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Evaluation of the Overall Potential Toxicity of Shallow Marine Sediment from Oran Bay by Means of Bioassays of Acute Toxicity

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Abstract

This study investigates the ecotoxicological evaluation of sediment contamination from west Algerian Mediterranean coast (Oran harbour-Ain Franin).The toxicity of sediment and interstitials waters was estimated using bioessays of acute toxicity with brine shrimp Arthémia salina. The bioessays reponses vary as a function of matrix and study area. The highest contamined samples (Oran harbour) are more toxic than the less contaminated samples (Ain Franin). The bioessay using interstitials waters was more sensible than those using contact sediment. The purpose of this study is to demonstrate the interest of ecotoxicological approach for assessing the quality of the coastal marine environment. The proposed approach is global, non-specific and gives elements to compares the sites between them in terms of quality of sediments and provide elements for classifying areas.

***Keywords*:** Acute toxicity; Ain Franin; Arthémia salina; bioessays; contact sediment; global contamination; interstitials waters; Oran harbor.

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1. Introduction

Sediments may act both as a sink and as a source of pollution [1]. Many pollutants can bind physically and chemically with sediments and persist for long periods of time to become bioavailable depending under certain hydrological conditions and exert adverse effects on aquatic organisms [2,3], sediment quality is crucial to the health of an aquatic ecosystem [4].

The determination of the real toxicity of sediments in aquatic ecosystems is challenging and necessary for an appropriate risk assessment. Different approaches have been developed and applied over the last several decades. Currently, the joint implementation of chemical, ecological and toxicological tools is recommended for an appropriate and successful toxicity risk assessment [5].

The aquatic environment usually represents the final destination of contaminants from problematic areas, where they can affect local biota, directly or indirectly [6]. The importance of sediment in aquatic systems is their role as supporting primary production as a substrate and a source of nutrients. This role is critical for organisms that represent the first links in the food chain, which depend all other aquatic organisms. Sediments are also a habitat and a source of organic matter for many burrowing and benthic invertebrates and benthic fish for a part of their life cycle. Sediments are both reservoirs and potential sources of pollution in aquatic ecosystems for many potentially toxic chemical pollutants for organisms [7]. For this reason, it is appropriate to monitor, evaluate and protect to ensure the integrity of the structure and functioning of aquatic ecosystems [8].

The development of bioassays for the assessment of the toxic potential of contaminated sediments is in constant evolution. These bioassays are generally used in the context of a battery. However, very few tools exist for the integration of their results for the comparison of sites and the priorization of sediment management actions. This study has been prepared in this context [9,10,11,12].

Several authors recommended the using of algae, crustaceans, insect larvae and fish as test species in aquatic ecotoxicology [13,14,15,16,17,18,19].

The aim of this study was to evaluate by bioassays the effects of global contamination of superficial marine sediment on a crustacean *Arthemia salina* including the time and the survival percentage as variables. The results are compared with those of the control cultures. To assess the potential toxicity of a marine sediment from two different sites in the Oran bay: (Oran harbour-Ain Franin) in controlled laboratory conditions the contact sediment and interstitials waters exposure tests were used.

The results show that the both bioassays can be used to measure the global toxicity of marine sediment, they can also be complementary. Bioassays applied to the interstitials waters are more sensitives than those applied on the contact sediment, pore water tests may complement the whole-sediment toxicity, because benthic organisms are exposed both to interstitials waters and sediment. According to the responses of organisms used, this work allowed us to classify the Oran harbour as a more affected site than that of Ain Franin.

2. Material and methods

The method used no specific, takes into account the total effect of these contaminants, interactions between compounds, their bioavailability, regardless of their nature and concentrations. Due to the fact that organisms differ in sensitivity to various substances, it is essential to select appropriate test organisms. It is important for organisms to belong to different taxonomic groups and represent different links of the trophic chain [20]. Artemia is one of the most used species in toxicity assessment because of its ease of culture, low cost, and its commercial availability in dry cysts.

2.1. Study site

In Oran bay, we selected two sampling stations (Figure 1):

Station 1: Oran harbour as affected site. These geographic coordinates (N 35° 42'663 "W 000° 39'320").

Station 2: Ain Franin (N00 ° 35'46'854 "W30 ° 768 '00") as a little impact site.

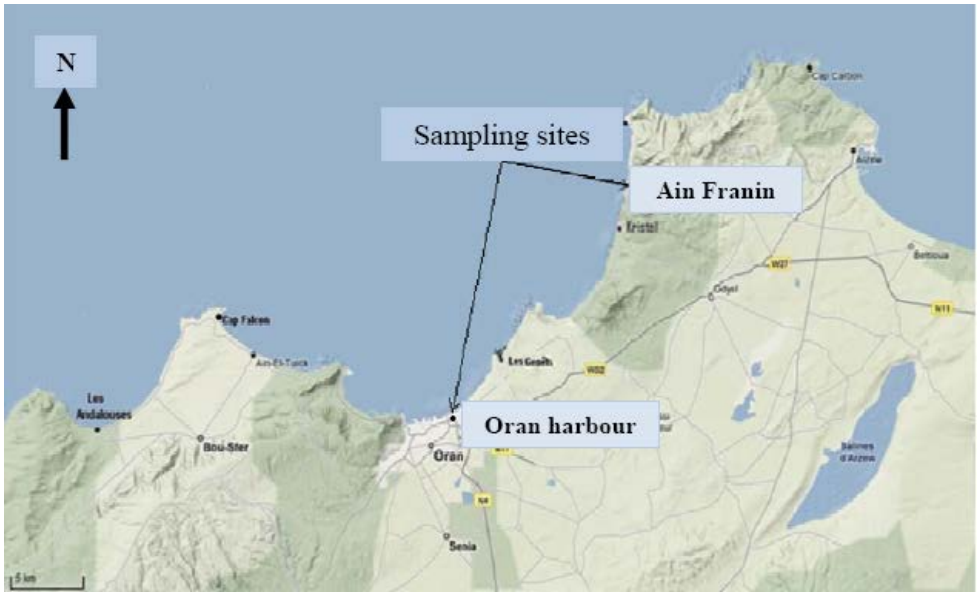

Figure1: Localization of sampling sites.

2.2. Collection, preparation of samples and mounting of bioassays

Our methodology following the chronological order of steps obviously start by:

A justified choice of sites and test organisms, culture of *Artemia salina* in controlled conditions of laboratory, sampling of superficial marine sediments, recolt of interstitials waters, and finally mounting of bioassays.Two sedimentary treatments were used for bioassays: Contact sediment and interstitials waters. A negative control, consisting of artificial substrate (using kaolnite clay), and artificial sea water were used.

2.3. Culture of Artemia salina in controlled conditions of laboratory

The hatching procedure followed the one described in ARC-test, standardized short-term toxicity test with *Artemia* nauplii [21]. For test approximately 0.5 g cysts of brine shrimp *A. salina* was incubated in 500 ml seawater in a cylindroconical tube at a temperature of 25 ± 1 C° and with lateral illumination by a light tube (1000 Lux) during the test period. All cysts were kept in continuous suspension with aeration provided by a small air tube extending to the bottom of the hatching device. After 18 to 24 hours, aeration was stopped and the hatched larvae (instar I) were transferred to new petri dish, each petri dish had ten individuals of nauplii and incubated at 25 C° for 24 hours, 48 hours and 72 hours. After 24, 48 and 72 hours from the start of the hatching, all larvae would have moulted to instar 2 or instar 3 stages.

2.4. The sampling of marine sediment

Sediment samples (first 2 cm) were collected from the study area (Oran harbour, Ain Franin) in plastic containers and transported to the laboratory. Sediment samples were visually checked and visible indigenous fauna and debris (leaves, etc.) removed with forceps [8], where they were stored at 4 °C in the dark (protected from light for two days, so let them settle). Following this settling, the supernatant was siphoned off, the sediments were then sieved to 2 mm and homogenized and placed at 4 ° C before starting the tests.

2.5. Collect of interstitials waters

Subsequent studies have demonstrated the suitability of the interstitial water for conducting tests with aquatic organisms such as gametes and embryos of sea urchins [22], benthic amphipods [23], fish embryos [22], naupliis copepods [24], algal zoospores [25] and other agencies lending to miniaturized tests [26]. Since testing interstitial waters employ a wide range of organizations and measure many effects parameters (including the survival, reproduction, fertilization, growth, and genotoxicity). They generally have the advantage of being faster, more sensitive and less expensive than trials with whole sediment using macrobenthic organisms.Pore water for testing was isolated from the whole sediment by centrifugation (3000 rpm, 30 min.), filtered on a 0.45 lm filter, and stored in the dark at 4 C°, until bioassays were performed (maximum storage time: 1 week).

2.6. Toxicity test

Glass flasks, (250 ml and 5.5 cm in diameter), with 10 organisms each (after hatching), were used in all treatments. The tests are conducted in 6 replicates. For contact sediment, organisms are contacted with overall sediment from each sampling site. Sediment and overlying water were added on the day before starting the test. Sediment was carefully placed at the bottom of the beakers and natural seawater was slowly added to minimize sediment disturbance [16]. The control test is carried out on artificial sediment (kaolnite clay).

For the test on the interstitials waters, the same protocol is followed, agencies are placed in beakers filled with interstitials waters. The control test was done on artificial seawater. In both bioassays, the survival rate of *Arthemia salina* is recognized after**:** 24h, 48h, 72h, and 96h.

3. Results and discussion

3.1. Contact sediment bioassay from Oran harbor

The monospecific test applied on the contact sediment reflects a disturbance after 48h which is due to a decrease of survival rate percentage of *Artemia salina* to 40%, in contrast the control culture maintained a satisfactory survival rates after 96h. Factors such as particle size distribution, organic carbon content (CO), salinity, and the presence (or absence) of nutrients can potentially affect the toxicity test results undertaken on whole sediments with benthic or pelagic organisms [27,28,29].

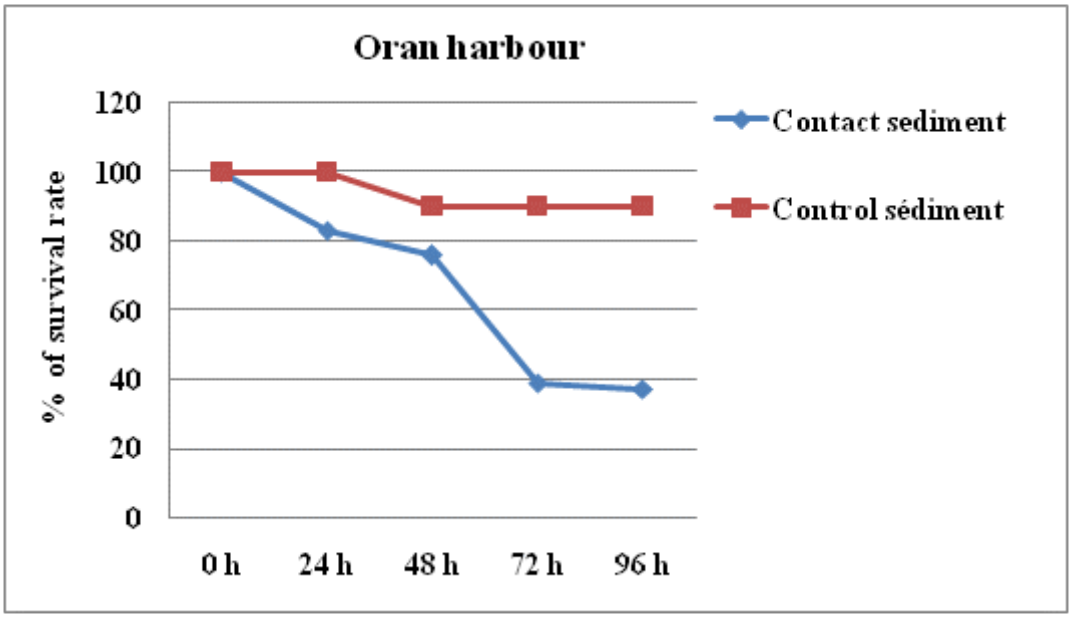

Figure 2: Percentage of survival rate of *Artemia salina* in contact sediment bioassay of Oran harbour.

3.2. Interstitials waters bioassay of Oran harbour

For equal exposure period of both crops of *Artemia salina* to the porous water of Oran harbour and water control, the results are differents. The effect of the potential toxicity of porous water causes a decrease in the survival percentage of organisms used from 24 h of exposure (60%) while it was (80%) for the previous experiment. Culture control using artificial seawater keeps almost the same kinetics than the contact sediment bioassay.

3.3. Sediment contact bioassay of Ain Franin

The comparison between both cultures of *Artemia salina* on the site of Ain Franin reflects a satisfactory survival rate after 96h of exposure agencies to contact sediments.

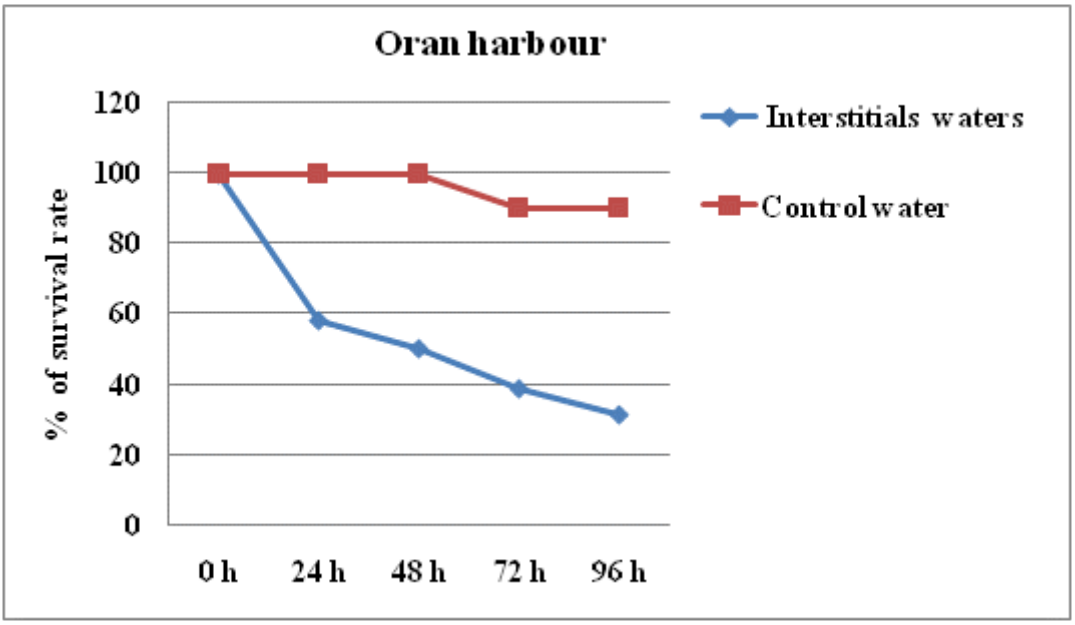

Figure 3: Percentage of survival rate of *Artemia salina* in interstitials waters bioassay of Oran harbour.

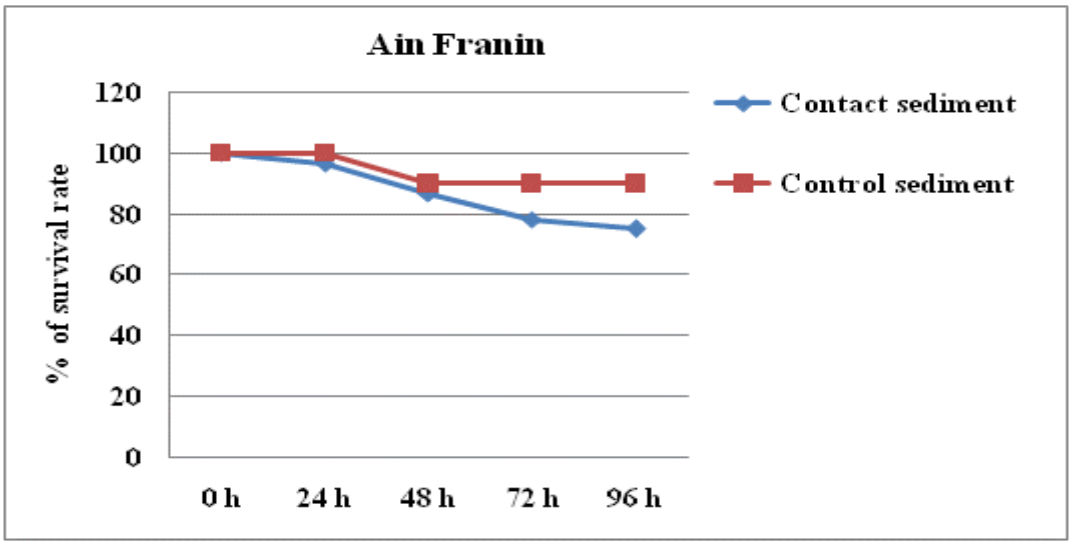

Figure 4: Percentage of survival rate of *Artemia salina* in contact sediment bioassay of Ain Franin.

3.4. Interstitials waters bioassay of Ain Franin

The same experimental scheme is shown in porous water of Ain Franin, which shows a much less impact than the harbour interstitials waters. Bioassays offer the advantage to demonstrate the presence of contaminants undetected by chemical analysis, and which reflect the toxicity of the bioavailable fraction of contaminants. Bioassays are the only tools that provide quantitative information on the toxicity of sediment. They take into account all the contaminants that are present and allow discrimination contaminated sediment samples from those who are not [30].

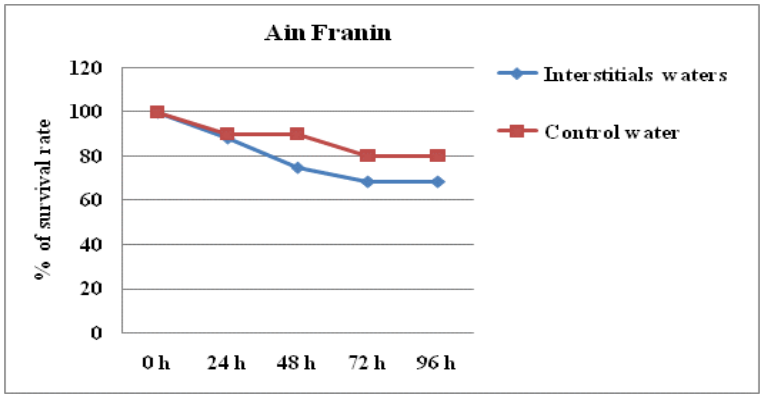

Figure 5: Percentage of survival rate of *Artemia salina* in interstitials waters bioassay of Ain Franin.

3.5. Intra-site and inter-matrix comparison

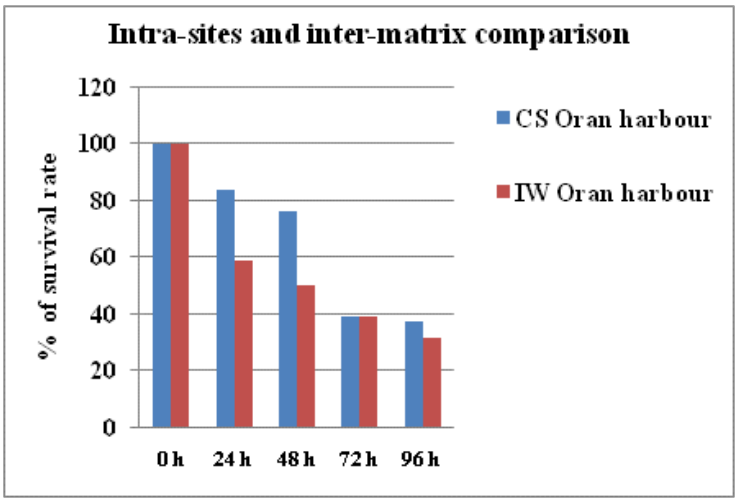

Figure 6: Intra-site and inter-matrix comparison (Oran harbour).

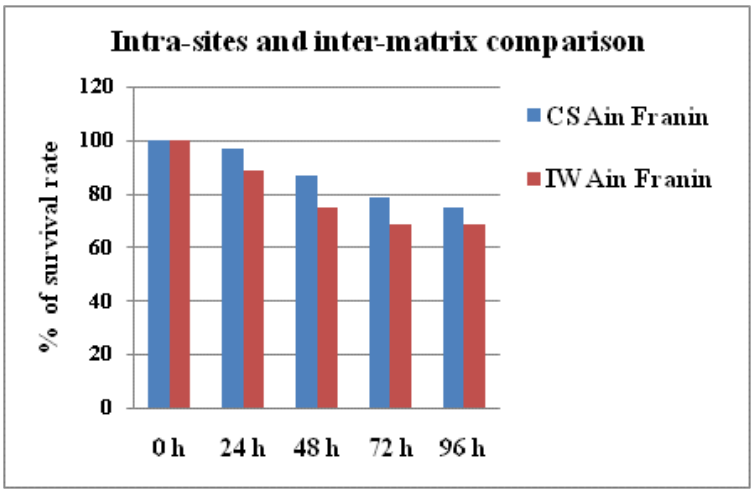

Figure 7: Intra-site and inter-matrix comparison (Ain Franin).

The intra-site comparison between responses species of the two matrices shows clearly that the tests applied to the porous water are more sensitives then those using the contact sediment. This corroborates with the results of many authors who argue that the bioavailable fraction of organic and inorganic contaminants in sediments is mainly found in the porous water sediment. This phase is considered the main route of exposure to aquatic organisms, pelagic and benthic by many authors [31, 32, 33]. Also, many bioassays are performed in aqueous phase on the porous water sediment or elutriates (including assessing the impact of dredging and / or delivery solution contaminants in the resuspension sediment [34].

3.6. Inter-site comparison for the same matrix: Contact sediment

Different exposure routes, modes of chemical action and different sensitivities may exist for benthic organisms [35,36]. From an ecotoxicological perspective, various approaches (interstitiel water quality, spiked sediment toxicity, tissue residue) were developed to detect the specific effects of chemicals on organisms living in sediment, but only whole-sediment tests using benthic organisms are suitable for a realistic risk assessment of the sediment compartment [37,38].

By comparing the survival rate of artemia in the same matrix (whole-sediment) of two sites in the Oran bay, it is clear that the harbour area is more affected than Ain Franin area, this contamination can be a real source of contamination for other coastal areas of the Oran bay in case of movement of superficial marine sediment or in the case of resuspension.

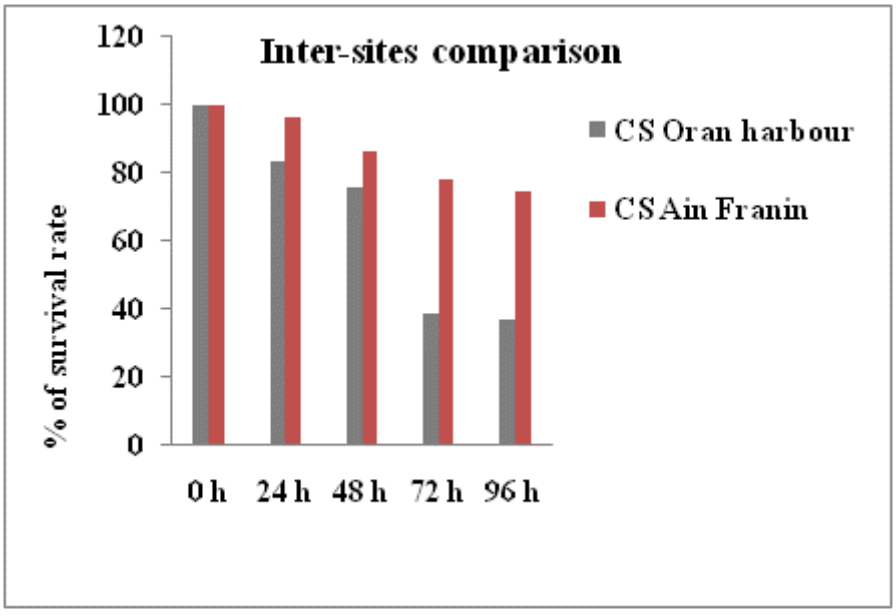

Figure 8: Inter-site comparison for the same matrix (Contact sediment).

3.7. Inter-site comparison for the same matrix: Interstitials waters

In the liquid phase of the sediment, also called interstitials waters, pollutants may be present in free form or complexed with inorganic or organic ligands (humic, fulvic ...). They can also be transferred to the solid phase

after precipitation reactions, substitution and / or adsorption on the particles. Even within these particles, resuspension of processes eg barge traffic on the channels can also modify the association of these contaminants to the solid phase [39]. Furthermore, the particles related pollutants can also pass into the liquid phase under the effect of some chemical processes (desorption or dissolution of phenomena caused for example when pH change) or biogeochemical (as the oxidation-reduction reactions caused by the bacteria). Finally, the distribution of metallic elements in liquid and solid phases of surface sediments is closely related to the composition of the particles, the bacterial activity and chemical reactions [39]. A parallel is drawn between the porous water contamination levels of our two sampling sites, and survival results of our test species is actually lower in contact with harbour porous water. This imperative reflects a higher pollution at the Oran harbour in opposition to the Ain Franin site.

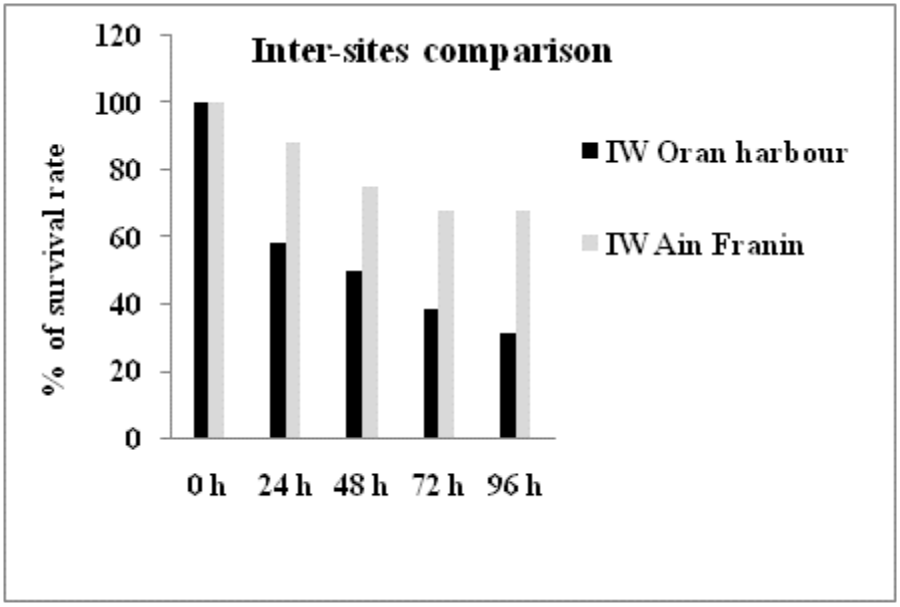

Figure 9: Cross-site for the same matrix comparison: Interstitials Waters.

4. Conclusion

Sediment toxicity is difficult to address because of the interaction of the chemicals with the sediment, which determines their bioavailability. The sorption strength of sediments may vary depending on the composition of sediments and the organic matter content [40]. The purpose of this study is to demonstrate the interest of ecotoxicological approach for assessing the quality of the coastal marine environment in order, firstly, to draw up an environmental assessment by a non-specific screening technical and, secondly, to give another opportunity for a long-term monitoring of sediment quality of the Mediterranean coast. The proposed approach is global, non-specific and shows toxicity bioavailable xenobiotic molecules of the superficial sediment layer. In this context we have chosen: To study the sediment compartment in his first 2 cm as a contact sediment bioassays and interstitials waters.

Our choice is focused on survival test using as reference species *Artemia salina* has a broad distribution, a good representation of the environment, economic interest and increased sensitivity to pollutants. The results obtained

affirm us a present and even some pollution in the Oran bay; but this contamination is greater in harbour sediments.

The use of two matrices (sediment, interstitials waters) in the bioassays allowed us to infer that the tests applied to the interstitials waters (IW) are more sensitives than those using the contact sediment (CS).

Control cultures have maintained a satisfactory survival rate of agencies criminalizing the effect of the potential toxicity of sediment sampled on our biological tool. The variety of contaminants on the coast of the studied area, the diversity of sources of inputs and contaminant transport pathways, as well as the variety of methodological approaches, make complex an environmental study on the scale of a large coast line. Using a holistic approach, not discriminating against pollutants aligns the measures. Although different method is sensitivity according to the contaminants, that interpretation is comprehensive and that the ecotoxicological approach is different from the direct measurement of contaminants, the present study is consistent with results from the literature regarding the contamination chemical. It compares the sites between them in terms of quality of sediments and provide elements for classifying areas.

References

[1] Hollert, H., Dürr, M., Erdinger, L., Braunbeck, T., 2000. Cytotoxicity of settling particulate matter and sediments of the Neckar River (Germany) during a winter flood. *Environ. Toxicol. Chem*. 19 (3), 528–534.

[2] Winkels, H.J., Kroonenberg, S.B., Lychagin, M.Y., Marin, G., Rusakov, G.V., Kasimov, N.S., 1998. Geochronology of priority pollutants in sedimentation zones of the Volga and Danube delta in comparison with the Rhine delta. *Appl. Geochem*. 13 (5), 581–591 (Jul).

[3] Zoppini, A., Ademollo, N., Amalfitano, S., Casella, P., Patrolecco, L., Polesello, S., 2014. Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. *Sci. Total Environ*. 484, 74–83 (Jun 15).

[4] Davoren, M., Ní Shúilleabháin, S., Hartl, M.G.J., Sheehan, D., O'Brien, N.M., O'Halloran, J., et *al*., 2005. Assessing the potential of fish cell lines as tools for the cytotoxicity testing of estuarine sediment aqueous elutriates. *Toxicol. In Vitro* 19 (3), 421–431.

[5] De Castro-Català, N., Kuzmanovic , M., Roig, N., Sierra , J., Ginebreda , A., Barceló, D., Pérez, S., Petrovic, M., Picó , Y., Schuhmacher , M ., Muñoz, I., 2016. Ecotoxicity of sediments in rivers: Invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools. *Science of the Total Environment* 540 297–306.

[6] Fleeger, JW., Carman, KR., Nisbet, RM., 2003. Indirect effects of contaminants in aquatic ecosystems. *Sci Total Environ* ;317:207–33.

[7] Chapman, P.M. , Long, E.R,.1983.The use of bioessays as a part of a comprehensive approach to marine pollution assessment. *Marine Pollution Bulletin*, 14, 81-84.

[8] Antunes, S.C., De Figueiredo, D.R ., Marques, S.M., Castro, B.B., Pereira, R., Gonçalves., 2007**.** *Science of the Total Environment* 374 (2007) 252–259

[9] Ingersoll, CG., Ankley, GT., Benoit, DA., Brunson, EL., Burton, GA., Dwyer ,FJ., et *al*., .1995. Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates: a review of methods and applications. *Environ Toxicol Chem*;14:1885–94.

[10] ISO, Water quality., 1996. Determination of the inhibition of the mobility of *Daphnia magna Straus* (Cladocera, Crustacea) — acute toxicity test. *ISO International Standard* 6341. *Geneva, Switzerland: International Organization for Standardization*.

[11] ASTM, 1997. Standard guide for *Daphnia magna* life-cycle toxicity tests., Report E1193-97. *Philadelphia, USA: American Society for Testing and Materilas.*

[12] ASTM, 2000. Test method for measuring the toxicity of sediment associated contaminants with freshwater invertebrates. *Annual Book of American Society for Testing and Materials Standards. Philadelphia, USA*: ASTM. E 1706-00.

[13] Nebeker, AV., Cairns, MA., Gakstatter, JH., Malueg, KW., Schuytema, GS., Krawczyk, DF., 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. *Environ Toxicol Chem* 1984;3:617–30.

[14] OECD, (Organisation for Economic Cooperation and Development)., 1984. Algal growth inhibition test. *OECD Guidelines for Testing of Chemicals, vol*. 201. Paris: OECD.

[15] OECD, Daphnia sp., 2000a. Acute immobilisation test. Revised Proposal for Updating Guideline, vol. 202. Paris, France: *Organization for the Economic Cooperation and Development.*

[16] OECD., 2000b. Sediment-water chironomid toxicity test using spiked water— draft document. OECD Guidelines for the Testing of Chemicals—*Proposal for a New Guideline;vol*. 219.

[17] Taylor, EJ., Maund, SJ., Pascoe, D., 1991. Evaluation of a chronic toxicity test using growth of the insect Chironomus riparius Meigen. In: Jeffrey DW, Madden B, editors. *Bioindicators and Environmental Management. London, UK: Academic Press*; p. 343–52.

[18] Environment Canada, 1992. Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum. *Report EPS 1/RM/25. Ottawa, ON, Canada: Environment Canada.*

[19] USEPA, 1994. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA 600/ 7-91-002. *US Environmental Protection*

Agency:Washington, DC.

[20] Baran, A.,Tarnawski, M., 2015. Assessment of heavy metals mobility and toxicity in contaminated sediments by sequential extraction and a battery of bioassays.*Ecotoxicology* DOI 10.1007/s10646-015-1499-4.

[21] Vanhaecke, P., Persoone, G., 1981. Report on an intercalibration exercise on a short-term standard toxicity test with *Artemia* nauplii (ARCtest). *Inserm*;**106**:359-76.

[22] Carr, R.S., et D.C. Chapman. 1992. Comparison of solid-phase and pore-water approaches for assessing the quality of estuarine sediments. Chem. Ecol. 7: 19-30.

[23]. Winger, P.V., et P.J. Lasier. 1991. A vacuum-operated pore-water extractor for estuarine

and freshwater sediments. Arch. Environ. Contam. Toxicol. 21: 321-324.

[24] Carr, R.S., E.R. Long, H.L. Winsdom, D.C. Chapman, G. Thurby, G.M. Sloane, et D.A.

Wolfe. 1996. Sediment quality assessment studies of Tampa Bay. Environ. Toxicol. Chem. 15: 1218-1231.

[25] Hooten, R.L., et R.S. Carr. 1998. Development and application of a marine sediment pore-water toxicity test using *Ulva fasciata* zoospores. Environ. Toxicol. Chem. 17: 932-940.141.

[26] Wells, P.G., K. Lee, et C. Blaise. 1998. Microscale Testing in aquatic Toxicology: Advances, techniques, and Practice. CRC Press, Boca Raton, FL. 679 p.

[27] DeWitt, T.H., G.R. Ditsworth, et R.C. Swartz. 1988. Effects of natural sediment features on survival of the phoxoxephalid amphipod *Rhepoxynius abronius*. Mar.Environ. Res. 25: 99-124.

[28] Ankley, G.T., N.A. Thomas, D.M. Di Toro, D.J. Hansen, J.D. Mahony, W.J. Berry, R.C.Swartz, R.A. Hoke, A.W. Garrison, H.E. Allen, et C. S. Zarba. 1994. Assessing potential bioavailability of metals in sediments: A proposed approach. *Environ. Manag*. 18: 331-337.

[29] Suedel, B.C. et J.H. Rodgers, Jr. 1994. Development of formulated reference sediments for freshwater and estuarine sediment testing. Environ. *Toxicol. Chem*. 13:1163-1175.

[30] Bombardier, M., 2007. Développement d'outils écotoxicologiques pour l'évaluation de sédiments. Thèse

UFR Sciences Fondamentales et Appliquées. Spécialité : *Toxicologie de l'Environnement*. *Universié de Metz*.153p.

[31] Di Toro, DM., Zarba, CS., Hansen, DJ., Berry, WJ., Swartz, RC., Cowan, CE., Pavlou, SP., Allen,

HE., Thomas, NA., Paquin, PR., 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environ Toxicol Chem* 10:1541- 1583.

[32] Ankley, G.T., Mattson, V.R., Leonard, E.N.,West, C.W., Bennet, J.L.,1993.Predicting the acute toxicity of Cu in freshwater sediments.Evaluation of the role of acid volatile sulfide.*Environmental Toxicity and Chemistry* 12,pp.315-320.

[33] Peterson, G.S., Ankley, G.T., Leonard, E.N., 1996. Effect of bioturbation on metal sulfide oxidation in surficial freshwater sediments. *Environmental Toxicology and Chemistry*, vol. 15, n°12, pp. 2147-2155.

[34] Burton, Jr,. A., Ingersoll, C.G., Burnett, L.C., Henry, M., Hinman, M. L., Klaine, S.J., Landrum, P. F., Ross, P., Tuchman, M., 1996. A comparison of sediment toxicity test methods at three Great lake areas of concern. *Journal of Great Lakes Research,* 22, 495-511.

[35] Rodriguez, P., Reynoldson, T.B., 1999. Laboratory methods and criteria for sediment bioassessment. *Manual of Bioassessment of Aquatic Sediments Qualitypp.* 83–133.

[36] Ingersoll, C.G., Kunz, J.L., Hughes, J.P., Wang, N., Ireland, D.S., Mount, D.R., et *al.*, 2015. Relative sensitivity of an amphipod Hyalella azteca, a midge Chironomus dilutus, and a unionid mussel Lampsilis siliquoidea to a toxic sediment. *Environ. Toxicol. Chem.* 34 (5), 1134–1144.

[37] OECD, 1992. Report on the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment. *Environment Monograph No*. 59.

[38] Vandegehuchte, M.B., Nguyen, L.T.H., De Laender, F., Muyssen, B.T.A., Janssen, C.R., 2013. Whole sediment toxicity tests for metal risk assessments: on the importance of equilibration and test design to increase ecological relevance. *Environ. Toxicol. Chem.* 32 (5), 1048–1059.

[39] Bonnet, C., 2000. Développement de bioessais sur sédiments et applications à l'étude, en laboratoire,de la toxicité de sédiments dulçaquicoles contaminés. *Thèse, Université de Metz,* ***France,*** 309 pp.

[40] Cornelissen, G., Gustafsson, O., 2005. Prediction of large variation in biota to sediment accumulation factors due to concentration-dependent black carbon adsorption of planar hydrophobic organic compounds. *Environ. Toxicol. Chem*. 24 (3), 495–498.