (LE): This effluent combines wastewater from several industrial units with highly heterogeneous domestic water flows, mainly wastewater from the production of agri-food oils and soap.
- Oukacha effluent (OU) from a group of companies in Aïn Sbaa discharges into the sea at the "Oukacha" industrial complex, through which very heterogeneous domestic and industrial water flows from the agri-food and chemical industries.
- Chahdia effluent (CH) collects wastewater from a number of companies in Ain Sbaa, mainly from the textile industry. It discharges into the sea at Chahdia Beach.
- Zenata effluent (ZE): The Zenata industrial zone is a very important industrial park in Morocco. The discharge is an emissary from several industrial units operating in several sectors, mainly the agri-food, chemical, mechanical and electrical industries. It is characterized by its strong odor.
- Sidi Abdellah Belhaj (SAB) effluent: The Sidi Abdallah Belhaj area is very active economically, especially for the inhabitants of the region's douars, as well as for a phosphate mining structure.

2.3. Safi area

- The Phosphate processing effluent (TP) comes from the chemical complex located 10 km from Safi, which belongs to the Office Chérifien des Phosphates Group and is used for the industrial manufacture of phosphoric acid and fertilizers through the transformation of phosphates and sulfur.
- -The Jorf Lihoudi effluent (JL) comes from a fish industry specializing in canned sardines.
- Discharge concentration ranges (in volume/volume percentage) obtained by dilution in reference seawater are 0.25%, 0.50%, 1%, and 2% for refinery and Sidi Abdellah belhaj discharges 11%, 22%, 45% and 90% for Lesieur and Oukacha discharges and 5%, 11%, 22% and 45% for other discharges.

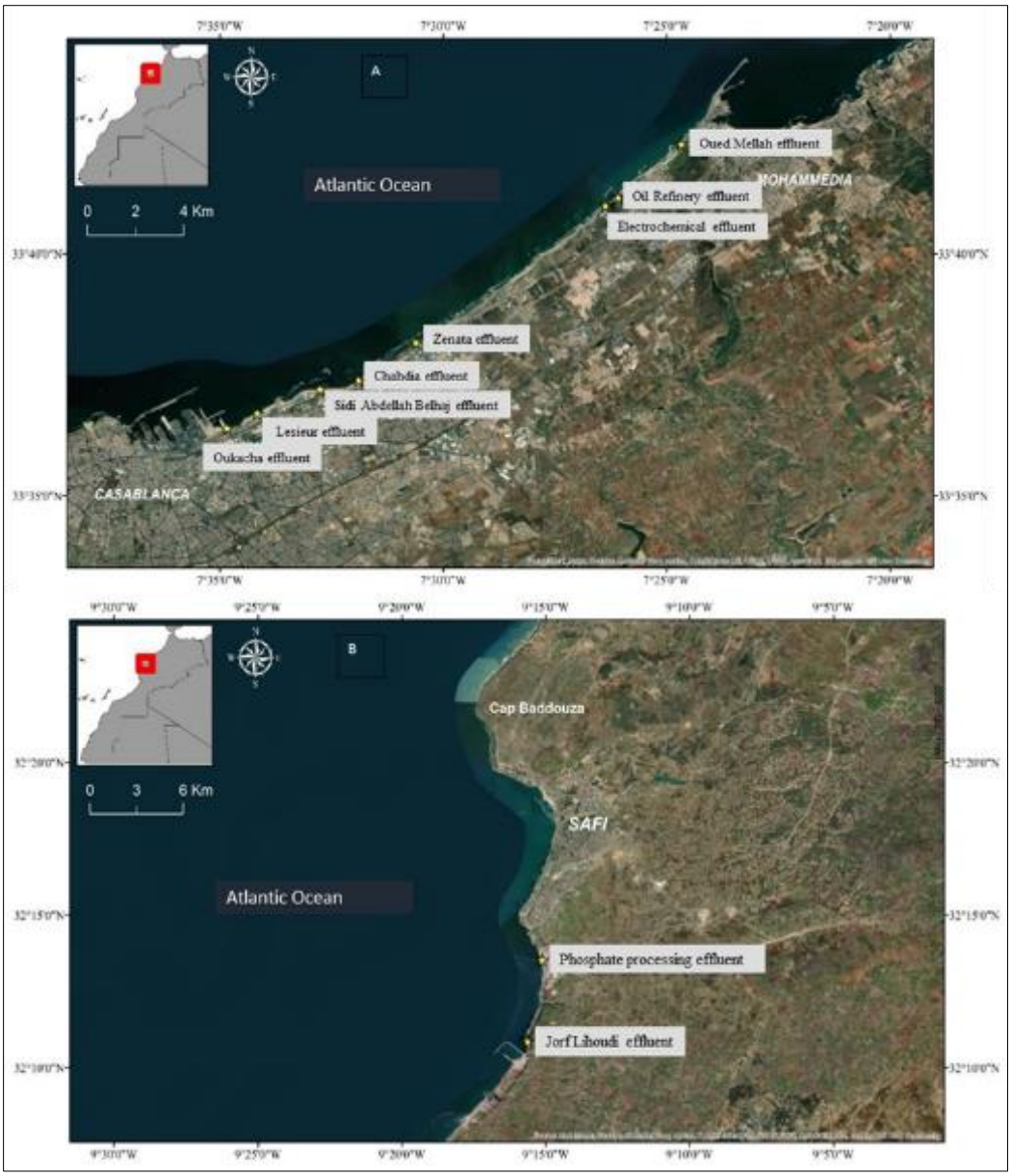

Figure 1 Map showing the location of effluents tested; A: Mohammedia-Casablanca area. B: Safi area

Four metals (mercury, copper, cadmium, and lead) were also tested in the form of nitrates according to the following concentration ranges (mg·L-1) :

- Hg(II) (Hg(NO3)2) : 0,125 – 0,25 – 0,5 - 1
- Cu(II) (Cu(NO3)2) - Cd(II) (Cd(NO3)2) - Pb (II) (Pb(NO3)2) : 0,625 – 1,25 – 2,5 - 5

The bioassay protocol used is inspired by NM ISO 11348-3 (2014). Freeze-dried bacteria, stored in a freezer at -18°C to -20°C, are rehydrated in a reconstitution solution corresponding to pure water and placed in a well at 4°C to 6°C in the Microtox. Samples are diluted in a 2% NaCl solution (diluent) after correction of their salinity with an osmotic adjustment solution (sterile 22% NaCl solution), according to the protocol described in the Microtox Omni software, which controls the measuring device. Samples are placed in wells at a temperature of 15°C. 10 µl of biological reagent are dispensed into the measuring cuvettes, and approximately 15 min later, the initial light emitted I_0 is measured; the time is noted. An aliquot of 0.5 ml of diluent or different dilutions of the sample is added to each measuring cuvette. The light emitted by each of these cuvettes is measured again 5, 15, and 30 min after the noted time of addition.

The percentage of effect, which corresponds to the percentage of light intensity inhibition, is determined by the software. Microtox's measurement of sample toxicity gives its IC50: the sample concentration, which, under the

conditions of temperature and duration of the experiment, produces a 50% reduction in the light emitted by the biological reagent solution compared with a control. This concentration is expressed in milligrams per liter for metal compounds and as a percentage of volume/volume concentration for effluents.

To classify the discharges tested according to their toxicity (Table 1), we used the classification specific to the Microtox test according to EC50 values established by Bennett and Cubbage (1992).

Table 1 Toxicity ranges based on 5 and 15-minute Microtox@ EC50 values (Bennett and Cubbage, 1992).

CE50 Microtox (%)	**Toxicity level**
0 - 19	Extremely Toxic
20 - 39	Very Toxic
40 - 59	Toxic
60 - 79	Moderately Toxic
80 - 99	Slightly Toxic
>100	Non Toxic

The validity of the tests is verified using a reference toxicant, zinc sulfate (ZnSO4, 7H2O). This compound was chosen because it is well-known, has a relatively stable chemical composition, and is one of the most problematic pollutants in the environment. A range of concentrations is prepared from a stock solution of 1g. L-1: 0.625 - 1.25 - 2.5 - 5 ppm.

3. Results

3.1. Assay validity

The toxic use of reference zinc sulfate allowed us to verify the sensitivity of the bacteria. The mean IC50 is 2.15 mg·L-1 of Zn++ after 15 minutes of exposure, a value which falls within the reference range of 06 - 2.2 mg/L specified by the manufacturer Microtox (Azur Environmental, 1995). These results validate the tests carried out, the results of which can therefore be analyzed.

3.2. Impact of metals

The results of the metal's effects on bacterial luminescence measured by Microtox (figure 2) show that they exert a toxicity proportional to the concentration of the metal in the medium at different exposure times, mercury being the most toxic.

A concentration of 1ppm Hg.L-1 causes 100% inhibition of bacterial luminescence. Lead is also highly toxic to bacterial luminescence, with inhibition rates of over 70% at 2.5 ppm and 100% at 5 ppm Pb.L-1. Cadmium also has a significant effect on bacterial luminescence, which decreases by over 80% at 2.5 ppm Cd.L-1. For copper, its toxicity on luminescence remains relatively lower than that observed with the two other metals (Figure 2). At a concentration of 2.5 ppm Cu.L-1, the percentage of inhibition does not exceed 60% after 5 and 15 minutes of exposure, but at 5 ppm, the effect is extreme (100% inhibition of luminescence at all exposure times).

A comparison of the IC50 calculated for the four tested metals shows that mercury is the most toxic metal for *V. fischeri* bacteria (IC50 = 0.63 mg·L-1), followed by lead (IC50 = 1.08 mg·L-1), cadmium (IC50 = 1.87 mg·L-1) and copper, which is the least toxic metal with an IC50 of 2.57 mg·L-1.

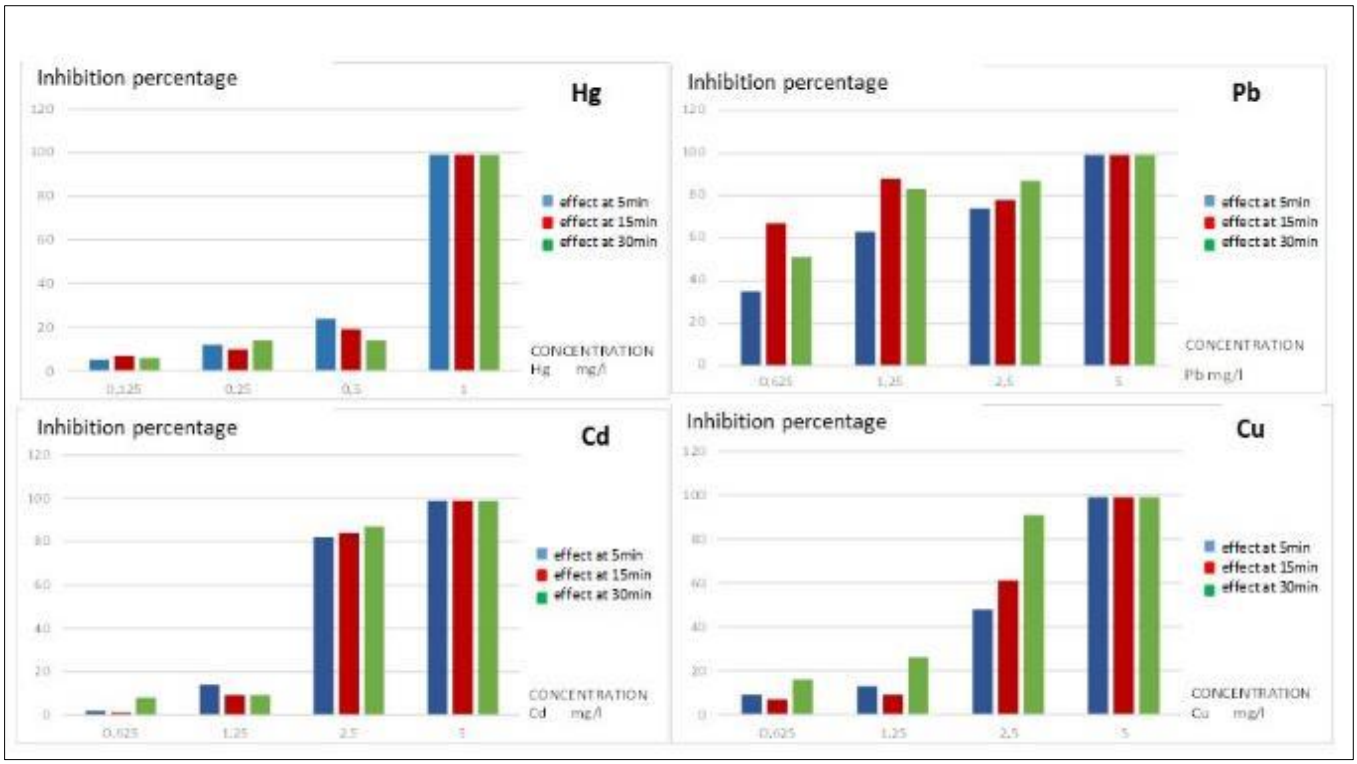

Figure 2 Effect of metals (Hg, Cd, Pb, Cu) on the luminescence of *Vibrio fischeri* bacteria at different exposure times. inhibition percentage

3.3. Impact of effluents

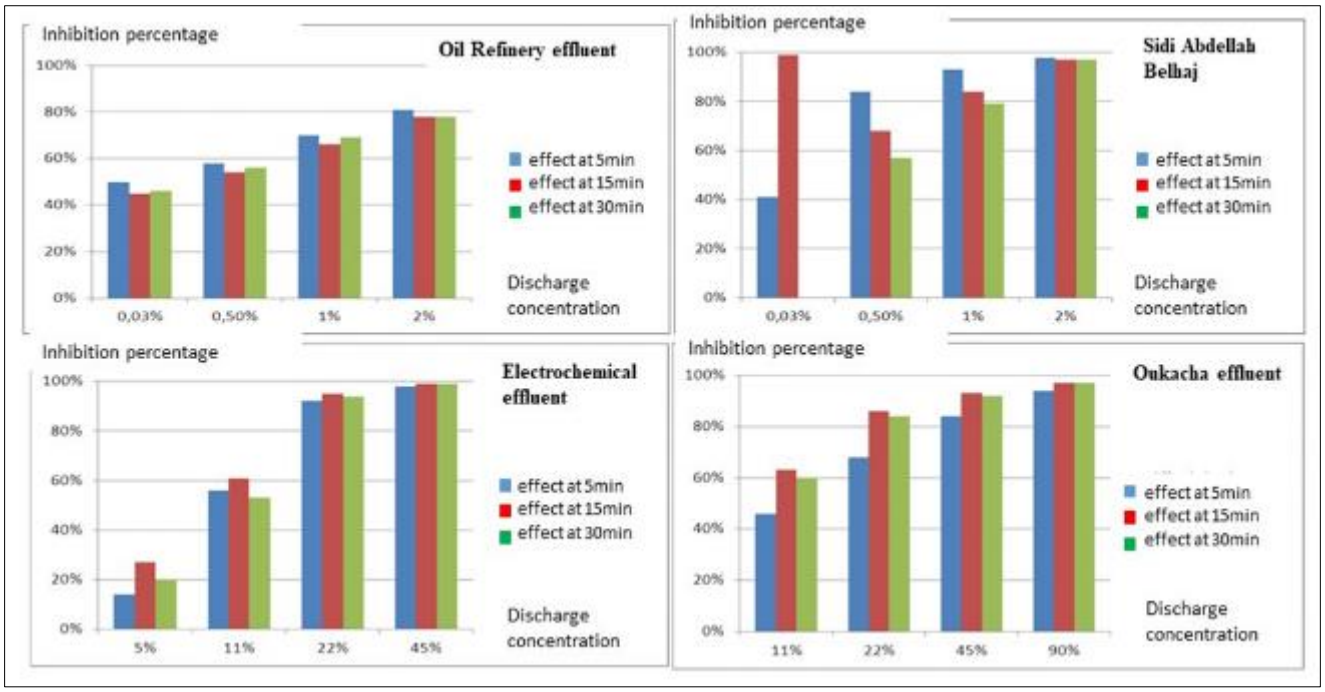

Figure 3a Impact of discharges from the Mohammedia-Casablanca area on the luminescence of *Vibrio fischeri* bacteria at different exposure times

The results of Microtox tests carried out on effluents from the Casablanca-Mohammedia coastal area and from a phosphate complex and fish industry based in Safi are shown in figures 3 and 4. Analysis of these results shows the existence of a dose-effect relationship at different exposure times. The discharge from Sidi Abdellah Belhaj exerts a

strong toxic effect on *V. fischeri*. Indeed, at 1% (v/v) of effluent in the test medium, inhibition percentages of bacterial luminescence exceed 60%, and with 2% effluent dilution, inhibition can reach 100%. The calculated IC50s are 0.28 and 0.25% for the oil refinery discharge and the Sidi Abdellah Belhaj discharge, respectively. For the Electrochemical discharge, we observed an effect starting at 5%, which increases with the discharge concentration in the medium and exceeds 50% with the 11% dilution; the calculated IC50 is 10%. Similarly, for the Oukacha discharge, the percentage of inhibition exceeds 80% from 45% of the discharge in the medium; the IC50 is 12% (figure 3a).

The toxicity of the Zenata effluent is not very pronounced, with an IC50 of 78%. The Lesieur, Chahdia, and Oued Mellah discharges have a negligible effect on bacterial luminescence; the IC50s calculated for these discharges are over 100% (figure 3b).

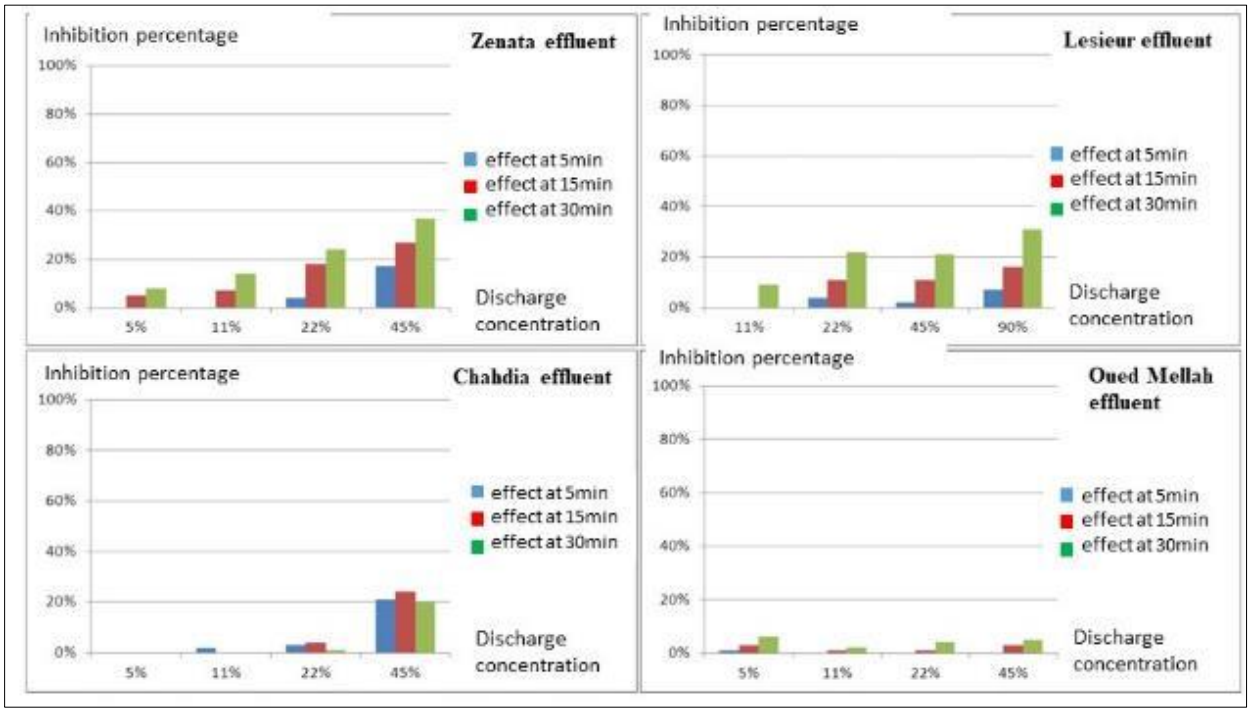

Figure 3b Impact of discharges from the Mohammedia-Casablanca area on the luminescence of *Vibrio fischeri* bacteria at different exposure times

Discharge from the Safi phosphate complex is highly toxic for bacteria, with luminescence inhibition exceeding 80% at a dilution of 12.5%; the calculated IC50 is 7.8%. The toxicity of the fish industry's effluent is also apparent at a dilution of 6.25%, with an effect of almost 60%; the IC50 calculated for this effluent is 13.8% (figure 4).

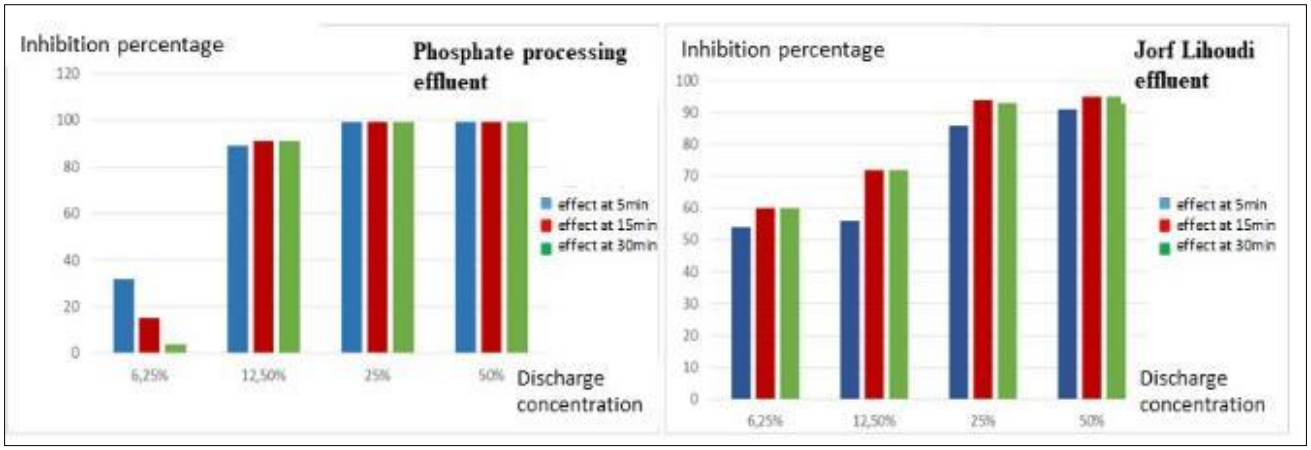

Figure 4 Impact of discharges from the Safi area on the luminescence of *Vibrio fischeri* bacteria at different exposure times

Based on the classification table specific to the Microtox test according to IC50 values (Bennett and Cubbage, 1992, Table 1), we were able to classify the discharges tested according to their toxicity to *V. fisheri* luminescence. Effluents RP, SAB, TP, EL, OU, and JL are classified as extremely toxic. Discharge ZE is moderately toxic, while discharges LE, OM, and CH are classified as non-toxic to bacterial luminescence.

4. Discussion

4.1. Test validity

Test validation is verified by the use of the reference toxicant zinc sulfate. The mean IC50 is 2.15 mg·L-1 of Zn++ after 15 minutes of exposure. This is in agreement with the IC50 value found in the reference document Azur Environmental (1995) and those recorded by other works in the literature (Table 2).

Table 2 Comparison of inhibitory concentrations (IC50) for zinc sulfate tested in *Vibrio fischeri*

Study reference	CI50-15mn (mg Zn++/L)
This study	2,15
Azur Environmental (1995)	0,6 – 2,2
Microbics (1992)	1,20
F.S. Mowat & K.J. Bundy (2001)	2,62
Ince et al. (1999)	1,62

4.2. Metal Toxicity

The results obtained show that *V. fischeri* bacteria are highly sensitive to metals. In fact, inhibition of luminescence was observed at very low concentrations of metals from 5 min exposure (from 0.125 ppm) and IC50 -5 min, varying between 0.6 and 2.5 ppm. Mercury is the most toxic of all metals, ranking at the top of the list in terms of toxicity to marine organisms. Galli et al. (1994) found a lower IC50 with Microtox (0.63 ppm Hg++). Grange and Pescheux (1985) recorded an even lower IC50 (0.065ppm) with HgCl2. The early larval developmental stages of *C. gigas* were shown to be more sensitive to this metal with an EC50- 24 h of 4.4 µg·L-1 (Bouhallaoui et al. 2017).

Lead also exerts a toxic effect on bacterial luminescence, with a noticeable effect recorded from 0.625 ppm. The IC50 is estimated at 1.08 ppm; Grange and Pescheux (1985) found an IC50 close to our own (1.4 ppm) with Pb(NO3)2.

Cadmium has a significant inhibitory effect on light intensity, with an IC50 of 1.87 ppm, but this is lower than that recorded with lead and mercury. In a study on Microtox, an IC50 of 1.2 ppm was calculated for cadmium nitrate (1%NaCl) (Quiniou and JUDAS, 1995). An experiment on the effects of Cd and Hg on the larval behavior of the crab *Eurypanopeus depressus* showed that Cd at sublethal concentrations disrupted larval swimming activity, with an increase in mortality (MIRKES et al., 1978).

The impact of copper on luminescent bacteria is not negligible but remains moderate compared to the other metals; the IC50 calculated is 2.57 mg·L-1 which is a low value compared to that recorded in other research work, which demonstrated higher toxicity of copper with an IC50 of 0.9 mg·L-1 (Petala et al., 2005). Indeed, salts of this metal (Cu oxide, Cu sulfate, and Cu chloride) are used in the composition of antifouling paints that profoundly disrupt aquaculture activities.

Estimating the potential toxicity of metals using this bioassay enabled us to establish a toxicity gradient compatible with that found in the literature. Indeed, based on IC50 values, the metals tested can be ranked from high to low toxicity in the following order: Hg > Pb > Cd > Cu; Jawecki et al.,(1997) recorded a similar gradient of toxicity of metals assessed by Microtox (Hg > Pb = Zn > SDS > Cd).

Furthermore, unlike the metallic elements lead and copper, for which the toxic effect generally increases with exposure time, the toxicity of mercury and cadmium is not influenced by increasing exposure time from 5 to 30 minutes; this result is in agreement with that found by Petala et al. (2005).

4.3. Effluents toxicity

The problem of urban and industrial waste is an ever-present priority. As people's standard of living increases, so do the quantities of waste they produce. It has long been recognized that assessing the ecotoxicity of complex liquid effluents, such as urban or industrial waste leachates, requires the simultaneous use of different bioassays (Sekkat et al., 2001). For routine monitoring, the Microtox test is the preferred choice, as it offers good sensitivity, is easy to use, and is perfectly suited to large sample runs.

This study assessed the impact of industrial effluents on the bacterial luminescence of *V. fischeri* using the Microtox test and showed that this indicator is highly sensitive to effluents since we observed inhibitory effects on luminescence (40%) at concentrations as low as 0.03% of the effluent in the environment (case of effluents from the oil refinery and Sidi Abdellah Belhaj). The IC50s recorded testify to the great harmfulness of effluents discharged into the marine environment, whose chemical composition of pollutants in sometimes varying proportions is often unknown.

The toxicity of the discharges studied is attributed to their load of chemical pollutants of different origins (Elhaimeur et al., 2013). The RP and SAB discharges proved to be the most toxic to bacteria (IC50s of 0.25 and 0.28%, respectively). The toxicity of the SAB effluent can be attributed to its high detergent load; a previous study showed that this effluent significantly inhibited the growth of the microalga *Tetraselmis sp*, with an IC50-96h of 0.49% (Menikher, 2009). The toxicity of petroleum refinery (RP) emissions is due to the toxic materials released into the air during oil production, such as sulfur oxides, nitrogen oxides, and volatile organic compounds, in addition to solid waste. The spatial evolution of aromatic hydrocarbons reveals high concentrations at the site near the refinery outfall, up to 150 ppm. Similarly, significant levels have been recorded at remote stations, indicating the impact of this activity on the surrounding coastal fringe (Berraho, 2006). The impact of the Refinery (RP) and Electrochemical (EL) discharges may also be due to their basic pH (9 and 12 respectively); the EL discharge emanates from a company known for its petrochemical activities, which use metals, mainly mercury, in production processes, and the results of heavy metal analysis (Cd, Pb, Hg, Cu) reveal significant contamination at the various sampling points, in some cases exceeding tolerable values (Berraho, 2006). The toxicity of this discharge is confirmed by the use of other bioindicators that have shown their high sensitivity to this discharge, such as *Paracentrotus lividus, Crassosrea gigas, Mitylus galoprovincialis* and *Tetraselmis sp* (Bouhallaoui et al., 2017; El Haimeur et al., 2013; Bouhallaoui et al., 2011; Menikher, 2009).

TP effluent also has an impact on bacterial luminescence. This effluent, classified as extremely toxic (IC50-5min = 7.78%), comes from an industrial phosphate processing complex. Physico-chemical characterization of the effluent shows abnormal values for a number of parameters (acid pH, high phosphorus and phosphate levels), indicating a considerable input of phosphates, which could lead to eutrophication of the environment (Berraho, 2006).

Oukacha (OU) discharge has shown significant toxicity on bacteria, with an IC50 of 12%; studies have revealed the impact of this discharge on the lethality and filtration behavior of bivalves (Berraho, 2006; Bouhallaoui, 2005). In addition, a study on the use of sea urchin embryolarvary development stages to assess environmental quality showed that the quality of marine waters sampled in the vicinity of the OU discharge is poor (El Haimeur et al. 2013); wastewater is indeed discharged directly into this area without any prior treatment.

JL discharge from a fish cannery also has a significant impact, inhibiting bacterial luminescence (IC50 =13.78%). Indeed, liquid waste discharges from canneries, which are dumped directly into the marine environment without prior treatment, are responsible for altering the quality of the city's environment (Ghil, 2008). Studies have also shown that fish processing industries generate effluents loaded with protein nitrogen, organic matter, and salt and contain significant quantities of fats and sulfates (Ziani et al., 2015).

The performance of a luminescence inhibition test for marine bacteria, *photobacterium phosphoreum,* was compared with that of the *Daphnia magna* microcrustacean mobility inhibition test, in a toxicity screening of 39 industrial effluents (Vasseur et al., 1984). The Microtox test has clear advantages over the Daphnia test and appears well suited to large-scale toxicity screening of complex industrial effluents.

Furthermore, Boillot (2008) found that the *Vibrio fischeri* luminescence inhibition test showed intermediate responses between the *Daphnia magna* test and the *Pseudokirchneriella subcapitata* test, and was able to classify organisms according to their sensitivity to the samples studied (from most to least sensitive): *Pseudokirchneriella subcapitata > Vibrio fischeri > Daphnia magna.*

5. Conclusion

The findings of this study confirm the suitability of the Microtox test as an effective tool for evaluating the toxicity of wastewater discharges and pure substances, particularly inorganic pollutants. The sensitivity of *Vibrio fischeri* to these discharges highlights its value as a bioindicator for wastewater. Additionally, the results underscore the risks posed by anthropogenic activities to coastal ecosystems, emphasizing the need for robust monitoring strategies.

This study reveals an improved environmental status at the Lesieur, Chahdia, and Oued Mellah sites, in contrast to a concerning state at locations impacted by petroleum refining and phosphate processing activities. The analysis also indicates a gradient of escalating toxicity from metals such as Cu, Cd, Pb, and Hg, revealing the need for mitigation efforts. The Microtox test, with its rapidity, simplicity, sensitivity, and cost-effectiveness, proves highly valuable for large-scale acute toxicity screening and comparative toxicity assessments. Furthermore, it demonstrates potential in sediment toxicity evaluations, including aqueous sediment extracts and solid-phase sediments.

For comprehensive ecotoxicological diagnostics, the integration of the Microtox test within a battery of assays that utilize organisms from different trophic levels is recommended. This multi-tiered approach will enhance the ability to assess toxicity across molecular, cellular, and community levels, ensuring a complete environmental evaluation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

References

[1] Azur Environmental, 1995. Microtox Acute Basic Test Procedures. Azur Environmental,. Carlsbad, California, USA.

[2] BECKMANN Instruments, 1982. Microtox System operating manual. Carlsbad, CA: Beckman Instructions Inc. Carlsbad, California 015-555879.

[3] Bennett. J , Cubbage .J, 1992. Review and Evaluation of Microtox Test for Freshwater Sediments ;Washington State Department of Ecology Environmental Investigations and Laboratory Services Program Toxics, Compliance, and Ground Water Investigations Section Olympia, Washington 98504-7710

[4] Boucheseiche, C., Cremille, E., Pelte, T., & Pojer, K. (2002). Rhône-Mediterranean-Corsica Basin. Technical Guide No. 7. Toxic and Ecotoxicological Pollution: Basic Concepts. 84p.

[5] Boillot, C. (2008). Assessment of Ecotoxicological Risks Related to Hospital Effluent Discharges into Aquatic Environments: Contribution to Improving the "Effect Characterization" Phase. INSA Lyon, 299p.

[6] Bouhallaoui, M., Benhra, A., El Haimeur, B., Quiniou, F., & Blaghen, M. (2017). Use of Embryonic-Larval Development of the Pacific Oyster *Crassostrea gigas* as a Tool for Diagnosing the Toxicity of Pure Substances and Complex Mixtures. *Journal of Water Science*, 30(3), 171–181.

[7] Berraho, A. (2006). Marine Environment Assessment. Internal Report, INRH. 144p.

[8] Bouhallaoui, M., Benhra, A., Nili, H., El Haimeur, B., Elamrani, S., & Chafik, A. (2011). Use of Embryonic-Larval Development Stages in *Mytilus galloprovincialis* for Assessing Marine Water Quality and Discharge Toxicity. *Bulletin of the French Zoological Society*, 136(1–4), 205–218.

[9] Galli, R., Munz, C. D., & Scholtz (1994). Evaluation and Application of Aquatic Toxicity Tests: Use of the Microtox Test for Predicting Toxicity Based on Contaminant Concentrations in Soil. *Hydrobiologia*, 273, 179–189.

[10] Ghil, A. (2008). Urban Governance of Water and Access to Basic Services in Vulnerable Areas: Case of Safi City (Morocco). Advanced Diploma in Planning and Urbanism (DESAU). National Institute of Urban and Regional Planning, INAU (Morocco).

[11] Grange, D., & Pescheux, F. (1985). Use of Luminescent Bacteria to Assess Water Toxicity: The Microtox Test. *Bulletin of Laboratory Communication*, 136p.

[12] Ince, N.H., Dirilgen, N., Apikyan, I.G., Tezcanli, G., & Üstün, B. (1999). Assessment of the Toxic Interactions of Heavy Metals in Binary Mixtures: A Statistical Approach. *Archives of Environmental Contamination and Toxicology*, 36, 365–372.

[13] Menikhar, N. (2009). Ecotoxicological Study of Complex Environments Along the Greater Casablanca Coastline. Master's Thesis, Faculty of Sciences Ben M'Sik, Casablanca. 60p.

[14] Mowat, F. S., & Bundy, K. J. (2001). Correlation of Field-Measured Toxicity with Chemical Concentrations and Pollutant Availability. *Environmental International*, 27(6), 479–489.

[15] Microbis (1992). Microtox Update Manual. Microbis Corporation, Carlsbad, CA, USA.

[16] Mirkes, D.Z., Wernberg, W.B., & De Coursey, P.J. (1978). Effects of Cadmium and Mercury on the Behavioral Responses and Development of *Eurypanopeus depressus* Larvae. *Marine Biology*, 47, 143–147.

[17] NM ISO 11348-3 (2014). Water Quality: Determination of the Inhibitory Effect of Water Samples on the Luminescence of *Vibrio fischeri* (Luminescent Bacteria Test). Part 2: Method Using Lyophilized Bacteria.

[18] Nałecz-Jawecki, G., Rudź, B., & Sawicki, J. (1997). Evaluation of the Toxicity of Medical Devices Using Spirotox and Microtox Tests: I. Toxicity of Selected Toxicants in Various Diluents. *Journal of Biomedical Materials Research*, 35, 101–105.

[19] Petala, M., Tsiridis, V., Kyrizis, S., Samaras, P., Kungolos, A., & Sakellaropoulos, G. P. (2005). Evaluation of Toxic Responses of Heavy Metals and Organic Pollutants Using the Microtox Test. *Proceedings of the 9th International Science and Technology Conference*. Rhodes Island, Greece, 1–3.

[20] Quiniou, F., & Judas, A. (1995). Toxicity Tests: Lethal and Sublethal Effects, A Bibliographic Synthesis. DEL Brest 95–17. Internal Report.

[21] Toussaint, M.W., Shedd, T.R., Van Der Schale, W.H., & Leather, G.R. (1995). A Comparison of Standard Acute Toxicity Tests with Rapid-Screening Toxicity Tests. *Environmental Toxicology and Chemistry*, 14, 907–915.

[22] Teodorovic, I., Planojevic, I., Knezevic, P., Radak, S., & Nemet, I. (2009). Sensitivity of Bacterial vs. Acute *Daphnia magna* Toxicity Tests to Metals. *Central European Journal of Biology*, 4, 482–492.

[23] Kungolos, A., Hadjispyrou, S., Petala, M., Tsiridis, V., Samaras, P.G., et al. (2004). Toxic Properties of Metals and Organotin Compounds and Their Interactions on *Daphnia magna* and *Vibrio fischeri*. *Water, Air, & Soil Pollution: Focus*, 4, 101–110.

[24] Sekkat, N., Guerbet, M., & Jouany, J.M. (2001). Comparative Study of Eight Short-Term Bioassays for Assessing the Toxicity of Leachates from Urban and Industrial Waste. *Journal of Water Science*, 14(1), 63–72.

[25] Vasseur, P., Ferard, J.F., Vial, J., & Larbaigt, G. (1984). Comparison of Microtox and Daphnia Tests for Assessing Acute Toxicity of Industrial Effluents. *Environmental Pollution Series A, Ecological and Biological*, 34(3), 225–235.

[26] Ziani, F., Bendou, A., Salghi, R., Bazzi, L., Ait Boughrous, A., & Aboulhassan, M. A. (2015). Electrochemical Oxidation of Fish Canning Wastewaters Using SnO2 Electrode. *Journal of Materials and Environmental Science*, 6(12).