# Assessment of seawater pollution by heavy metals in the neighbourhood of Algiers: use of the sea urchin, *Paracentrotus lividus*, as a bioindicator

Dina Soualili, Philippe Dubois, Pol Gosselin, Philippe Pernet, and Monique Guillou

Soualili, D., Dubois, P., Gosselin, P., Pernet, P., and Guillou, M. 2008 Assessment of seawater pollution by heavy metals in the neighbourhood of Algiers: use of the sea urchin, *Paracentrotus lividus*, as a bioindicator. – ICES Journal of Marine Science, 65: 132–139.

The objective of the study was to assess the levels of contamination by heavy metals near the Algiers metropolis, using a combination of chemical and toxicological data gained from analyses of sediments and biological parameters characteristic of the sea urchin, *Paracentrotus lividus*. Zinc, lead, cadmium, copper, and iron concentrations were determined in sediments and in sea urchin gonads. Sediment toxicity was assessed by bioassay based on the larval development of sea urchins. The most numerous larval abnormalities were found in a site near Algiers identified as highly polluted by lead. The levels of the other metals across the study area fell within the background concentrations reported in the literature for the Mediterranean Sea, with the exception of zinc, which showed high values in female gonads.

**Keywords:** Algeria, bioassays, gonads, heavy metals, *Paracentrotus lividus*, sediment.

Received 27 August 2007; accepted 14 November 2007; advance access publication 4 January 2008.

*D. Soualili: Faculté des Sciences de la Nature, Laboratoire de Biologie et d'Ecologie Marine, Université des Sciences et Technologie Houari Boumedienne, Bab-Ezzouar, Algiers, Algeria. P. Dubois and P. Pernet: Laboratoire de Biologie Marine, Université Libre de Bruxelles, CP 160/15, Av. F.D. Roosevelt, B-1050 Bruxelles, Belgium. P. Gosselin: Laboratoire de Biologie Marine, Université de Mons-Hainaut, Av. du Champs de Mars, Pentagone, 7000 Mons, Belgium. M. Guillou: Laboratoire des Sciences de l'Environnement Marin, UMR CNRS 6539, Institut Universitaire Européen de la Mer, Place N. Copernic, 29280 Plouzané, France. Correspondence to M. Guillou: tel: +33 298 498634; fax +33 298 498645; e-mail: mguillou@univ-brest.fr*

## Introduction

The Atlantic–Mediterranean sea urchin *Paracentrotus lividus* is distributed from Ireland to southern Morocco, and throughout the Mediterranean Sea. Its ecological role as an opportunist is important in different ecosystems. For example, in the Mediterranean Sea, through its grazing activity, *P. lividus* locally controls the dynamics of seaweeds (see review by Lozano *et al.*, 1995) and seagrasses (see review by Tomas *et al.*, 2004). However, in coastal zones, its morphology, biology, and demography (Harmelin *et al.*, 1981; Delmas and Régis, 1984; Pancucci *et al.*, 1993) and therefore its long-term ecological role can be influenced by human activities. Because of its sedentary habits and acknowledged sensitivity to pollutants, it has been used in several studies as a biological–biochemical indicator of local pollution (Warnau *et al.*, 1998; Coteur *et al.*, 2001; Bayed *et al.*, 2005). Moreover, the sensitivity of its embryos and larvae has prompted its use in embryotoxicity tests (Hagström and Lönning, 1973; Pagano *et al.*, 1986; Warnau *et al.*, 1996). Additionally, assessing the embryonic and larval development of sea urchins is among the toxicity assays used in monitoring and risk assessment programmes (see review of Beiras *et al.*, 2003).

Along the coast of Algeria, *P. lividus* is a dominant species of shallow-water ecosystems, and up to 25 animals can be found per square metre (Semroud, 1993). It colonizes different biotopes, such as *Posidonia oceanica* and *Cymodocea nodosa* meadows, rocky substrata with photophile algae, and overgrazed rocky substrata (Semroud, 1993; Guettaf *et al.*, 2000), and it lives from the low-water limit to a depth of ~10 m (Dieuzeide, 1933; Semroud, 1993).

Around the Bay of Algiers, the human population is exposed to anthropogenic disturbance of different origins. Indeed, more than 4.3 million people (950 habitants km$^{-2}$) live within the metropolitan district of Algiers in a coastal zone 115 km long. Moreover, 1000 companies have activities in the fields of metalworking, building materials, petrochemistry, pharmaceuticals, mechanical, electrical and electronic engineering industries, food and paper production (Larid, 2003; PAC, 2005). In other respects, two rivers flow into the Bay of Algiers, El Hamiz and El Harrach (970 km$^2$; 6 m$^3$ h$^{-1}$); the latter drains the domestic and industrial wastewaters of the city of Algiers itself, and just 8% of the wastewater discharged into the bay (225 million m$^3$ year$^{-1}$) is treated (PAC, 2005). The greatest pollutant flow along the Algerian coasts (100 000 t year$^{-1}$ of organic chemical compounds, 175 000 t year$^{-1}$ of suspended matter, 1500 t year$^{-1}$ of nitrogen, and 4000 t year$^{-1}$ of phosphorus) is discharged into the marine area impacted by the Algiers metropolis (Larid, 2003). In 2004, bathing was prohibited from 46 beaches inside the bay. The marine ecosystem is seriously affected by the pollution, and a decrease in biodiversity of 14% for the species of major ecological interest has been documented (PAC, 2005).

The objective of this study was to assess marine pollution by heavy metals in the area from a combination of chemical and toxicological data acquired through analyses of sediments and the gonads of the sea urchin, *P. lividus*, supplemented with embryo/larva sediment toxicity bioassays. The toxicity of the metal bioavailable fraction was detected by abnormalities in the development of *P. lividus* because the species is a known indicator of metal contamination and can accumulate metals as a function of the contamination level of the environment (Warnau *et al.*, 1998). The results of bioassays were compared with those from the analysis of metal contamination in the sediments and in the gonads of adult sea urchins inhabiting the sediments, which had been tested previously. Three field sites near the Bay of Algiers were chosen according to potential sources of pollution, to classify them as a function of environmental quality.

## Material and methods

### Sampling sites

Two of the three field sites, Algiers Beach and Tamentfoust, are located in the Bay of Algiers; the third, Sidi-Fredj, is situated at the north of the Bay of Bou-Ismail (east of the Bay of Algiers) (Figure 1). Of the three, Algiers Beach is the closest to the Algiers metropolis; its habitat consists of a mixture of photophile algae-covered rocks and degraded *P. oceanica* meadows. Tamentfoust is situated in a semi-closed creek with overgrazed rocks and a few, sparse *P. oceanica* beds. Sidi-Fredj shelters a thalassotherapy centre; the site is less impacted by pollution than the Bay of Algiers (PAC, 2005), because it is farther from the very industrialized area and from El Hamiz and El Harrach rivers. Although the *P. oceanica* beds present there are in better health than those in the Bay of Algiers, they are regressing progressively (PAC, 2005).

### Collection and preparation of samples

In March 2002, 40 sea urchins 45–65 mm in diameter were sampled from each field site by scuba diving at 1–5 m. March falls in the time of year when gonads start to mature (Semroud and Kada, 1987; Guettaf *et al.*, 2000), before the spawning which tends at least partially to eliminate the metal load in the gonad (Warnau *et al.*, 1998).

Immediately following their collection, sea urchin test diameters were measured in the laboratory and dissected. After their sex had been determined, the gonads were dried at 60°C for 48 h, then stored separately in hermetically sealed polyethylene containers. All contact with the urchins was with stainless steel instruments.

At the same time as the sampling, the upper 2-cm layer of the sediments inhabited by sea urchins was collected by coring (5 cm diameter). Then, the samples were placed into sealed polyethylene bags placed on ice to be brought back to the laboratory where they were immediately dried at 60°C for 48 h until constant weight before storage, at room temperature, in hermetically-sealed polyethylene containers.

### Metal analysis

The concentrations of zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu), and iron (Fe) were measured in urchin gonads and in sediments according to the method described by Coteur *et al.* (2003). As the sediment was heterogeneous and comprised mainly large gravel, only the portion with grains $<1$ mm was kept; here we term it the "total fraction". Metal concentrations were also measured in the fraction containing grains $<63$ µm (the silt-clay fraction) known to accumulate organic matter and heavy metals preferentially. Pb, Cd, and Cu concentrations were determined by graphite-furnace electrothermic atomic absorption spectrometry performed on a Varian GTA100 SpectrAA640Z spectrometer. Zn and Fe concentrations were measured by flame atomic absorption spectrometry on a GBC 906AA spectrometer. The accuracy of the method was assessed using certified reference material (*Mytilus edulis* tissues, BCR number 278R from the Community Bureau of Reference, Brussels, Belgium). Detection limits for Zn, Pb, Cd, Cu, and Fe were 0.002, 0.014, 0.001, 0.002,

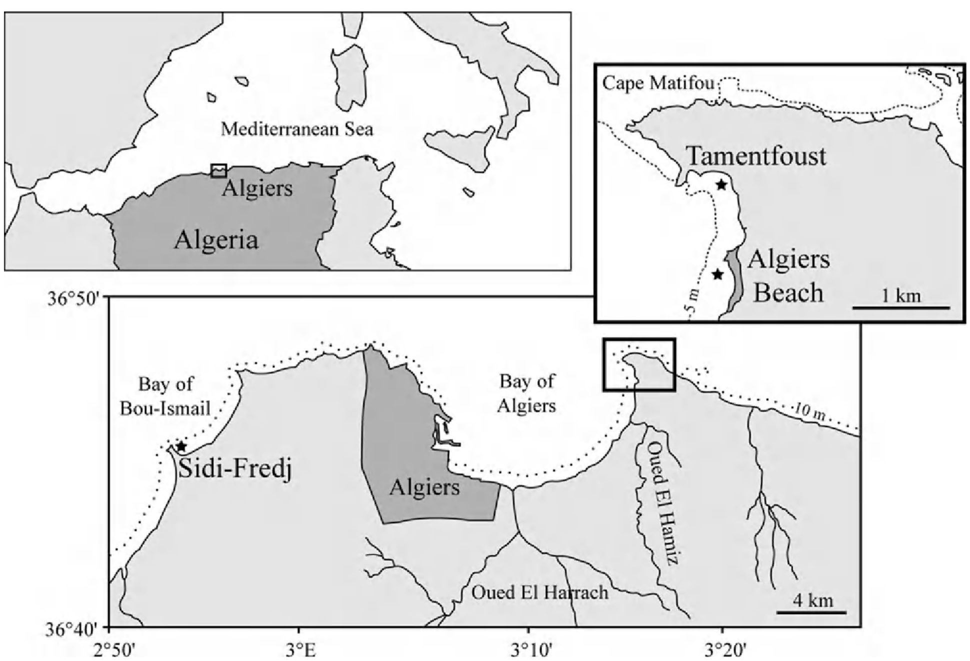


**Figure 1.** Location of the three sampling sites: Algiers Beach, Tamentfoust, and Sidi-Fredj.

and 0.004 μg of metal per millilitre of digested sample, respectively.

The gonads of ten male and ten female urchins per site were analysed. For the sediment, 3–8 replicates of the total fraction and 0–3 replicates of the fraction <63 μm were used because the silt-clay fraction was very low in the sediment of some sites.

### Bioassays of embryos and larvae

The toxicity of the sediments at the three field sites was assessed by embryo–larval bioassays in May 2003. The seawater used in the tests was collected at Luc-sur-Mer (Normandy, France) in front of the marine station, because this site is used as reference by the scientists of the Laboratory of Marine Biology of ULB (the Free University of Brussels, Belgium), where the metal analyses were performed. The seawater salinity was 33. Before analysis, the seawater was allowed to decant for 48 h, then filtered through a 0.22-μm membrane and stored at 20 ± 1°C (i.e. it was filtered seawater, FSW). Adult *P. lividus* were collected intertidally from a reference population inhabiting the rocky basins of Morgat (Bay of Douarnenez, Brittany, France), where the salinity was 34 in May 2003. Individuals were transferred to the cultivation system of the marine laboratory of the University of Mons-Hainaut, Belgium (UMH). For the embryotoxicity test, the reference sediment was sampled at Wimereux (Nord-Pas-de-Calais, France) in the same way as the Algerian sediment samples.

The embryo–larval bioassays were performed at the Mons-Hainaut laboratory; they were based on the method described by Coteur *et al.* (2003). Spawning was induced by injection of 20 μl $g^{-1}$ of KCl 0.5 N through the peristomial membrane. Gametes from three female and three male sea urchins were collected in FSW, and their quality (i.e. the overall shape of eggs, and the sperm motility) was checked under an Olympus TO41 inverted light microscope. Eggs from each female were fertilized with sperm taken from a pool of sperm from the three males. Embryos at the early gastrula stage (4–5 h after fertilization at 14 ± 1°C) were mixed before the bioassays. Experiments with embryos were performed in 4 × 6-well plates (Falcon, ref. 35-3046) with one plate per site [Alger Plage, Tamentfoust, Sidi-Fredj, and Wimereux (reference)]; each well was filled with 0.1 g of dried sediment before addition of 10 ml of FWS before the bioassay. One plate filled with only FWS was used as control. At the beginning of the bioassay, batches of 250–300 embryos were transferred to each well. After 72 h at 14 ± 1°C, larvae were fixed by addition of 1 ml of formalin (commercial solution, 35%) to each well before storage of the plates for 2 h at 70°C. This protocol leads to perfect preservation of the larvae. The plates with fixed larvae were then stored at 4°C. The frequency of developmental stages was scored on a random sample of 100 larvae per well under an inverted light microscope. When the sediment is relatively coarse, larvae are easily distinguishable between the grains; in fine sands, larvae were characterized after re-suspension by shaking the sample. The larvae were then classified into four groups according to the morphological criteria adapted from Warnau and Pagano (1994): normal plutei ("Normal", N), retarded plutei presenting a delayed development ("Retarded", R), abnormal plutei with skeletal malformations and/or gut abnormalities ("Pathologic 1", P1), and embryos whose development ended at the blastula or gastrula stage ("Pathologic 2", P2). The rates of "Viable" larvae given later were obtained by summing the rates of "Normal" and "Retarded" larvae from each batch.

### Statistical analyses

After arcsine transformation, the developmental rates of normal and viable larvae were compared using a one-way analysis of variance (ANOVA), followed with a Bonferroni test (Zar, 1996). Dunnett's tests were used for comparing the rates measured with the rates of the corresponding controls (Zar, 1996). Significant differences were determined at the 95% level. Contamination levels in gonads were compared by two-way ANOVA and a Tukey HSD (honestly significant difference) multiple mean comparison test (effects: sex and sampling site).

Existence of a relationship between the levels of contamination by metals in the gonads and sediments and the percentage of abnormal and viable larvae was investigated by factor analysis, using a principal-component method with an extraction matrix based on Pearson correlation coefficients and the "varimax" method of factor rotation.

## Results

### Levels of contamination in sediment and sea urchin gonads

The importance of the fraction of sediment <63 μm depended on the site; it accounted for 0.03%, 0.26%, and 1.56% of the total fraction at Tamentfoust, Sidi-Fredj, and Algiers Beach, respectively, explaining the lack or scarcity (1) of replicates at Tamentfoust and Sidi-Fredj (Table 1). The sediment from Algiers Beach, as expected, contained more silt-clay than the other ones.

Tables 1 and 2 list the concentrations of heavy metals found in the sediments (total fraction and fraction < 63 μm). Statistical analyses for further comparison of metal contents were made on only the total fraction because of the scarcity of replicates at Tamentfoust and Sidi-Fredj. Fe, Pb, and Zn concentrations in the total fraction of sediments differed with sampling site (Table 2). Algiers Beach was the site most significantly contaminated by Pb, but it also had the lowest concentrations of Fe and Zn. Fe concentrations were significantly higher in the sediments from Sidi-Fredj.

Table 3 shows the concentrations in heavy metals measured in the gonads of sea urchins. Table 4 lists the results of the two-way ANOVA by metal, with sex and sampling site as factors, and of

**Table 1.** Metal concentration in the total fraction ($n = 3-8$) and in the <63 μm grain-size fraction ($n = 0-3$) of the sediment collected at three Algerian sites.

| Site | Parameter | Metal concentration (μg $g^{-1}$ dry wt) | | | | |
|---|---|---|---|---|---|---|
| | | Zn | Pb | Cu | Cd | Fe |
| Total fraction | | | | | | |
| Algiers Beach | Mean | 0.023 | 39.63 | 4.08 | 0.76 | 7.11 |
| | s.d. | 0.005 | 7.93 | 0.62 | 1.05 | 2.18 |
| Tamentfoust | Mean | 0.045 | 14.59 | 5.73 | 0.15 | 17.85 |
| | s.d. | 0.010 | 1.55 | 1.65 | 0.06 | 3.71 |
| Sidi-Fredj | Mean | 0.050 | 12.37 | 6.43 | 0.12 | 31.32 |
| | s.d. | 0.006 | 4.07 | 1.32 | 0.01 | 6.49 |
| <63 μm fraction | | | | | | |
| Algiers Beach | Mean | 0.081 | 23.76 | 20.89 | 1.22 | 19.70 |
| | s.d. | 0.008 | 0.75 | 0.75 | 0.04 | 2.08 |
| Sidi-Fredj | Mean | 0.08 | 40.63 | 10.48 | 0.25 | 41.50 |

**Table 2.** Comparison of the metal concentrations in the total fraction of the sediments collected from the three Algerian sites, AB, Algiers Beach; TM, Tamentfoust; and SF, Sidi-Fredj.

| Metal in sediments total fraction | ANOVA *p*-value | Level of contamination[a] + ⟵ − |
|---|---|---|
| Zn | 0.03 | SF TM AB |
| Pb | $>10^{-2}$ | AB TM SF |
| Cu | NS[b] | TM SF AB |
| Cd | NS | AB TM SF |
| Fe | $>10^{-2}$ | SF TM AB |

[a]Stations showing no statistical difference between metal concentrations are underlined ($p > 0.05$, Tukey HSD test).
[b]NS, not statistically significant.

the Tukey HSD test carried out after the ANOVA to rank the sites by increasing level of contamination. There was no significant variation in the concentrations of Pb and Cu by sex. However, Zn and Cd levels, unlike Cu, were significantly higher in female gonads than in male gonads. Moreover, the results highlight the fact that Pb, Fe, and Cu concentrations in sea urchin gonads are dependent on field site. Our finding a sex-dependence in terms of Cu concentration led us to perform a one-way ANOVA with sex as factor for intercomparison of the field sites. From that test, we concluded that sea urchins from Sidi-Fredj were the most contaminated, with concentrations significantly higher than in female sea urchins from Tamentfoust ($p < 0.05$) and male sea urchins from Algiers Beach ($p < 0.005$).

## Development of embryos and larvae

Figure 2 is a comparison of the rates with which normal (N) and viable (B) larvae are found at the three sites. The percentages of normal and viable larvae found decreased when embryos were exposed to sediments from Algiers Beach (one-way ANOVA followed by a Bonferroni's test, $p = 0.05$). Table 5 lists the frequencies

**Table 3.** Metal concentrations ($n = 10$) in the gonads of *Paracentrotus lividus* collected from the three Algerian sites.

| Site and metals in gonads | Parameter | Metal concentration ($\mu g\ g^{-1}$ dry wt) | | | | |
|---|---|---|---|---|---|---|
| | | Zn | Pb | Cu | Cd | Fe |
| Algiers Beach | | | | | | |
| Female | Mean | 385.5* | 6.14 | 2.84 | 0.14 | 73.8 |
| | s.d. | 344.1 | 3.46 | 0.97 | 0.08 | 35.5 |
| Male | Mean | 32.9 | 7.78 | 3.19 | 0.08 | 19.3 |
| | s.d. | 13.5 | 8.77 | 0.83 | 0.04 | 19.7 |
| Tamentfoust | | | | | | |
| Female | Mean | 538.2* | 1.5 | 2.49* | 0.12 | 113 |
| | s.d. | 324.3 | 1.72 | 0.47 | 0.08 | 37.6 |
| Males | Mean | 76.1 | 0.88 | 3.88 | 0.05 | 112.6 |
| | s.d. | 172.2 | 0.44 | 0.84 | 0.01 | 66 |
| Sidi-Fredj | | | | | | |
| Female | Mean | 366.9 | 0.68 | 3.42 | 0.14* | 71.1 |
| | s.d. | 178.3 | 0.12 | 0.85 | 0.09 | 54.8 |
| Male | Mean | 52.9 | 0.90 | 4.42 | 0.05 | 92.7 |
| | s.d. | 73.2 | 0.41 | 0.56 | 0.03 | 78.8 |

*Significant difference between sexes.

**Table 4.** Comparison of the metal concentrations in the gonads of *Paracentrotus lividus* from three Algerian sites, AB Algiers Beach, TM Tamentfoust, SF Sidi-Fredj (two-factor ANOVA: sex and site).

| Metal in gonads | ANOVA *p* | | | *n* | Level of contamination[a] + ⟵ − |
|---|---|---|---|---|---|
| | Sex | Site | Interaction | | |
| Zn | $<10^{-4}$ | 0.26[b] | 0.53 | 60 | TM SF AB |
| Pb | 0.69[b] | $<10^{-4}$ | 0.66 | 60 | AB TM SF |
| Cu | $<10^{-4}$ | $<10^{-2}$ | 0.11 | 60 | SF TM AB |
| Cd | $<10^{-4}$ | 0.45[b] | 0.80 | 60 | SF AB TM |
| Fe | 0.40[b] | $<10^{-3}$ | 0.06 | 60 | TM SF AB |

[a]Stations showing no statistical difference between metal concentrations are underlined ($p > 0.05$, Tukey HSD test).
[b]Not statistically significant.

with which the different classes of larvae were found by site and in the controls. The low rate at which normal larvae were found after exposure to sediments from Algiers Beach (Dunnett's test, $p < 10^{-4}$) was related to an increase with which abnormal plutei (P1) and retarded plutei (R) were found; the high rate with which retarded plutei were found ($47.8 \pm 13.5$) explains why the rate of viable plutei (N+R) in Figure 2 was rather high (68.33%).

## Integrated data analysis

The factor analysis presented in Figure 3 was carried out to investigate possible relationships between metal concentrations in sediments as well as in male and female gonads, and the percentages of "viable" (healthy) and abnormal (P1) larvae. Succinctly, two factors are represented by the *x*- and *y*-axes; the variables were distributed along the axes according to their correlation coefficients for each factor. The first two factors accounted for 99.9% of the total variance in the data. Figure 3 shows that along the *x*-axis, Fe, Cu, and Zn concentrations in sediments opposed Cd and Pb concentrations in sediments. Moreover, depending on which metal was being studied, the strength of the relationship between the concentration of metal in sediments and the concentrations in male and female gonads was more or less marked. For example, Figure 3 indicates a strong relationship between Pb levels in sediments and in both types of gonads, whereas for Cd and Cu concentrations, the relationship is strong only between the male gonads and the sediments. Along the *x*-axis, the percentage of viable larvae is related to Fe, Cu, and Zn concentrations in the sediments, but less to Pb and Cd concentrations in sediments and gonads. This suggests a strongly negative interaction between Pb and Cd and the percentage of viable larvae. In terms of abnormal larvae, there is a strong relationship between Pb and Cd concentrations in the sediments and the gonads.

Zn concentrations in male and female gonads and Fe concentrations in female gonads lie at the negative end of the *y*-axis, whereas Cd and Cu concentrations in female gonads are at the opposite end of the axis. The percentage of viable larvae lies along the negative part of this axis, suggesting negative intercorrelation between this parameter and Cd and Cu concentrations in female gonads.

## Discussion

Our study aimed to assess the extent of marine pollution in the neighbourhood of the Bay of Algiers from a set of chemical and

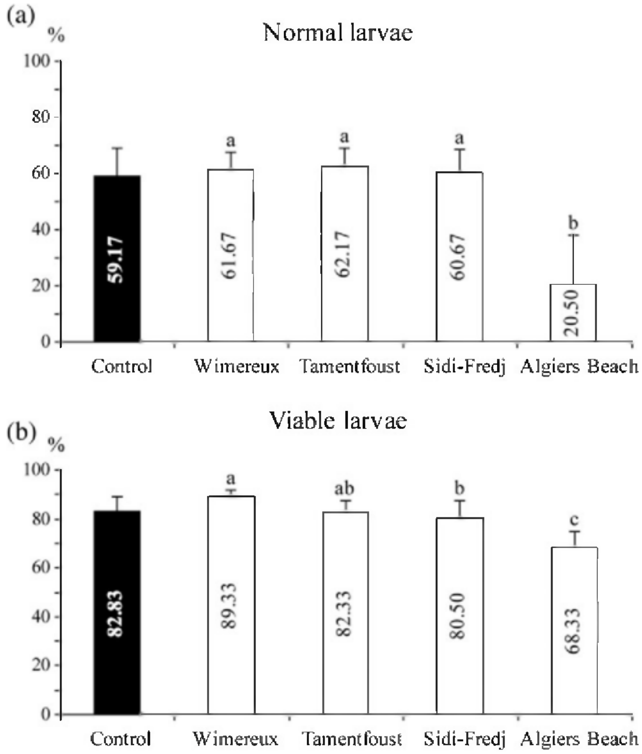


**Figure 2.** Percentages (mean ± s.d.) of (a) normal and (b) viable larvae of *Paracentrotus lividus* after exposition to dried sediments and control throughout embryogenesis (72 h). Six replicates by exposure, 100 larvae scored by replicate. For each species, there were no significant differences between the series designated by the same letter (one-way ANOVA followed by a Bonferroni's test, $p = 0.05$).

toxicological data and using the sea urchin *P. lividus* as a bioindicator.

None of the three sites stood out from the others as having higher concentrations of all metals in the total sediments or in sea urchin tissues. The sediments from Algiers Beach had the highest concentration of Pb but the lowest concentrations of Zn and Fe. In contrast, the highest concentration of Fe was at Sidi-Fredj. Of the three sites, Algiers Beach is richest in silt, and usually the higher the percentage of the fine fraction, the greater the load of heavy metals in the sediments. However, at Algiers Beach, only Pb was at a high concentration. The concentrations of heavy metals in the gonads of sea urchins from Algiers Beach confirmed the results for the sediments: a particularly high concentration of Pb and a low concentration of Fe. Sidi-Fredj had the greatest concentrations of Cu. Cd levels did not vary significantly between field sites.

The Cd and Cu concentrations measured in sediments can be considered as close to the background concentrations reported in the literature for the Mediterranean Sea. The total fraction contained 0.12–0.76 $\mu g\, g^{-1}$ dry wt of Cd compared with 0.05–1 $\mu g\, g^{-1}$ dry wt background concentration of Cd in the Mediterranean Sea (EEA, 1999). However, at Algiers Beach, Cd concentration was 1.22 $\mu g\, g^{-1}$ dry wt in the fraction of grains <63 μm. Cu concentration was 4.1–6.4 $\mu g\, g^{-1}$ dry wt in the total fraction of sediments and 20.9 $\mu g\, g^{-1}$ dry wt in the fraction of grains <63 μm, compared with 5–30 $\mu g\, g^{-1}$ dry wt background concentration in the Mediterranean Sea (EEA, 1999). Zn and Fe concentrations, respectively, fell within the ranges 0.02–0.08 and 7.1–41.5 $\mu g\, g^{-1}$ dry wt; these data are far lower than the values given in the literature for the Mediterranean Sea, i.e. Zn concentrations in the range 35–150 $\mu g\, g^{-1}$ dry wt (Saad *et al.*, 1981; van Hoogstraten and Nolting, 1991; Storelli *et al.*, 2001) and Fe concentrations >$10^3$ $\mu g\, g^{-1}$ dry wt (Saad *et al.*, 1981; Storelli *et al.*, 2001; Menchi *et al.*, 2002). Only the Pb values in the total fraction of sediments from Algiers Beach (39.6 $\mu g\, g^{-1}$ dry wt) and in the fraction of grains <63 μm at Sidi-Fredj (40.6 $\mu g\, g^{-1}$ dry wt) exceeded the background concentrations of 5.2–23.2 (EEA, 1999) or 4–17 $\mu g\, g^{-1}$ dry wt reported in the US NOAA-issued reference tables (Buchman, 1999).

In the gonads, other than for Zn, our conclusions on sediment contamination by heavy metals were confirmed by comparing our data with literature data on *P. lividus* (Table 6), especially the study by Warnau *et al.* (1998). The Cd, Cu, and Fe levels in sea urchin gonads from all three sampling sites averaged the same as background concentrations reported for *P. lividus* gonads. Our study showed no significant difference in Zn concentrations among sites; all values were higher than concentrations estimated for female gonads of *Sphaerechinus granularis* in the Bay of Brest (between 190 and 700 $\mu g\, g^{-1}$ dry wt) (Guillou *et al.*, 2000), a bay known to be contaminated by Zn-loaded run-off water because most of the roofs of buildings are made of Zn (Troadec, 1995). Under such conditions, the low Zn levels found in sediments compared with those in female gonads are intriguing. However, Zn is not a good indicator of pollution because it is an essential element for animal metabolism and can exist at temporarily high concentrations in tissues (Hambridge *et al.*, 1986). In contrast, the high Pb levels in gonads match the high concentrations in sediments. Of the sites compared in Table 6, only Rabat (Morocco) had concentrations higher than ours, probably because of the extensive pottery activity there and the absence of efficient wastewater treatment (Bayed *et al.*, 2005).

The geographical gradient we found in Pb in the sediments agrees with the gradient of metal accumulation in the gonads; both highlight Algiers Beach as the most heavily contaminated site in terms of Pb. However, the differences between sites in

**Table 5.** Frequencies (mean ± s.d.) of developmental stages of *Paracentrotus lividus* larvae exposed to sediment samples throughout embryogenesis.

| Site | N | R | P1 | P2 | Du1 | V | Du2 |
|---|---|---|---|---|---|---|---|
| Control | 59.2 ± 9.7 | 23.7 ± 5.9 | 8.7 ± 4.7 | 8.5 ± 3.2 | – | 82.8 ± 6.3 | – |
| Wimereux | 61.7 ± 5.8 | 27.7 ± 6.2 | 5.0 ± 2.3 | 5.7 ± 2.2 | = 0.6 | 89.3 ± 2.4 | =0.05 |
| Sidi-Fredj | 60.7 ± 8.1 | 19.8 ± 2.6 | 10.7 ± 3.1 | 8.8 ± 4.2 | = 0.6 | 80.5 ± 6.8 | =0.4 |
| Tamentfoust | 62.2 ± 6.6 | 20.2 ± 3.9 | 8.2 ± 3.3 | 9.5 ± 2.6 | = 0.6 | 82.3 ± 4.8 | =0.5 |
| Algiers Beach | 20.5 ± 17.4 | 47.8 ± 13.5 | 24.3 ± 7.9 | 7.3 ± 3.3 | <0.0005 | 68.3 ± 6.4 | <0.0005 |

N, normal plutei; R, retarded plutei; P1, abnormal plutei; P2, Blastula; V, viable plutei. Du1, result of the Dunnett test used to compare the rate of normal plutei to control plutei. Du2, result of the Dunnett test used to compare the rate of viable plutei to control plutei.

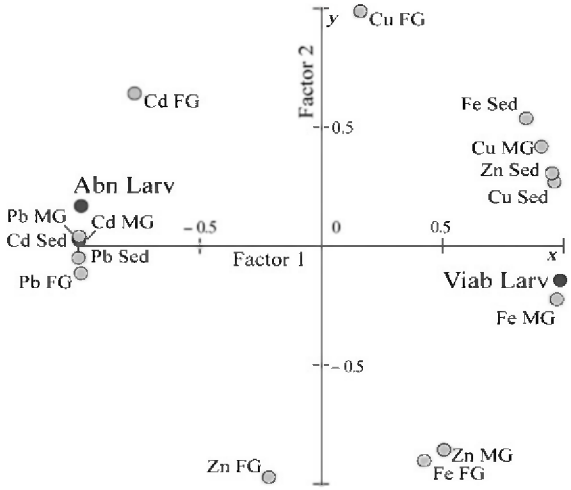


**Figure 3.** Factor analysis (principal component method) showing the relationships between the metal concentrations in the sediment (metal and SED), in the male gonads (metal and MG), in the female gonads (metal and FG), and the percentages of abnormal (Abn Larv) and viable larvae (Viab Larv). The first ($x$-axis) and the second ($y$-axis) factors account for 70% and 29.9% of the total variance, respectively.

terms of Fe and Zn concentrations in tissues and those in sediments are anomalous. In our opinion, the differences result from (i) a difference in the bioavailability of these metals, (ii) differences in the accumulation patterns of essential elements such as Fe and Zn against other metals, or (iii) the high risk of error in measuring metal concentrations in biota when the concentrations of Fe, Zn, and Cu in the sediments are very low.

The high contamination of Pb at Algiers Beach shown by sediment and gonad analyses is confirmed by the results of a recent study of the water and stream sediments of Oued El Harrach (Yoshida *et al.*, 2005). According to those authors, Pb concentrations are in the range 21–41 $\mu g\ g^{-1}$ dry wt in the sediments from the Oued mouth, close to the values we found in terms of the total fraction of Algiers Beach sediments (39.6 $\mu g\ g^{-1}$ dry wt). It is therefore likely that the Pb pollution results from the discharge of untreated industrial wastewater.

The results of the bioassays showed that Algiers Beach was the only site where toxins affected sea urchin embryos, with 24.3% being abnormal larvae (vs. 7.9 $\pm$ 2.8% for the other sites) and 47.8% of retarded larvae (vs. 22.56 $\pm$ 4.4% for the other sites). No difference was detected between the two other Algerian sites, the reference site (Wimereux) and the control. According to Kobayashi's (1991) criteria, the level of normal plutei from Algiers Beach (20.5%) indicates strong inhibition of sea urchin development attributable to a marked environmental disturbance. The viability of larvae appeared to be negatively dependent on Pb concentrations in male and female gonads, which were positively correlated with the Pb concentration in the total fraction of the sediments. The positive relationship deduced from the integrated data analysis found between the rates at which viable larvae were found and Cu, Fe, and Zn concentrations would not be sufficient to demonstrate a positive effect of these metals on larval

**Table 6.** Comparison of the mean concentrations of metals in the gonads of *Paracentrotus lividus*.

| Source and sampling date | Sites | Mean concentration ($\mu g\ g^{-1}$ dry wt) | | | | |
|---|---|---|---|---|---|---|
| | | Zn | Pb | Cu | Cd | Fe |
| Present study March 2002 | Algiers Beach | | | | | |
| | Females | 385.5 | **6.14** | 2.84 | 0.14 | 73.8 |
| | Males | 32.9 | 7.78 | 3.19 | 0.08 | 19.3 |
| | Tamentfoust | | | | | |
| | Females | **538.2** | 1.5 | 2.49 | 0.12 | 113 |
| | Males | 76.1 | 0.88 | 3.88 | 0.05 | 112.6 |
| | Sidi-Fredj | | | | | |
| | Females | 366.9 | 0.68 | 3.42 | 0.14 | 71.1 |
| | Males | 52.9 | 0.90 | 4.42 | 0.05 | 92.7 |
| Bayed *et al.* (2005) March 2000 | Atlantic Morocco | | | | | |
| | Rabat | | | | | |
| | Females | – | **35.32** | 3.34 | **25.15** | – |
| | Males | – | 12.41 | 0.52 | 2.58 | – |
| | Bouznika | | | | | |
| | Females | – | **11.3** | **5.56** | 2.21 | – |
| | Males | – | 7.18 | 1.52 | 1.51 | – |
| | Mohammedia | | | | | |
| | Females | – | 5.22 | 2.51 | 2.24 | – |
| | Males | – | 6.14 | 1.18 | 2.32 | – |
| Warnau *et al.* (1998) | Calvi | 124 | 2.25 | 0.15 | 3.47 | 51 |
| | Ischia | 140 | 3.02 | 0.41 | 3.41 | 90 |
| | Marseille | 109 | 3.68 | 0.19 | 3.51 | 39 |
| Storelli *et al.* (2001) April 1998 | Adriatic Sea | 157.1 | 0.86 | 0.24 | 5.19 | 18.37 |

High values are emboldened and the maxima underlined.

development, but rather the lack of a negative effect attributable to low concentrations of these metals.

At Tamentfoust, located in a semi-enclosed creek a little farther than Algiers Beach from the Oued El Harrach, the slight accumulation of Pb in sediments and tissues and the lack of larval abnormalities both support the hypothesis of local pollution of Algiers Beach by Pb. Moreover, because of its location, Tamentfoust may be protected from marine and sediment flows from the Oued. The criteria in use in this study did not allow us to discriminate between Sidi-Fredj and Tamentfoust, although the former was expected to be less polluted by heavy metals because of its location far from the highly industrialized area impacted by the Algiers metropolis.

In conclusion, our study has confirmed the relationships between developmental abnormalities of sea urchins, the bioaccumulation of metals, and their contamination of sediments, at least where metal concentrations in the sediments were sufficiently high. Our investigations led us to discriminate one toxic metal (Pb) at concentrations sufficiently high in sediments and gonads of sea urchins from Algiers Beach to explain the poor sea urchin larval development observed *in situ* (DS, pers. obs.). However, the intensity of the biological impact at that site does not rule out the possibility that other pollutants could be involved. Our method could be improved by increasing the number of toxins analysed, and by extending metal analysis to other sea urchin storage organs, such as the gut, to determine whether heavy metals are also accumulated there (Warnau *et al.*, 1998; Guillou *et al.*, 2000).

## Acknowledgements

This paper forms part of DS's thesis. The study was carried out within the "Laboratoire de Biologie Marine de l'Université Libre de Bruxelles, ULB" and the "Laboratoire de Biologie Marine de l'Université de Mons-Hainaut, UMH" (Belgium), where PhD is a senior research associate of the FRS–FNRS. The final part was conducted within the "Laboratoire des Sciences de l'Environnement Marin de l'Université de Bretagne Occidentale, UBO" (France). The study in Belgium was funded by a grant from the Institut des Sciences Agronomiques et Vétérinaires de l'Université de Blida (Algeria), and the stay in France by a grant from the Algerian Department of Higher Education and Research. DS gratefully acknowledges the hospitality and access to facilities of, and helpful discussions with, M. Jangoux (ULB). All authors also thank F. Boukroufa for assistance in the field, M. Briand (UBO) for help in the preparation of the artwork, and M. P. Friocourt (UBO) for help with drafting the manuscript in English.

## References

Bayed, A., Quiniou, F., Benrha, A., and Guillou, M. 2005. The *Paracentrotus lividus* populations from the northern Moroccan Atlantic coast: growth, reproduction and health condition. Journal of the Marine Biological Association of the UK, 85: 999–1007.

Beiras, R., Fernandez, N., Bellas, J., Besada, V., Gonzalez-Quijano, A., and Nunes, T. 2003. Integrative assessment of marine pollution in Galician estuaries using sediment chemistry, mussel bioaccumulation, and embryo-larval toxicity bioassays. Chemosphere, 52: 1209–1224.

Buchman, M. F. 1999. NOAA Screening Quick Reference Tables, NOAA HAZMAT Report 9-1, Seattle, WA. *In* Coastal Protection and Restoration Division, NOAA. 12 pp.

Coteur, G., Danis, B., Fowler, S. W., Teysiié, J. L., Dubois, P., and Warnau, M. 2001. Effects of PCB's on reactive oxygen species (ROS) production by the immune cells of *Paracentrotus lividus* (Echinodermata). Marine Pollution Bulletin, 42: 667–672.

Coteur, G., Gosselin, P., Wantier, P., Chambost-Manciet, Y., Danis, B., Pernet, P., Warnau, M., *et al.* 2003. Echinoderms as bio-indicators, bioassays and impact assessment tools of sediment-associated metals and PCBs in the North Sea. Archives of Environmental Contamination and Toxicology, 45: 190–202.

Delmas, P., and Régis, M. B. 1984. Influence d'une pollution complexe à dominante domestique sur les populations de l'échinoïde comestible *Paracentrotus lividus* (Lamarck.). Vie Marine, 6: 63–72.

Dieuzeide, R. 1933. Les Echinoides réguliers de la baie de Castiglione. Thèse de Doctorat d'Etat, Biologie des populations et Ecotoxicologie, Université d'Aix-Marseille II, France. 205 pp.

EEA. 1999. Environmental state and threats. *In* State and Pressures of the Marine and Coastal Mediterranean Environment, pp. 76–104. UNEP. 137 pp.

Guettaf, M., Gustavo, A., San Martin, G. H., and Francour, P. 2000. Interpopulation variability of the reproductive cycle of *Paracentrotus lividus* (Echinodermata: Echinoidea) in the south-western Mediterranean. Journal of the Marine Biological Association of the UK, 80: 899–907.

Guillou, M., Quiniou, F., Huart, B., and Pagano, G. 2000. Comparison of embryonic development and metal contamination in several populations of the sea urchin *Sphaerechinus granularis* (Larmarck) exposed to anthropogenic pollution. Archives of Environmental Contamination and Toxicology, 39: 337–344.

Hagström, B. E., and Lönning, S. 1973. The sea urchin egg as testing object in toxicology. Acta Pharmacologica and Toxicologica, 13: 7–49.

Hambridge, K. M., Casey, C. E., and Krebs, N. F. 1986. Zinc. *In* Trace Elements in Human and Animal Nutrition. Volume 2, 5th edn, pp. 1–137. Academic Press, Orlando, FL. 480 pp.

Harmelin, J. G., Bouchon, C., and Hong, J. S. 1981. Impact de la pollution sur la distribution des échinodermes des substrats durs en Provence (Méditerranée Nord-Occidentale). Téthys, 10: 13–36.

Kobayashi, N. 1991. Marine pollution bioassay by using sea urchin eggs in the Tanabe Bay, Wakayama Prefecture, Japan, 1970–1987. Marine Pollution Bulletin, 23: 709–713.

Larid, M. 2003. Analyse de la durabilité dans le cadre du PAC "Zone côtière algéroise" (Algérie). Rapport de la première étape. 36 pp.

Lozano, J., Galera, J., Lopez, S., Turon, X., Palacin, C., and Morera, G. 1995. Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. Marine Ecology Progress Series, 122: 179–191.

Menchi, V., Balocchi, C., Pozo, K., Perra, G., Graziozi, M., and Focardi, S. 2002. Distribution of PCBs, PAHs and heavy metals in bottom sediments of the eastern Mediterranean Sea. *In* Challenges in Environmental Risk Assessment and Modelling, Proceedings of the SETAC, Europe, 12th Annual Meeting, Vienna, Austria, p. 147. SETAC Europe, Brussels.

PAC (Rapport d'Aménagement Côtier). 2005. "Zone côtière algéroise". Protection des sites sensibles naturels marins du secteur Cap Djinet au Mont Chenoua. Impact des activités anthropiques. Ministère de l'Aménagement du Territoire et de l'Environnement. République Algérienne Démocratique et Populaire. 88 pp.

Pagano, G., Cipollaro, M., Corsale, G., Esposito, E., Ragucci, E., Giordano, G. G., and Trieff, N. M. 1986. The sea urchin: bioassay for the assessment of damage from environmental contaminants. *In* Community Toxicity Testing, pp. 66–92. Ed. by J. J. R. Cairns. American Society for Testing and Materials, Philadelphia. 345 pp.

Pancucci, M. A., Panayotidis, P., and Zenetos, A. 1993. Morphological changes in sea urchin populations as a response to environmental stress. *In* Quantified Phenotypic Responses in Morphology and Physiology, pp. 247–257. Ed. by J. C. Aldrich. JAPAGA, Ashford, Ireland. 323 pp.

Saad, M. A., El-Rayis, O. A., and El-Nady, F. E. 1981. Occurrence of organic matter and heavy metals in sediments from the Mediterranean. *In* Management of Industrial Wastewater in Developing Nations, pp. 127–139. Ed. by D. Stuckey, and A. D. Hamza. Pergamon Press, Oxford.

Semroud, R. 1993. Contribution à la connaissance de l'écosystème à *Posidonia oceanica* (L) Delile dans la région d'Alger (Algérie): étude de quelques compartiments. PhD thesis, Institut des Sciences de la Nature, Université des Sciences et de la Technologie Houari Boumediene, Algeria. 129 pp.

Semroud, R., and Kada, H. 1987. Contribution à l'étude de l'oursin *Paracentrotus lividus* (Larmarck) dans la région d'Alger (Algérie): indice de réplétion et indice gonadique. *In* Colloque International sur *Paracentrotus lividus* et les Oursins Comestibles, pp. 117–124. Ed. by C. F. Boudouresque. GIS Posidonie Publications, Marseille, France. 433 pp.

Storelli, M. M., Storelli, A., and Marcotrigiano, G. O. 2001. Heavy metals in the aquatic environment of the southern Adriatic Sea, Italy. Macroalgae, sediments and benthic species. Environment International, 26: 505–509.

Tomas, F., Romero, J., and Turon, X. 2004. Settlement and recruitment of the sea urchin *Paracentrotus lividus* in two contrasting habitats in the Mediterranean. Marine Ecology Progress Series, 282: 173–184.

Troadec, P. 1995. La qualité du milieu marin: la rade de Brest. Communauté urbaine de Brest, rapport interne. 165 pp.

van Hoogstraten, R. J., and Nolting, R. F. 1991. Trace and major elements in sediments and inporewaters from the North Western basin of the Mediterranean Sea. Netherlands Institute for Sea Research (NIOZ). Rapport 1991. 72 pp.

Warnau, M., Biondo, R., Temara, A., Bouquegneau, J. M., Jangoux, M., and Dubois, P. 1998. Distribution of heavy metal in the echinoid *Paracentrotus lividus* (Lmk) from the Mediterranean *Posidonia oceanica* ecosystem: seasonal and geographical variations. Journal of Sea Research, 39: 267–280.

Warnau, M., Iaccarino, M., De Biase, A., Temara, A., Jangoux, M., and Dubois, P. 1996. Spermiotoxicity and embryotoxicity of heavy metals in the echinoid *Paracentrotus lividus*. Environmental Toxicology and Chemistry, 15: 1931–1936.

Warnau, M., and Pagano, G. 1994. Developmental toxicity of PbC12 in the echinoid *Paracentrotus lividus* (Echinodermata). Bulletin of Environmental Contamination and Toxicology, 53: 434–441.

Yoshida, M., Moali, M., Houas, O., Lakhdari, M., Nechaoui, L., Guerrida, D., Chatal, A., *et al.* 2005. Environmental pollution in Oued El Harrach area, Algeria. A preliminary report on mercury and heavy metals contaminations. *In* Compte-Rendu du Séminaire "Pollution et Protection de l'Environnement en Algérie", pp. 19–37. ONEDD, JICA, Alger.

Zar, J. H. 1996. Biostatistical Analysis. Prentice-Hall, Upper Saddle River, NJ.

doi:10.1093/icesjms/fsm183