Use of benthic population and embryo-larval development stages of the sea urchin *Paracentrotus lividus* for evaluating the ecological quality of the west Moroccan Mediterranean coast

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Abstract
Moroccan Mediterranean coast is tending to become overused due to urbanization, industrial, fishing and leisure activities; thus, the sea is the final receptacle for nuisances developed. Ecological assessment of aquatic systems using biological indicators has been frequently used to assess water quality. Benthic populations are good indicators of the state of their environment because of their sedentary lifestyle and the diversity of their quick responses to different sources of disturbance. Biological tests on sea urchins are widely used to assess contaminants in sediment and effects on health status. Their sensitivity is manifested by disturbances in the reproductive cycle, fertility and larval development.

The main objective of this study is to contribute to the evaluation of quality of West Moroccan Mediterranean coast by studying benthic population's organization and calculation of biological indices first, then studying sediment toxicity using embryotoxicity bioassay on sea urchin Paracentrotus lividus. Results of the evaluation of the environment impact and toxicity of sediments of West Moroccan Mediterranean coast showed that with the exception of a few stations which are of good quality, the ecological status and the quality of the sediments tested range from moderate to poor.

Keywords: Bio-evaluation, benthic population, biological index, bio-indicator, embryotoxicity test, Mediterranean, sea urchin, ecotoxicology, AMBI, trophic index.

Introduction

Coastal line undergoes high strategic challenges for environment and economy. It is a very complex ecosystem subject to multiple interactions between physical, chemical and biological factors which are expressed on different spatial and temporal scales[13]. All aquatic ecosystems undergo a degradation of their quality after being exposed to pollution, which seems to affect all the compartments of the ecosystem[6]. In the Mediterranean, discharges of liquid and solid wastes into marine environment from urban and industrial areas are high and can cause a lasting deterioration in environment state[24]. All these effluents bring pollutants of various natures in significant quantities which inevitably end up in the Mediterranean[3]. Water then becomes unsuitable for life for aquatic organisms[18].

Bio-indicators are used to collect predictive signals of potential changes in response to stress in the marine environment. The benthic stand is a good indicator of the state of its environment due to its sedentary lifestyle, its longevity and the diversity of its responses to different sources of disturbance (pollution, organic enrichment etc.)[28].

Sediments are commonly used as an environmental matrix in monitoring programs, as they act as major reservoirs of persistent pollutants in coastal and estuarine systems. Sediment, being an important compartment of the aquatic ecosystem, serves as habitat and food source for many species. Various tools, precise, fast, expensive and giving results easy to interpret, exist to assess the quality of a sediment[5].

Sea urchin biotests constitute a recognized and widely used element of the researchers' toolbox for assessing contaminants in sediments and impacts on modified health status[31]. The common sea urchin *Paracentrotus lividus* is an echinides of separate gonochoric sexes showing no sexual dimorphism. It has a wide distribution all along the coasts of Mediterranean basin[35].

Sea urchins have the characteristics sought in a bio-indicator. Their sensitivity to environmental conditions is manifested by disturbances in the reproductive cycle, reduced fertility or disturbances in larval development and effects observed in disturbed areas on sea urchin populations have often been described[14,21]. These indications motivated the choice of *Paracentrotus lividus* as a bio indicator of the environment quality.

The main objective of our study is to contribute to the evaluation of quality of Mediterranean coastal waters by assessing quality of the environment using biological indices linked to the benthic population of the mediolittoral and to evaluate the quality of coastal waters using the embryotoxicity in sea urchin "*Paracentrotus lividus"* bioassay.

Material and Methods

Study area: Our study area is located in west Moroccan Mediterranean façade from Tetouan to Al Hoceima on the oceanographic ship "Al Amir Moulay Abdellah". Six stations were sampled (Table 1) during the period of July 2017 on the oceanographic boat "Al Amir Moulay Abdellah".

Sediments sampling: Sediments samples were taken from different stations using Van-Veen bucket. About 6 kilograms of sediment were sampled in each station. In order to study benthic population, 5Kg of sediment were preserved in a polyethylene jar fixed on the field with 8 % formol solution, the rest of sediment is kept for embryotoxicity test.

Once in the laboratory, fixed samples are washed using 1 mm mesh screen; the refusal is recovered and then sorted under a binocular magnifying glass. Individuals are kept then in vials labelled containing 8 % formol solution until identification of the species was made using many keys of fauna identification (Fauna of France, Worms etc.). Samples intended to embryotoxicity test are kept at -20°C until analysed.

As for embryotoxicity test, sediment is mechanically stirred with filtered seawater in a ratio of 1:4 at 100 rpm for 8h (®TAITEC Rotary Shaker NR-20), then left to settle for 8h before recovering the supernatant water: this is the raw elutriate. The seawater comes from an unpolluted reference site and in this study, it is Dar Bouazza site (33°33´38.0 "N - 7°49´00.8"W).

Physico-chemical parameters: Physico-chemical parameters of marine water (water temperature (T°C), salinity (sal g/L) and dissolved oxygen are measured *in situ*.

Benthic population analysis

Structural parameters: After identifying fauna recovered from sediment, several structural parameters have been calculated to evaluate the structure of the ecosystem:
- Specific richness S: number of species for each sample.
- Abundance N: number of individuals for each sample.
- Shannon and Weaver index (H') [37].
- Pielou equitability Index (J') [32].

Biological indexes - TI, AMBI, M-AMBI and BENTIX: Evaluation of the functional structure of the study area is based on classification of species as reported by Grall and Glémarc[19] according to trophic levels. Different trophic groups were established according to nature, size and state of food: predators (C), scavengers (N), herbivores (H), detritivores (Dt), suspensivores (S), selective deposit feeders (Ds), non-selective deposit feeders (Dss or L) and micrograzers (µB).

Different biological indexes were calculated namely Trophic Index (TI), Azti Marine Biotic Index (AMBI), Modified Azti Marine Biotic Index (M-AMBI) and BENTIX. Trophic index (TI) reflects the diet of the species. Four trophic groups were determined to allow the calculation of this index expressed as a percentage and interpreted as described as follows[40]:
IT> 60: The population is normal
30> IT> 60: The population is unbalanced
IT <30: The population is disturbed

Determination of ecological groups and biotic indexes was according to Grall method[20], this latter was carried out to evaluate the degree of degradation of macro-benthic populations; it is based on grouping species into five groups of different pollu-sensitivity. Species of each group have the same ecological behavior regarding organic pollution and have similar profiles of abundance regarding overload gradient of organic matter.

Calculation of biotic coefficient for AMBI[9] is based on the relative abundance of each ecological group compared to its total abundance in the sample, the values obtained for this index range from 0 to 7. As for M-AMBI, this latter is based on a factor analysis according to AMBI, Shannon index (H') and specific richness (S) (Table 2). AMBI and M-AMBI calculations are carried out by software available on the AZTI laboratory site.

BENTIX is another biotic index developed by Simboura et al[38]; this index reduces the number of ecological groups to only 3, which reduces the uncertainty associated to the transition class and simplifies its calculation, its range is in the following interval [0, 6] (Table 3).

Table 1
Geographic coordinates of sampling sites in the Mediterranean Moroccan West

Station	Latitude N (DMS)	Longitude W (DMS)
St 1	35°12.33	004°14.47
St 2	35°14.61	004°33.28
St 3	35°13.84	004°43.00
St 4	35°18.50	004°51.59
St 5	35°28.9	005°05.66
St 6	35°34.01	003°50.33

Table 2
Reference values of the AMBI and M-AMBI biotic indices for defining the ecological status of benthic habitats[9]

Ecological status	AMBI	M-AMBI
High	0 – 1,2	0,8 – 1
Good	1,2 – 3,3	0,5 – 0,8
Moderate	3,3 – 4,3	0,4 – 0,5
Poor	4,3 – 5,5	0,2 – 0,4
Bad	5,5 - 7	0 – 0,2

Table 3
Thresholds and classification of populations from BENTIX[38]

Classification-Pollution	Biotic coefficient (BC)	Ecological status
Normal	$4,5 < BC \leq 6$	High
Slightly polluted, transition	$3,5 < BC \leq 4,5$	Good
Moderately polluted	$2,5 < BC \leq 3,5$	Moderate
Very polluted	$2 < BC \leq 2,5$	poor
Azoic	0	bad

Table 4
Ecotoxicological assessment criteria (EAC) for the sea-urchin embryo test (SET)

Quality	High	Good	Moderate	Poor	Bad
EAC	EAC0	EAC1	EAC2	EAC3	
TU	0	0,27	0,86	1,73	

Bio-test: Embryotoxicity test on sea urchin: The method in this study was adopted from[1] "Standard guide for conducting static acute toxicity test with echinoid embryos". Mature individuals of sea urchin *Paracentrotus lividus* (diameter ranges between 3 and 5 cm) are sampled from reference site (Dar Bouazza). After acclimatization of individuals under laboratory conditions, the emission of gametes was induced by injection of 0.5 M of KCl (potassium chloride) solution in the coelomic cavity. The sperm of several males was collected, dried up and stored, females are collected in Erlenmeyer containing filtered seawater, the sperm and oocytes were checked under microscope to choose the best females and males[23].

The recovered oocytes are filtred and then washed twice by decantation in order to remove poor quality oocytes. The fertilization process was checked under a microscope and the couple (one male and one female) with fertilization rate up to 90% was selected for the experiment[1]. The eggs are fertilized with 1 ml of a sperm suspension prepared from 40 µl of dry semen diluted in 2 ml of EMF. The fertilized eggs were incubated in different concentrations of sediment extract (5%, 25%, 50%, 75% and 100%) for 48 h at 25 °C. Embryo-larval development was stopped at pluteus stage by the addition of 8 % neutral formol and the percentages of larval anomalies were determined according to ASTM[1].

The toxic effect of different studied sediments was assessed by using the rate of abnormal pluteus larvae. EC50 corresponding to the concentration inducing 50% of anomalies was calculated using Log Probit software[17]. The EC50 was used for the calculation of the toxicity unit (TU) according to the following formula[15]:

$$TU = 100/EC50.$$

Depending on the value of the toxicity unit (TU) calculated and the ecotoxicological evaluation criteria (EAC), the studied site can be classified from good to poor quality (Table 4).

Results and Discussion

Impact of sediment on the benthic population: The choice of studying benthic population of west Mediterranean region finds its originality in the fact that this type of data is lacking in this part of the Moroccan coast[7]. Through this study, classification of Moroccan zone of the western Mediterranean was possible according to the quality of sediments collected in this zone and its ecological status. Benthic communities are considered to be major tools for bio-monitoring of aquatic environments in different countries[22]. Furthermore, understanding the functioning of a marine system and the cause-effect relationship with ecosystem responses are challenges today[8].

Physicochemical parameters: Figure 2 illustrates the physico-chemical parameters variation (measured *in situ*). Temperature ranges from 16.08 C ° to 24.74 C °, the slight variation was showed in salinity 36.42 g/L and 36.79 g/L. Dissolved oxygen values range from 3.75 mg/L to 4.26 mg/L, it did not show significant changes across the entire study region.

Taxonomic structure: As shown in figure 3, a total of 162 individuals were enumerated in the studied area. Analysis of taxonomic structure of studied area allowed us to identify 33 species belonging to 3 taxonomic groups: 67% are annelids, 21% molluscs and 12% arthropods (Figure 3). This structure shows a similarity with studies carried out in the Mediterranean area where annelids are the main faunal group (49%) compared to the amphipod group (24%) and molluscs (17%) [30].

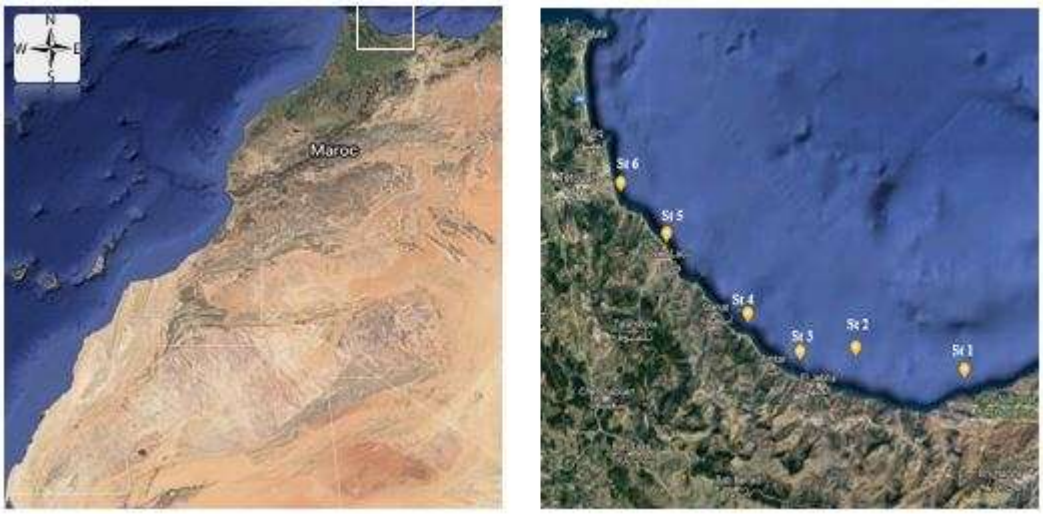

Figure 1: Geographical locations of sampling sites in the Mediterranean Moroccan West

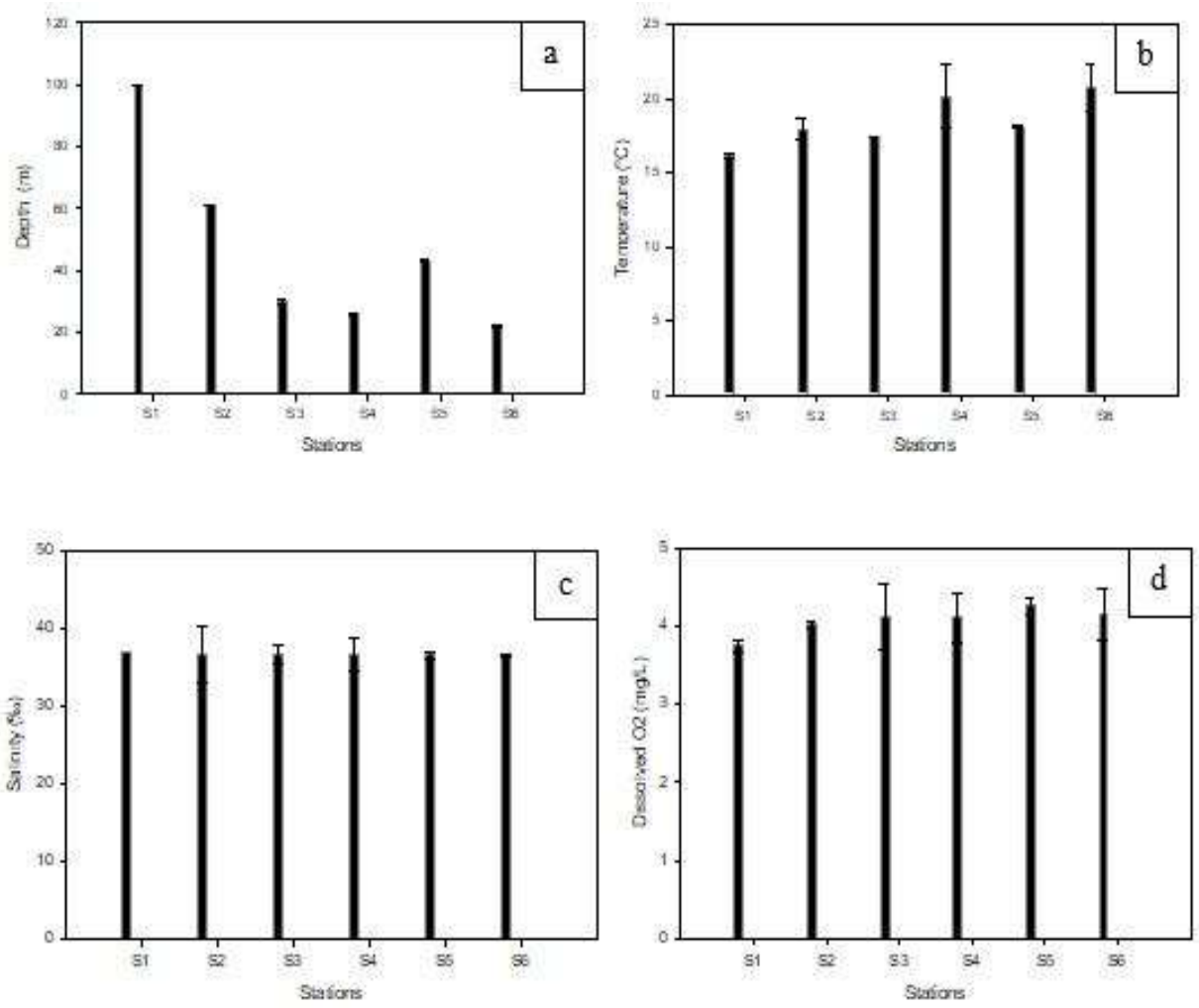

Figure 2: Physico-chemical parameters of the sampling stations of Mediterranean western Moroccan coast. (a) depth, (b) Temperature, (c) Salinity, (d) Dissolved O_2

Specific richness S, abundance, Shannon diversity index H' and Pielou index J': Identification showed the presence of 33 species with a total abundance of 162 according to the analysis of specific richness (S) and abundance in each station. The results of this study demonstrated spatial variability between stations in terms of species richness and abundance, indeed, 12 species were recorded for St 5 and St 6 with an abundance of 28 and 29 respectively. This testifies to a good diversity at the level of these stations while the other stations record a lower specific richness, as for station 3 witch records only 5 species with an abundance of 13 (figure 4a).

Biological diversity of the population was studied by using the Shannon H 'index, the diversity values are variable reaching a maximum of 3.4 reported for station 6 while the minimum value (2.13) was recorded in station 3. Calculation of Pielou index J' showed a maximum value of (0.95) in station 6 (figure 4b).

Trophic organization of the benthic population: The evaluation of trophic organization is illustrated in figure 5. Results showed 7 trophic groups. Suspensivores dominate in St 1 with 39%; Wave action is one of the main factors influencing benthic communities, this factor represents a source of constant stress that forces populations to have specific adaptations to survive, one of these adaptations is the mode of suspension which allows for suspensivores to feed on matter brought by movements of the water[33]. Detritivores are the dominant group in St 2, St 3 and St 6 with a percentage of 44%, 55% and 53% respectively.

A study regarding Smir lagoon located in the Mediterranean region showed that the same trophic group is also dominant in the lagoon[12]. Surface depositors dominated in station St 4 with a percentage of 34% according to Bazairi et al.[4] Their presence could be favored by the weak currents which promote sedimentation. Organic enrichment could also be a factor favoring surface depositors[33]. The taxonomic structure of this station is characterized by reduced number of species with high abundance, which means the dominance of a few species. These observations reflect a disturbed ecological status of this station since specific richness is low, same case was recorded in Moulay Bousselham lagoon[18].

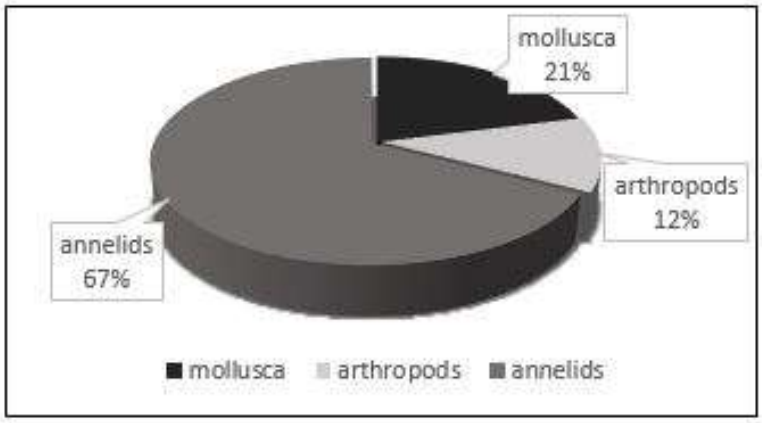

Figure 3: Taxonomic structure of the study area

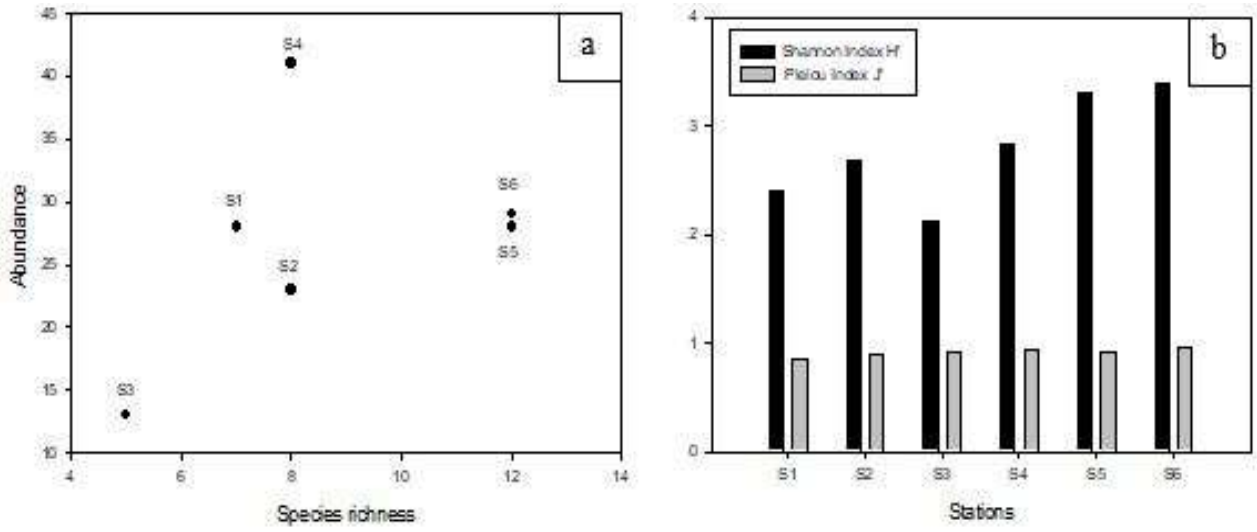

Figure 4: (a) Variation in species richness and abundance and (b) Variations in the Shannon H 'index and the Pielou J' index in different studied stations.

Trophic index (TI), AMBI, M-AMBI and BENTIX: Results obtained for trophic index (TI), which linked to the diet of species[40], are shown in figure 6. Only station 4 showed a value below 60% showing a disturbed ecological state, slightly affected by an organic matter enrichment. The other stations showed values above the threshold (60%).

The classification of the six stations according to the AMBI index is shown in figure 7. The analysis of these results showed that stations 1, 3, 4 and 6 were classified as slightly polluted, while station 2 and station 5 were classified as unpolluted. The diagnostic of the stations state did indeed reveal a variation of the population state between impoverished (station 2 and station 5) and unbalanced for other stations (Table 5). Ecological status of St 2 is normal, unpolluted. Indeed, this station is located at a distance from the coast (Takmout region), it is also little sheltered. Station St 5 also showed a normal unpolluted state, although it is close to the village of Oued Laou. These results can be explained by the fact that the region has only one artisanal fishing site[27] and the tourist activity is limited to the summer period.

St 6 is classified as slightly polluted, its ecological status is described as impoverished and this could be due to the influence of pollution from Martil's city known for its intense summer tourist activity. As for St 1, which is located in AL Hoceima province, it is classified as slightly polluted with an impoverished state.

This station is characterized by a depth of 100 meters which slightly disturbs the sediment. St 3 and St 4 recorded close values, they have a poor state of health and are slightly polluted. This state could be due to the high intensity of artisanal fishing in the area (El Jebeha-Stehat region) or also by the strong seaside potential and influence of this region[29].

M-AMBI is a factor analysis that associates AMBI, diversity (H') and specific richness. Results obtained (figure 8) according to this index and its reference values as in table 2 showed that stations 1, 3 and 4 indicate a good quality, whereas the other stations are indicating very good quality. Ecological status according to BENTIX is described in table 6. Stations 3, 4 and 5 have a good ecological status with a slightly polluted population in transition while stations 1, 2 and 6 express a high ecological state with a normal population.

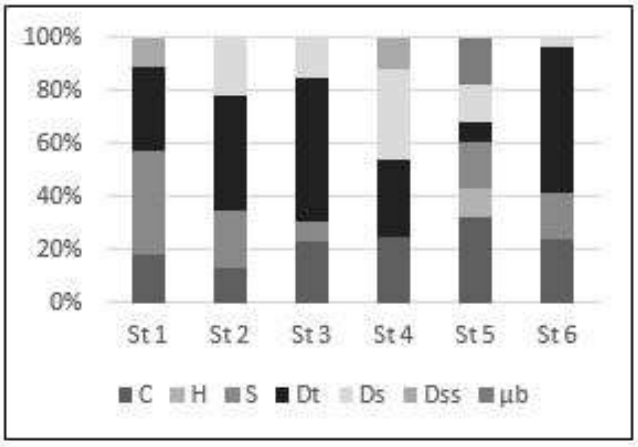

Figure 5: Trophic structure of the population of each station sampled

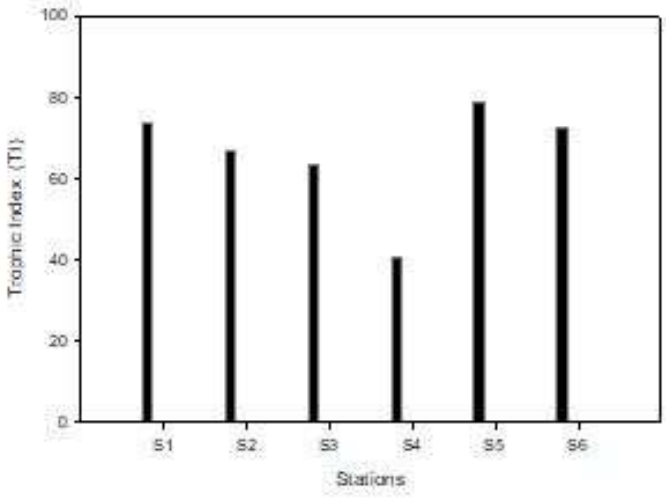

Figure 6: Trophic Index (TI) values for each station

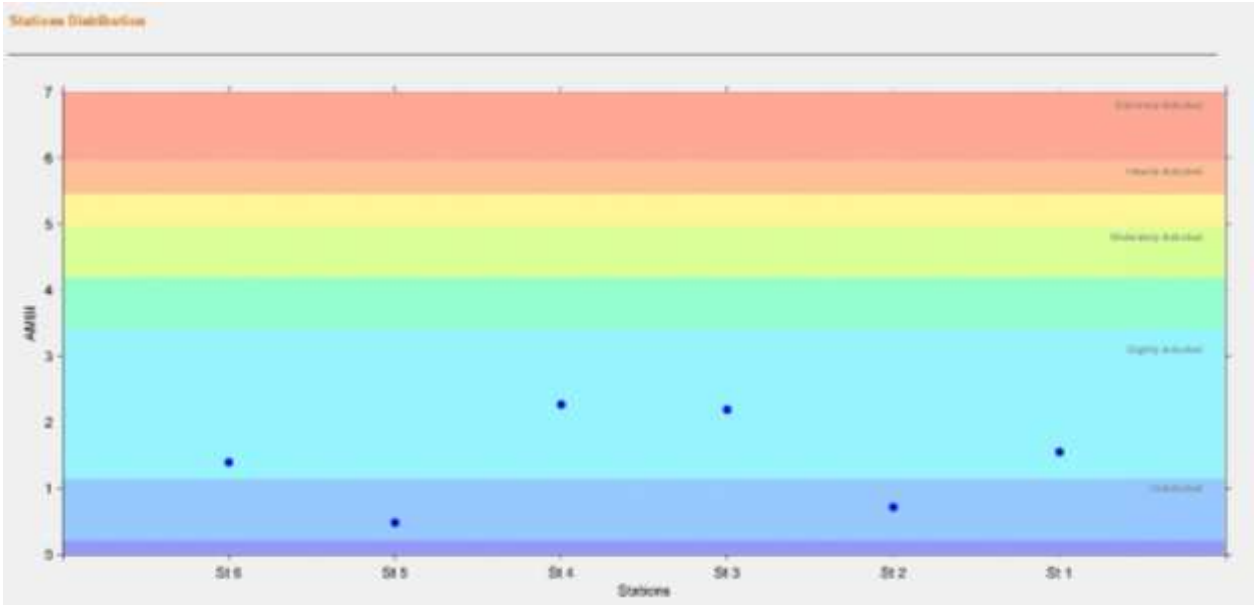

Figure 7: The quality of studied stations according to AMBI

Table 5
State of the population in different sites according to AMBI index

Station	AMBI	BI	Ecological status	Pollution scale
St 1	1,5	2	Imbalance	Slightly polluted
St 2	0,7	1	Normal	Unpolluted
St 3	2,2	2	Imbalance	Slightly polluted
St 4	2,3	2	Imbalance	Slightly polluted
St 5	0,5	1	normal	Unpolluted
St 6	1,3	2	imbalance	Slightly polluted

Table 6
Diagnosis of the ecological status and classification of the benthic population according to BENTIX

Station	BENTIX	Classification-pollution	Ecological status
St 1	5,05	Normal	High
St 2	5,65	Normal	High
St 3	4	Slightly polluted-transition	Good
St 4	4,05	Slightly polluted-transition	Good
St 5	4	Slightly polluted-transition	Good
St 6	4,8	Normal	High

Both M-AMBI index and BENTIX did not discriminate any station. All stations are rated from good to very good quality; according to BENTIX, stations St 3, St 4 and St 5 reflected a good state of health, they are slightly polluted in a transition phase while other stations indicate a very good state.

Embryo-toxicity of the sediment: In another way we tried to assess the quality of the medium in the West Mediterraneean zone using a bioessay of general toxicity, it is a study of the impact of the quality of the sediment on the embryolarval development of sea urchin. The results generally revealed a notable impact on the quality of the environment and demonstrated the great sensitivity of the early stages of embryolarval development versus the state of the environment in which they live. Indeed, it has been shown that low concentrations of toxicants can modify the physiological functioning of both larval stage and adult.

Also, embryos and larvae have a higher rate of accumulation in connection with their rapid development which allows observations of shorter durations[34]. Previous studies on the quality of marine water and discharges using the larval development stages of *P. lividus* showed the sensitivity of embryos and larvae to discharges[16].

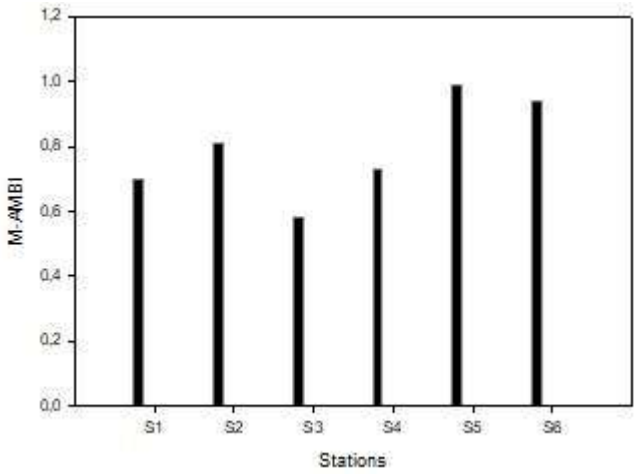

Figure 8: Classification of stations quality according to M-AMBI index.

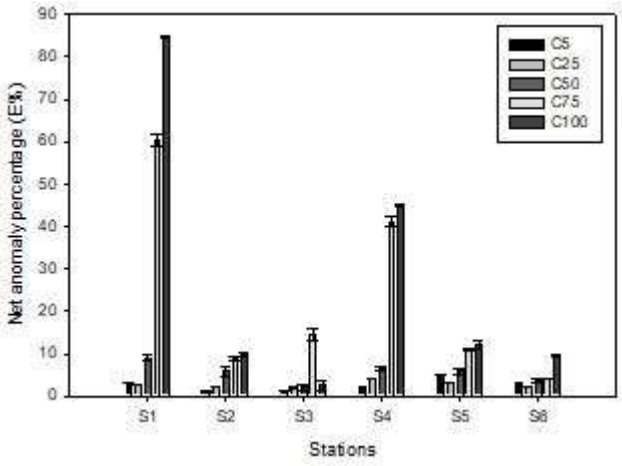

Figure 9: Toxicity of sediment on the embryolarval development of sea urchins (rate of abnormal larvae)

Table 7
Toxicity units (TU) values and quality of tested sediments

Stations	TU	Quality
St 1	1,35	Poor
St 2	Too high to be calculated	Good
St 3	1,08	Poor
St 4	0,97	Poor
St 5	Too high to be calculated	Good
St 6	0,67	Moderate

Figure 9 illustrates results of the effect of sediment on embryo-larval development of sea urchin represented as net percentage of abnormal larvae as function of the concentration of the extracts sediment in the test medium. For all sediments, the percentage of abnormal larvae increases with the increase of concentrations of sediments extract in the medium. However, St 2, St 3, St 5 and St 6 showed a low percentage of anomalies even at 100% of sediment in the medium (figure 9).

Sediments extracts belonging to station 1 and station 4 exert notable toxicity with an effect that often exceeds 80% at a concentration of 100% of the extract in the medium. Sediments from stations 2, 3, 5 and 6 show a low impact on development with percentages of larval anomalies witch do not reach 20% even at 100% of the extract sediment in the medium.

Table 7 gives the values of the toxicity units (TU) of all stations calculated from the EC50 and gives the classification of the sediments quality of each station determined according to the criteria of Duran and Beiras[15]. According to the results of the toxicity units, sediments from stations 2 and 5 are classified as good quality; sediment for station S6 is classified as moderate quality and sediments for the other stations are qualified of poor quality. Results of the toxicity study agree with the results obtained by AMBI index. Indeed, the sediment of St 5 does not exhibit any

toxicity on the medium with a toxicity unit of zero (TU = 0). Only the EC10 could be estimated (67.25%) thus showing that it will take at least 68% concentration of sediment extract in the medium to achieve a 10% effect. This made it possible to classify this station as being of "Good" quality. St 2 was also qualified as good quality according to its toxicity unit which is equal to zero; EC10-48h=82.62%, demonstrating that 83% sediment extract in the medium induces only 10% larval abnormalities.

St 6 is of "Moderate" quality with a toxicity unit of TU = 0.67. St 1 is of "Poor" quality with a toxicity unit of 1.35. Previous results had, however, shown the absence of contamination in this region[2]. Sediments of St 3 and St 4 are of "poor" quality with toxicity units of 1.09 and 0.97 respectively. Studies carried out have highlighted contamination in the same area by PAH (polycyclic aromatic hydrocarbon) as well as contamination by OCPs (organochlorine pesticides)[24,36]. Toxicity bioassays using gametes and sea urchin embryos have often been used as rapid biological tools and have demonstrated their sensitivity and cost-effectiveness for assessing the quality of marine sediments and seawater worldwide[1,10,11,16,26,39].

The results of this work showed that the quality of sediments exerts an impact on the ecosystem. The ecological status as well as the quality of the sediments tested range from good to poor, this same status was recorded in a similar study in the Mediterranean region using ecological indicators[7].

Conclusion

Assessment of water quality of West Moroccan Mediterranean coast is carried out by biological indicators of contamination of the marine environment, specifically benthic populations, which are used for calculation of biological indices. The results were confirmed by the embryotoxicity bioassay based on the use of sea urchin larvae of *Paracentrotus lividus* detecting the presence of toxic substances in sediments. Ecotoxicological diagnosis of the biological impact of pollutants shows that sediment compartment in our study indicates the presence of toxicity in this latter.

Indeed and with the exception of 2 stations which are considered to be of good quality, the quality of the population and the sediment for other stations range is between moderate and impoverished. These results confirm that benthic population as a bioindicator and the embryotoxicity bioassay constitutes the material of choice for monitoring quality of the marine environment.

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