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Assessment of sediment/water contamination by in vivo transplantation of the cockles *Cerastoderma glaucum* from a non contaminated to a contaminated area by cadmium

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Abstract In this study the cockle *Cerastoderma glaucum*, a filter-feeding bivalve living in the upper layer of sediment was used to investigate the cadmium contamination at a heavily urbanised and industrial area, with a view to using them as an indicator of water/sediment contamination. To this end, cockles collected from indigenous population in a relatively uncontaminated site (Ras Ungha) were in vivo transplanted into sediment and water removed from cadmium contaminated site (El Hofra) for 45 days. The manipulative experiment was undertaken in order to examine the trace metal bioavailability in the contaminated area and to establish an analytical framework between the bioaccumulation of cadmium in the tissues and their biological effect in transplanted cockles. For this purpose, a range of sublethal stress biomarkers were selected on the basis of their potential to provide relevant information. Cadmium concentrations were determined in the sediment and in the soft tissue of the cockles from the two studied stations at time 0. Compared to the reference site, cadmium concentrations in the contaminated site were 53 higher in the sediment and 15 higher in the whole soft tissues. The variation of cadmium concentrations and biomarkers responses in transplanted cockles were determined as a function of exposure time. After 45 days' experience, cadmium concentrations increased by a factor of 5 compared with time 0. No significant change could be detected in controls. In the digestive gland of exposed cockles cadmium was mainly associated with the cytosolic fraction. The significant increase of cadmium concentration in the soluble fraction was followed by a significant increase in the concentration of the Sulphydryl-Containing Heat Stable Compounds (SCHSC) including metallothionein like proteins by approximately 86%. This is led to investigation into possible existence of an induction of MT-like proteins in relation to cadmium accumulated by exposed cockles. Transplanted cockles were also subjected to some stress effect consisting in significant inhibition of cholinesterase activity by 26.5% when compared with control cockles. Increased levels of malondialdehydes (MDA) following cadmium exposure have been also reported, suggesting that exposed cockles have been submitted to an oxidative stress probably due to the presence of high cadmium contamination in the sediment. Principal component analyses showed that cockles in vivo transplanted into cadmium contaminated sediment and water were standing out progressively from the control group as a function of exposure time. A clear separation of the transplanted cockles from their controls was observed after 45 days' experience. But, the transplanted cockles appeared not having yet reached the same characteristics as the resident cockles from the contaminated site.

Keywords Acetylcholinesterase · Cadmium · *C. glaucum* · In vivo transplantation · Malondialdehyde · Metallothionein like proteins · Subcellular distribution · Sulphydryl-Containing Heat Stable Compounds (SCHSC)

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Introduction

Coastal area in the gulf of Gabès, serve as reproductive and nursery grounds for a number of economically important fish and shellfish species. This ecosystem is known to be

very rich in aquatic resources and contributes to about 65% of the national production in Tunisia (Hamza-Chaffai et al. 2003). Unfortunately, important industrial activities developed along this coastal area lead to the increasing of the contamination risk in the marine environment, and as a consequence to change in phytoplankton community composition and in the bottom, subtidal and epibentic habitats. The major cause of this change is thought to be metallic pollution and especially cadmium contamination. In fact the gulf of Gabès is characterized by an important cadmium contamination of the sediment, which originated from the crude phosphate treatment plants localised in this area (Illou 1999; Serbaji 1991, 2000; Abdellaoui and Jaballi 2005). Cadmium concentrations in bivalves collected from some localities along this area exceeded the consumption safety level currently authorized by OMS (Smaoui-Dammak et al. 2003; Machreki-Ajmi and Hamza-Chaffai 2006).

In this perspective, an active biomonitoring studies were performed in order to measure and compare the concentrations of pollutant in space and in time (Hamza-Chaffai 1993; El-Menif 1995; Machreki-Ajmi 2002; Smaoui-Damak et al. 2003). In situ and in vivo studies dealing with biomarkers concept relying on the interaction between a toxic metals and biological receptor in living organisms were performed to detect the effects of metal on organisms (Hamza-chaffai et al. 1995, 1997, 2000; Smaoui-Damak et al. 2004). Transplantation experiment from a non polluted to a contaminated area can be also a feasible strategy for monitoring the effect of environmental change in the coastal area (Amiard-Triquet et al. 1998; Geffard et al. 2001). Such approach provides direct information on the level of, or exposure to contaminant (Langston 1984; Riedel et al. 1995), and can also determine the sublethal effect of contaminant on marine organisms. The promise for transplanting organisms from "clean" environment to those containing contaminant, is that individuals have not previously had the opportunity to regulate their physiology in order to withstand contaminant stress. Consequently, we can estimate the initial stress response, which may be correlated with levels of bioaccumulated contaminants (Luca-Abbott 2001). In the present study, the suspension and deposit feeder bivalves *C. glaucum* collected from indigenous populations in a relatively uncontaminated area were in vivo transplanted into a heavily cadmium contaminated area in order to investigate the water/sediment pollution. Such transplantation experiment provides a combination of the experimental control of laboratory bioassays with the environmental realism of field monitoring.

Cerastoderma glaucum are a burrowing species living just under the sediment surface, and maintaining contact with water column via short siphon. Their ability to accumulate high concentration of trace metals makes them suitable for both field and laboratory experiments (Chabert 1984; Szefer and Szefer 1985; Szefer and Wolowicz 1993; Szefer et al. 1999). It has been recently used at various stations within the coastal area of the gulf of Gabès, this species showed measurable differences in body condition in broad to the level of site contamination (Machreki-Ajmi and Hamza-Chaffai 2006; Machreki-Ajmi et al. 2008). In this study, uptake rates of cadmium by transplanted *C. glaucum* were determined. The accumulation experiment was carried to examine the trace metal bioavailability in the contaminated area, and to establish an analytical framework between the bioaccumulation of cadmium in the tissues of organisms and their biological effects. The primary objectives of this study were to determine the effectiveness of using *C. glaucum* as an indicator of water/sediment contamination and to investigate the possibility of using in vivo transplanted species if organisms in the contaminated area are not be able to recover.

Materials and methods

Experimental designs

Specimens of *C. glaucum* were collected in November 2004 from indigenous population from a reference site (Ras Ungha (RU)). The cockles were calibrated according to the maximum antero-posterior length of their shell (29 mm ± 1.5). In the laboratory, cockles were kept in aerated sea water over a sediment layer collected from the same area. After acclimatizing period (3 days), cockles were assigned to polyethylene tanks (60 × 60 × 40 cm) containing a layer of sediment covered by aerated sea water; one group of animals (RU/EH) was incubated into water and sediment collected from the heavily contaminated site (EL Hofra) (transplanted animals). Another group of cockles (RU/RU) was incubated into sediment and water collected from their site of origin (Ras Ungha) (control animals). Water in each tank was changed twice a week. Seawater was carefully introduced, so as not disturb the sediment structure. The water column was permanently aerated by a diffuser connected to an air pump, and was controlled for temperature (17 ± 0.5°C) and salinity (36 ± 0.5‰). Experiments occurred under natural light condition and animals were fed with a solution of Pro-coral-phyton.

Cadmium and biochemical analyses for control and transplanted cockles were performed after 10, 20, 30 and 45 days. Cockles originating from the contaminated and uncontaminated sites were also analyzed at the time of sampling ($t = 0$) and cadmium concentrations in the sediment were determined at each site. At the time of

sampling, local water characteristics (temperature, pH, salinity) were similar in both contaminated and uncontaminated sites.

Sites descriptions

The sampling sites were chosen on the basis of their trace metal contaminants level, reflecting different anthropogenic impact. El Hofra (EH) site is affected by both urban and industrial pollution. Effluents include domestic sewage and industrial wastes from the surrounding areas were discharged into sea, without any preliminary treatment. Metal contamination is mainly discharged from crude phosphate treatment plant located in the studied area. Station Ras Ungha (RU) was located at 55 Km to the south of (EH) station. It is relatively far from known local source of industrial and urban pollution, and considered, as consequence, a non contaminated site.

Cadmium analysis in the sediment

Surface sediments were collected in the same time with the biological samples from the contaminated and an uncontaminated site. Sediments were collected manually and to a depth no greater than 3 cm as cockles live in the superficial sediment. Sediment samples ($n = 5$) were dried at 50°C. After calcinations for 4 h at 1,000°C, sediment samples were mineralised in a mixture of HNO_3–HF–HCL (1:1:2) acids at 150°C. The resulting residue was diluted in deionised water and the metal analysis was carried out by atomic Absorption Spectrophotometry using graphite furnace instrumentation.

Internal quality controls were based on the analysis of Cd in standard reference materials (marine sediment, SD-MEDPOL-1/TM). Data obtained were 183 ± 5.2 μg g^{-1} compared with a certified value of 189 ± 2.3 μg g^{-1}.

Physiological status of control and transplanted animals

The condition index (CI) was measured on 15 animals for each sampling period for both control and transplanted cockles. It was calculated according the method of Amiard et al. (1998). (CI) values are expressed as the ratio between the fresh weight of soft tissues and the total weight, multiplied by 100.

Preparation of samples for chemical and biochemical analyses

At each sampling time, 15 specimens were used for cadmium analysis in the whole soft tissues, and 15 others for biochemical analysis. For in toto cadmium analysis, each individual was weighed [total mass (g) and wet weight soft tissues (g)] to determine its condition index. The soft tissues were then frozen until analysis. For biochemical analysis, each animal was measured (Shell length in mm) and weighed. Cockles were then rapidly dissected; gills and the digestive gland were dissected and weighed. The digestive gland of each animal was divided in two parts. One part was used for malondialdehyde quantification. The other part was analysed for SH-Heat Stable Compounds and sub-cellular partitioning. Acetylcholinesterase activity was determined in the gills.

Cadmium and sulphydryl-containing heat stable compounds (SCHSC) analyses

Compartmentalization

The tissues (digestive gland) were homogenized in ice-cold 50 mM Tris with 4.4 mM β-mercaptoethanol buffer adjusted to pH 8.6, the ratio of Tris solution to the fresh tissue mass was 4 ml/g. The homogenates were then submitted to a 10-min sonication in order to break the cell membrane and release the remaining metals. The soluble (S1) and insoluble (P1) fractions were separated by centrifugation (25,000 × g, 60 min, at 4°C). The soluble heat-stable compound including MTLPs was isolated by centrifugation of the S1 fraction (20,000 × g, 30 min, at 4°C) after heat treatment (80°C for 15 min).

Sulphydryl-containing heat stable compounds (SCHSC) analysis

SH-Containing Heat Stable Compounds (SCHSC) concentrations were measured by spectrophotometric determination of S-H groups using Ellman's reagent, according to the procedure of Viarengo et al. (1997). The heat stable fractions (the supernatant of the second centrifugation) of digestive gland were added to 4.2 ml of 0.43 mM DTNB in 0.2 M phosphate buffer. The concentration of reduced sulfhydryls was evaluated by reading the absorbance at 412 nm by using glutathione (GSH) as a reference standard. The results were expressed as concentration of SCHSC (mg/g of fresh tissue).

Cadmium analysis

Cadmium analysis was carried out in the whole soft tissue (in toto) and in the soluble (S1) and insoluble (P1) fractions of the digestive gland. The determination of trace element was performed with extreme caution to avoid secondary contamination. All labware was soaked in 10% hydrochloric acid, rinsed three times with deionised water and dried in a desiccator sheltered from atmospheric dust. The soluble (S1) and insoluble fraction (P1) were digested with

suprapure nitric acid at 80°C for 7–8 h (1 ml nitric acid per 0.5 ml cytosol and 1 ml nitric acid per 0.5 g pellet). For cadmium analysis in toto, the soft tissues of each specimens were oven-dried (80°C) to constant weight. The samples were then digested with concentrated nitric acid (2 ml per 0.1 g of dry tissue). After digestion, cadmium levels in the acid solutions were determined, after dilution with deionised water, by flameless atomic absorption spectrophotometry using the Zeeman correction. Internal quality controls were based on the analysis of Cd in standard reference materials (mussel tissue, SRM-2976) from the National Institute of standard and technology. Data obtained were 0.75 ± 0.12 μg g^{-1} compared with a certified value of 0.82 ± 0.16 μg g^{-1}.

Malondialdehyde analysis

Lipid peroxidation was evaluated by measurement of malondialdehyde (MDA) concentration according to the colorimetric method of Sunderman et al. (1985), which is based on the reaction of thiobarbituric acid with MDA. Manual homogenisation was performed with 150 mM KCl at 4°C with a dilution ratio of 1:10 (wet weight tissue: volume of buffer) for the digestive gland. Malondialdehyde levels were estimated at 532 nm using 1,1,3,3-tetraethoxypropane as a standard. The concentration of lipid peroxidation in the digestive gland is expressed as μM of MDA per gram of fresh tissues.

Acetylcholinesterase analysis

For acetylcholinesterase measurements, the tissues (gills) were homogenized in phosphate buffer (0.1 M, pH = 7.4) at a ratio of 3 ml of buffer for 1 g of tissues. The homogenate obtained was then centrifuged at 9,000 *g* for 20 min at −4°C. An aliquot of the supernatant was used for measuring AChE according to Ellman method (Ellman et al. 1961), adapted to the microplate reader by Bocquené and Galgani (1998). The extracts were incubated in the presence of acetylthiocholine iodide as substrate and 5.5′-dithiobis-2-dinitrobenzoic acid (DTNB). The reaction was carried out at 25°C and the absorption was measured by a spectrophotometer at 412 nm. The enzymatic reaction rate was quantified against a blank without substrate for each activity measurement. A second blank was performed without sample to substract the spontaneous hydrolysis of the substrate. AChE activity is expressed as nmol of the product developed per minute and per mg of proteins. The quantity of protein present in the homogenate was determined according to the methods of Bradford (1976) at 595 nm, using bovine serum albumin (BSA) as a reference standard.

Statistical analysis

One way ANOVA was used to reveal the effect of the time factor for all the statistical results a probability of $P < 0.05$ was considered significant. Statistical analyses were carried out using the SPSS software. Normality and homogeneity of variances were verified and LN transformation where determined when it is necessary. Significant differences were located using a Tukey multiple comparison test for a normal distribution.

Principal compound analysis (PCA) was used to discriminate the transplanted organisms from the control ones; six variables were taken into consideration: Cd (S1), Cd (P1), SCHSC and MDA concentrations, AChE activity as well as the condition index of cockles.

Results

Chemical and biological characteristics of studied sites

Cadmium concentrations in sediments

Cadmium analyses in the sediment showed that cadmium in El Hofra sediments (3.046 ± 0,509 μg/g dry wt) were 53 higher than those of Ras Ungha (0.057 ± 0,004 μg/g dry wt). Cadmium levels in the sediment from the contaminated site (El Hofra) were higher compared the natural concentration in uncontaminated sediment (0.2 μg/g) (Boutier et al. 1991) and exceeds the maximum permitted levels (3 μg g^{-1}) (Laurent et al. 1977).

Cadmium concentrations and biomarker levels in C. glaucum

Significant differences in condition index, cadmium concentrations and in each biomarker (Tukey, $P < 0.05$) were observed between the two studied sites. The in toto concentrations of cadmium in *C. glaucum* from El Hofra (2.78 μg g^{-1} dry wt) were 15-fold higher than those of Ras Ungha (0.18 μg g^{-1} dry wt) (Tukey, $P < 0.05$). In agreement with cadmium variation, the biomarkers level varied significantly between the two studied sites (Table 1). In the cockle from the contaminated site cholinesterase activities (AChE) and the condition index (CI) were significant lower and the MDA levels were significant higher compared to those from the reference site (Ras Ungha). For The Sulphydryl-Containing Heat Stable Compounds, higher concentrations were observed in the contaminated cockles. Difference between contaminated and uncontaminated site was most pronounced for SCHSC and AChE (2.5-fold and 2.1-folde higher, respectively) and least obvious for MDA (1.5-fold higher).

Table 1 Chemical (Cd), biochemical (SCHSC, MDA, AChE) and physiological (CI) characteristics of cockles *C. glaucum* originating from the two studied sites: contaminated site (El Hofra) and uncontaminated site (RU)

	(Cd) in toto µg/g dry wt	(Cd) DG µg/g wet wt	(SCHSC) (mg/g) wet wt	MDA (µmol/g) wet wt	AchE (nmol/ min/mg Prot)	CI (%)
El Hofra (EH)	2.78 ± 0.4	1.06 ± 0.07	1.22 ± 0.09	158.66 ± 19.5	3.34 ± 0,64	13.06 ± 0.69
Ras Ungha (RU)	0.181 ± 0.08	0.112 ± 0.03	0.48 ± 0.05	95.88 ± 8	7.15 ± 0,78	20.22 ± 1.5

Cadmium and biomarkers variation during the in vivo transplantation experiment

Condition index of cockles

The condition index (CI) values of the control and transplanted cockles are shown in Fig. 1. The condition index of transplanted cockles showed a tendency to be lower than those of control cockles ($t = 0$ day) but did not vary significantly (Tukey, $P < 0.05$). In the control cockles, the condition index did not vary significantly as a function of time.

Cadmium accumulation

The accumulation of cadmium in the whole tissues of *C. glaucum* exposed to cadmium contaminated sediment and water for 45 days is shown in Fig. 2. Cadmium content in the control cockles did not change during the course of experiment and remained at a constant level. In the transplanted cockles, cadmium levels increased regularly with time of exposure (ANOVA, $P < 0.05$). Significant increase in cadmium concentration in the whole soft tissues was observed after 20 days (Tukey, $P < 0.05$), regular increase in cadmium level was after observed as a function of time. After 45 days, the concentration of cadmium at transplanted cockles (1.01 ± 0.12 µg g^{-1} dry wt) was 5 times higher than those in the tissue of initial cockles (0.18 ± 0.09 µg g^{-1} dry wt), but this concentration

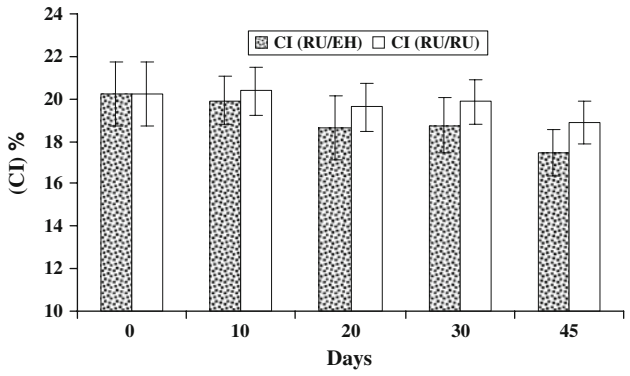

Fig. 1 Evolution of condition index (CI) (%) of control and transplanted cockles *C. glaucum* as a function of exposure time ($n = 15$)

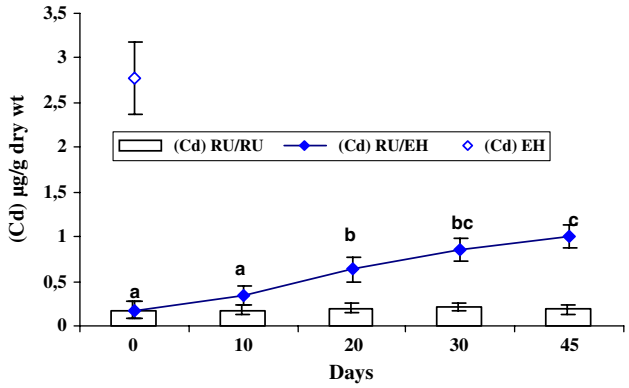

Fig. 2 Evolution of cadmium concentrations (µg/g dry wt) in the whole soft tissues in the control and transplanted cockles *C. glaucum* as a function of exposure time. The same superscripts indicate that is not differ significantly at 95% level (Tukey test), ($n = 15$)

remained lower compared to native specimens (cockles from the contaminated site) (2.78 ± 0.41 µg g^{-1} dry wt).

In the digestive gland of the transplanted cockles, cadmium concentration in both subcellular compounds of the digestive gland increased significantly as a function of time (ANOVA, $P < 0.05$). The partitioning of cadmium between soluble and insoluble fractions suggests change during experiments (Fig. 3A). At the beginning of bioaccumulation phase there is an equal distribution between S1 and P1 fractions. The cadmium associated to soluble fraction increased with exposure time, giving 68.5% of cytosolic cadmium at the end of exposure time.

Biomarker levels in transplanted and control cockles

The evolution of Sulphydryl-Containing Heat Stable Compounds (SCHSC) containing MT- like proteins at the digestive gland is represented in Fig. 3B. Mean SCHSC concentrations in transplanted cockles increased significantly during the exposure time (Tukey, $P < 0.05$), however no significant variation was detected in SCHSC levels in control cockles.

Compared with time zero, a significant increase in SH-Containing Heat Stable Compounds levels was observed after 20 days of exposure to cadmium contaminated environment (Tukey, $P < 0.05$). SCHSC content increased from 0.489 ± 0.057 mg g^{-1} ($t = 0$ days) to 0.74 ± 0.074 mg g^{-1} ($t = 20$ days) after that SCHSC concentrations

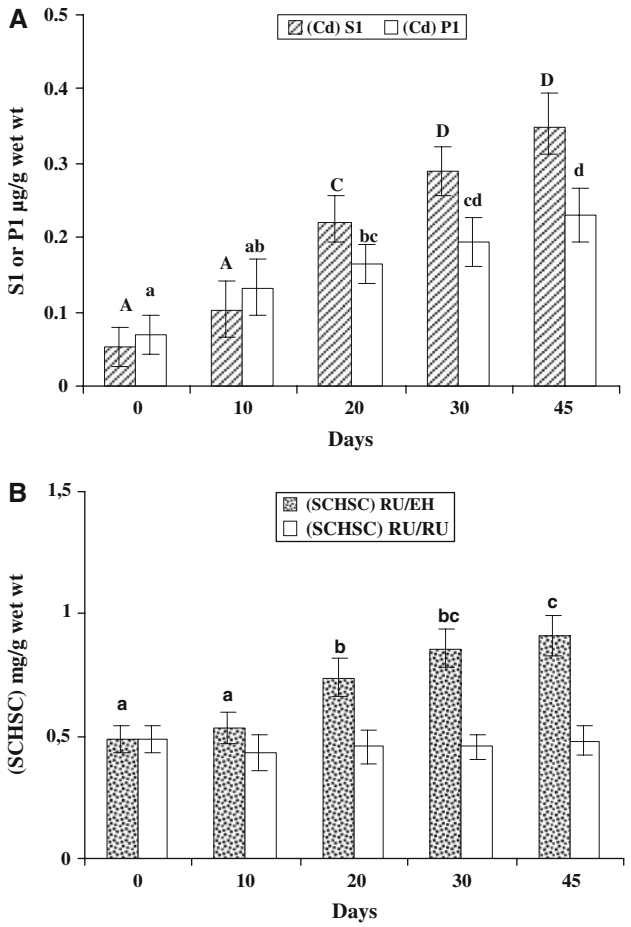

Fig. 3 Percentages of accumulated Cd in the soluble (S1) and insoluble (P1) fractions in the digestive gland of transplanted cockles (**A**) and (SCHSC) (mg/g wet wt) variation in the digestive gland of control and transplanted cockles (*C. glaucum*) (**B**) at different exposure time (confidence intervals at 95% level). The same superscripts indicate that is not differ significantly at 95% level (Tukey test), ($n = 15$)

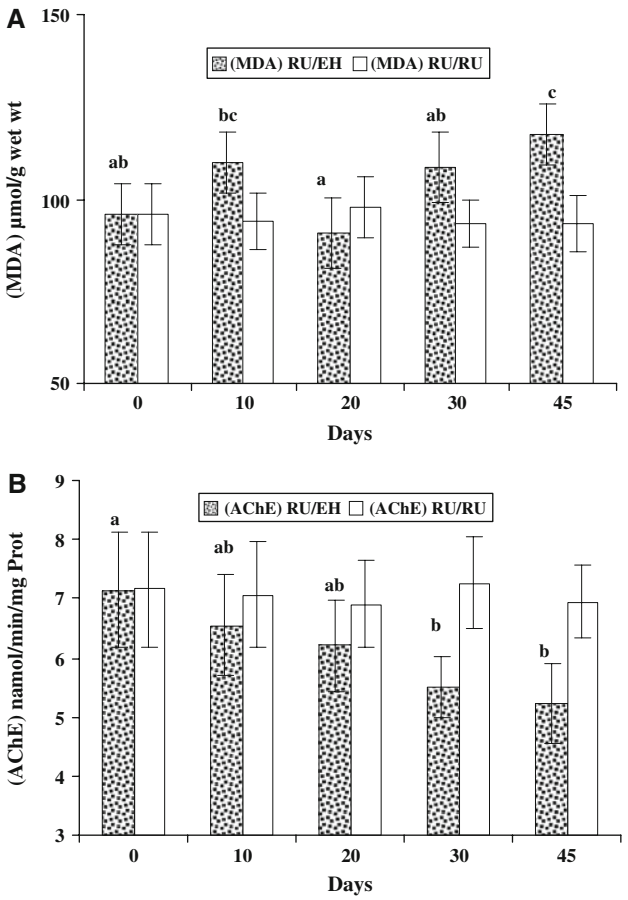

Fig. 4 Evolution of MDA (µmol/g wet wt) in the digestive gland (**A**) and AChE (nmol/min/mg Prot) in the gills (**B**) of control and transplanted cockles (*C. glaucum*) as a function of exposure time. The same superscripts indicate that is not differ significantly at 95% level (Tukey test), ($n = 15$)

increased slowly to reach 1.8-fold higher values by the end of the transplantation experiment.

The lipid oxidative damage was also studied (Fig. 4A). Malondialdehyde (MDA) levels have a tedency to fluctuate as a function of exposure time but no significant increase in the MDA levels was found during the first 30 days of transplantation. A significant increase in MDA levels was observed after 45 days. Malondialdehyde levels in control cockles remained unchanged along the transplant.

Acetylcholinesterase activity in transplanted and control cockles was recorded in Fig. 4B. Cholinesterase activity appeared to be decreased as compared to those recorded in control animals. A significant decrease in AChE activity was observed after 30 days of exposure time (Tukey, $P < 0.05$). Cholinesterase activity remained, then, constant between 30 and 45 days. During the exposure experiment, the mean activity of this enzyme was reduced by 26.5% when compared with control cockles.

Principal component analyses (PCA)

Principal component analyses (PCA) were performed to discriminate between the transplanted cockles (RU/EH), control cockles (RU/RU) and resident cockles from the contaminated site (EH). The parameters taken into consideration were: Cd (S1), Cd (P1), SCHSC and MDA concentrations measured in the digestive gland, AChE as well as the condition index. Correlations between variables and principal component are shown in Table 2, the first two axes represented 73.7% of the total variance. The horizontal axis PC1 represented 63.65% of total variation; it correlated with cadmium concentrations, SCHSC and MDA in the positive part and with the condition index (CI) and AChE in the negative part. The vertical axis PC2 represented 10.05% of total variation; it was associated with condition index in the positive part. The distribution diagram (Fig. 5) give the positions of the three studied groups of organisms in the co-ordinates of the two component axis PC1/PC2 provided in Table 2. The first

Table 2 PCA: Parameter variability resolved in two principal components

Experiments variables	Component 1	Component 2
Cd S1 (µg/g)	0.855	0.175
Cd P1 (µg/g)	0.811	0.313
SCHSC (mg/g)	0.892	0.222
MDA (µg/g)	0.751	−0.191
AChE (nmol/min/mg prot)	−0.754	0.002
IC (%)	−0.709	0.623
% of variance	63.65	10.05

component (PC1) evidenced two clusters; one in the left (RU/RU) and other in the right (EH). The control samples (RU/RU) scored negatively on the first axis (PC1) indicating lower cadmium concentration and higher (CI) and AChE activity. The samples from the contaminated site (EH) scored positively on the PC1 indicating high cadmium concentration and high values of MDA and SCHSC levels. Between these two clusters appeared the transplanted animals (RU/EH). The transplanted group interfered with the cluster (RU/RU) at time 10 and 20 days. For T30 and T45, points go toward the cluster (EH) (Fig. 5). After 45 days (T45) of exposure, transplanted cockles were nearer to contaminated (EH) than to uncontaminated group (RU/RU).

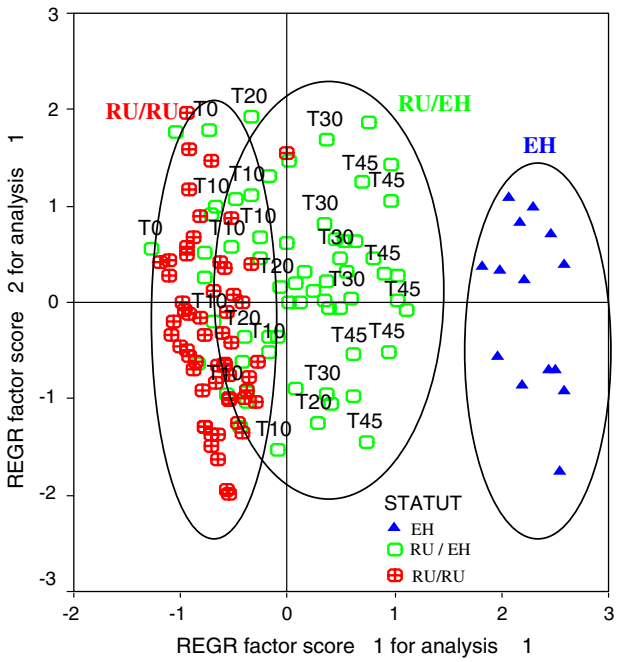

Fig. 5 The distribution diagram of the different groups of organisms ((RU/RU), (RU/EH), (EH)) as a function of the two principal component axis. Numerical codes represent time sample identification labels. Principal component loading and total variance associated with each axis are provided in Table 2

Discussion

In field condition animals are influenced by random factors even in transplantation experiments. The originality of this study is to stimulate field condition (water and sediment) without interfering with such factors. Our multimarker approach with in vivo transplanted cockles was performed to evaluate water and sediment quality in a cadmium contaminated area. For this purpose, animals were collected from a relatively non contaminated site (Ras Ungha) and were exposed, in laboratory, to cadmium contaminated sediment and water collected from a heavily cadmium contaminated site (EL Hofra).

The Chemical analyses highlighted significant difference between the two studied sites as to the degree of cadmium contamination of surface sediment. Compared to reference site cadmium levels observed in sediment from El Hofra displayed approximately 53 times higher. Cadmium levels observed in sediment and in organisms from the contaminated site may be essentially due to effluent from crude phosphate treatment plant located at proximity of this area. In agreement with metal contamination, biomarkers responses showed that El Hofra station compared to the reference site was under sever anthropogenic pressure.

Transplantation of *C. glaucum* from the uncontaminated site to cadmium contaminated sediment and water results in a significant increase of cadmium concentrations in the whole soft tissues. After 45 days, cadmium levels were 5 time higher in exposed cockles as compared to their controls. The present result lead to the conclusion that there are naturally elevated levels of bioavailable cadmium in the environment at the studied area and confirms the high potential of cadmium accumulation by *C. glaucum*.

Evolution of cadmium concentrations as a function of exposure time was also studied in the soluble (S1) and insoluble fractions (P1) of the digestive gland. Analysis of the proportional distribution of the total cadmium levels in this two fractions indicated that 68.5% of total accumulated cadmium is found in the cytosolic fraction (S1) containing MT-like proteins. Predominant accumulation of cadmium in cytosol fraction compared to the insoluble fraction is often interpreted as an indication of efficient detoxification of cadmium in aquatic organisms (Bebianno et al. 1993; Hamza-Chaffai et al. 2000, 2003). Most of the cytosolic cadmium in bivalves is typically bound to metallothioneins (Roesijadi 1996; Giguère et al. 2003; Hamza-Chaffai et al. 2003). In this study, the significant increase of cadmium concentrations in the soluble fraction was followed by a significant increase in the concentrations of the Sulphydryl-Containing Heat Stable Compounds (SCHSC) including metallothionein like proteins. The significant increase of SCHSC concentrations in the digestive gland of the

transplanted cockles compared with control animals could support the idea of induction of MT-like proteins in relation to cadmium accumulation. We can then hypothesis that metallothionein like proteins are involved in cadmium accumulation processes in transplanted *C. glaucum.* The importance of cadmium at the transcriptional level of MTs (mRNA) was recently evidenced in *C. glaucum* (Ladhaar-Chaabouni et al. (in press)), but no evidence that only MTs were produced in response to elevated tissue levels of cadmium. For this reason further studies remained necessary in order to investigate the behaviour of accumulated cadmium in *C. glaucum* and their distribution among cytosolic macromolecules.

The analysis of biomarkers of stress such as malondialdehyde (MDA) and cholinesterase activity (AChE) was also studied to assessing the possibility toxic impact of polluted sediment and water on transplanted organisms. The significant fluctuation of malondialdehyde concentrations as a function of exposure time implies that transplanted cockles have been exposed to an oxidative stress probably due to the presence of high cadmium contamination in the sediment. Several studies have shown a lipid peroxidation increase under cadmium contamination in molluscs (Geret et al. 2002; Company et al. 2004; Machreki-Ajmi et al. 2008). Company et al. (2004) showed that cadmium was the only metal leading to lipid peroxidation in the gill membrane of the mussels *Bathymodiolus azoricus*, and the inhibition of the most antioxidant enzyme appeared to be an effect of cadmium exposure.

Cholinesterase activity decreased significantly after 30 days of exposure. This is proving that transplanted organisms were exposed to some pollutants causing this inhibition. Inhibition of AChE by heavy metals was suggested in several studies (Dellali et al. 2001; Lionetto et al. 2003). In our study the cadmium quantity accumulated by transplanted organisms (1.01 μg g^{-1}) does not provide a valuable explanation for the depletion of cholinesterase activity. This would support the hypothesis that either pollutant other than cadmium is responsible for these toxic effects. The decrease in cholinesterase activity may be also related to general stress and decrease in condition index. However the condition index of the exposed cockles did not exhibit significant change with respect to controls. This was perhaps due to particular environment condition (food availability). Although the real meaning of the inhibition of AChE activity is not completely clear, the measure of this parameter remained useful because it is a marker of the early effect of a mixture of different chemical pollutant.

Chemical and biochemical parameters considering in the PCA analyses (Fig. 5) allowed a clear separation between control cockles (RU/RU) and cockles originating from the contaminated site (EH). For the transplanted cockles (RU/RU), we can be seen that after 20 days of transplantation, animals still had the same characteristics as their controls. Transplanted cockles were standing out progressively from the cluster of control group (RU/RU). A clear separation of the transplanted animals from their controls was observed after 45 days' experience, but the transplanted cockles appeared not having yet reached the same characteristics as the resident cockles from the contaminated site. An extended field study of long-term accumulation experiment should be carried out to better understand the regulation process of trace metal in *C. glaucum*.

As conclusion, in the course of this in vivo study some interesting variations of cadmium and biochemical responses were observed in the transplanted cockles from a relatively uncontaminated area into sediment and water removed from cadmium contaminated site. These responses suggested the potential application of biochemical response in *C. glaucum* as useful biomarkers and reinforce the validity of *C. glaucum* as a bioindicator organism useful for monitoring purposes that could be recommended in Tunisia national monitoring.

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References

Abdellaoui D, Jaballi Y (2005) Etude sédimentologique et géochimique des sédiments marins de la zone côtière de Sfax. Mémoire de projet de fin d'études en géologie d'environnement et d'aménagement. Faculté Des Sciences De Sfax

Amiard-Triquet C, Altmann S, Amiard JC, Ballan-Dufrançais C, Baumard P, Budzinski H et al (1998) Fate and effect of micropollutants in the Girond estuary, France: a multidisciplinary approach. Hydrobiologia 373/374:259–279. doi:10.1023/A:1017055118218

Bebianno MJ, Nott JA, Langston WS (1993) Cadmium metabolism in the clam *Ruditapes decussata*: the role of metallothioneins. Aquat Toxicol 27:315–334. doi:10.1016/0166-445X(93)90061-5

Bocquené G, Galgani F (1998) Biological effect of contaminants: cholinesterases inhibition by organophosphate and carbamate compounds. ICES Tech Mar Environ Sci 22:1–13

Boutier B, Alzieu C (1991) Les contaminants des sediments littoraux: les niveaux et leur signification. In: L'Ecotoxicologie des sédiments, SEFA, La Rochelle 5–7 Juin 1991, pp 38–51

Bradford M (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Company R, Serafim A, Bebianno MJ, Cosson R, Shillito B, Fiala-Médioni A (2004) Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel Bathymodiolus azoricus. Mar Environ Res 58:377–381. doi:10.1016/j.marenvres.2004.03.083

Chabert D (1984) Bioaccumulation du cadmium chez un mollusque bivalve: *Cerastoderma glaucum*, Poiret 1789, après contamination. Vie mar 6:57–61

Dellali M, Gnassia Barelli M, Romeo M, Aissa P (2001) The use of acetylcholinesterase activity in *Ruditapes decussatus* and *Mytilus galloprovincialis* in the biomonitoring of Bizerta logoon. Comp Biochem Physiol 130C:227–235

Ellman GL, Courtney KD, Andreas V, Featherstone RM (1961) A new and rapid colorimetric determination of AChE activity. Biochem Pharmacol 7:88–95. doi:10.1016/0006-2952(61)90145-9

El Mnif-Triqui N (1995) La palourde *Ruditapes decassatus* (Linnee, 1758) des côtes Tunisiennes. Biométrie, reproduction et impact de l'environnement sur la bioaccumulation de métaux traces. Thèse de troisième cycle. Université de Tunis II,Tunisie

Geffard A (2001) Réponse du biota à la contamination polymétallique d'un milieu estuarien, la Gironde, FR: Exposition, impregnation, induction d'une protéine de détoxication, la métallothionéines, impact au niveau individual et populationnel. Thèse de Doctorat, Université de Nante, France

Geret F, serafim A, Barreira L, Bebianno MJ (2002) Effect of cadmium on antioxidant enzyme and lipid peroxidation in the gills of the clam (*Ruditapes decassatus*). Biomarkers 7:242–256. doi:10.1080/13547500210125040

Giguère A, Couillard Y, Campbell PGC, Perceval O, Hare L, Pinel-Alloul B et al (2003) Steady-state distribution of metals among metallothionein and other cytosoic ligand and links to cytotoxicity in bivalves living along a polymetallic gradient. Aquat Toxicol 64:185–200. doi:10.1016/S0166-445X(03)00052-3

Hamza-Chaffai A (1993) Etude de la bioaccumylation métallique et métallothionéines chez des poissons de la côte de Sfax (Golfe de Gabès). Thèse de doctorat, University de Sfax, Tunisia

Hamza-Chaffai A, Cosson RP, Amiard-Triquet C, El Abed A (1995) Physico-chemical form of storage of metal (Cd, Cu and Zn) and metallothionein-like proteins in gills and liver of marine fish from the Tunisian cost: ecotoxicological consequences. Comp Biochem Physiol 111:329–341. doi:10.1016/0305-0491(94)00216-H

Hamza-Chaffai A, Amiard-Triquet C, El Abed A (1997) Metallothionein-Like protein: is it an efficient biomarker of metal contamination? A case study based on fish from the Tunisian coast. Arch Environ Contam Toxicol 33:53–62. doi:10.1007/s002449900223

Hamza-Chaffai A, Amiard JC, Pellerin J, Joux L, Berthet B (2000) The potential use of metallothionein in the clam (*Ruditapes decassatus*) as a biomarker of in situ metal exposure. Comp Biochem Physiol 127:185–197

Hamza-Chaffai A, Pellerin J, Amiard JC (2003) Health assessment of marine bivalve *Ruditapes decassatus* from the gulf of Gabès (Tunisia). Environ Int 28:609–617. doi:10.1016/S0160-4120(02)00102-2

Illou S (1999) Impact des rejets telluriques d'origines domestiques et industrielles sur les environnements côtières: cas du littoral Nord de la ville de Sfax (Tunisie). Doctorat de spécialité, Université de Tunis II, Tunisie

Machreki-Ajmi M (2002) Etude d'une contamination par le cadmium et le plomb chez le mollusque bivalve *(Cerastoderma glaucum)* issus de la région de Sfax: Etude in situ et in vivo. Diplôme d'études approfondies d'écologie générale. Faculté Des Sciences De Sfax

Machreki-Ajmi M, Hamza-Chaffai A (2006) Accumulation of cadmium and Lead in (*Cerastoderma glaucum*) originating from the Gulf of Gabès (Tunisia). Bull Environ Contam Toxicol 3:529–537. doi:10.1007/s00128-006-0952-8

Machreki-Ajmi M, Ketata I, Ladhar-Chaabouni R, Hamza-Chaffai A (2008) The effect of in situ cadmium contamination on some biomarkers in *Cerastoderma glaucum*. Ecotoxicology 17:1–11. doi:10.1007/s10646-007-0166-9

Ladhar-Chaabouni R, Mokdad-Gargouri R, Denis F, Hamza-Chaffai A (In press) Cloning and characterization of cDNA probes for the analysis of metallothionein gene expression in the Mediterranean bivalves: *Ruditapes decussatus* and *Cerastoderma glaucum.* Mol Biol Rep

Langston WJ (1984) Availability of arsenic to estuarine and marine organisms: a field and laboratory evaluation. Mar Biol 80:143–154

Laurent R, Fernex F, Baglinière C (1977) Contribution de la géologie sédimentaire à l'étude de la pollution du golfe de la Napoule. Rev Intern Océanogr Médic XL VII:97–100

Lionetto MG, Caricato R, Giordano ME, Pascariello MF, Marinosci L, Schettino T (2003) Integrated use of biomarkers (acetylcholinesterase and antioxidant enzyme activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. Mar Poll Bull 46:324–330

Luca-Abbott S (2001) Biomarkers of sublethal stress in the soft-sediment bivalve *Austrovenus stutchburyi* exposed in-situ to contaminated sediment in an urban New Zealand harbour. Mar Poll Bull 42:817–825

Riedel GF, Abbe GR, Sanders JG (1995) Silver and copper accumulation in two estuarine bivalves, the eastern oyster (*Crassostrea virginica*) and the hooked mussel (*Ischadium recurvum*) in the Patuxent river estuary, Maryland. Estuaries 18:445–455

Roesijadi G (1996) Metallothionein and its role in toxic metal regulation. Comp Biochem Physiol 113:117–123

Serbaji MM (1991) Contribution à l'étude de l'impact des rejets industriels et urbains sur l'environnement marin de la région de Sfax. Essais de traitement. Mémoire de DEA, Université Tunis II, Tunisie

Serbaji MM (2000) Utilisation d'un SIG multi-sources pour la compréhension et la gestion intégrée de l'écosystème côtier de la région de Sfax (Tunisie). Doctorat de spécialité, Université de Tunis II, Tunisie

Smaoui-Damak W, Hamza Chaffai A, Berthet B, Amiard JC (2003) Preliminary study of the clam *(Ruditapes decassatus)* exposed in situ to metal contamination and originating from the Gulf of Gabès, Tunisia. Bull Environ Contam Toxicol 71:961–970

Smaoui-Damak W, Hamza Chaffai A, Bebianno MJ, Amiard JC (2004) Variation of metallothioneins in gills of the clam *(Ruditapes decassatus)* from the Gulf of Gabès, (Tunisia). Comp Biochem Physiol 139:181–188

Sunderman FW, Marzouk A, Hopfer SM, Zaharia O, Reid MC (1985) Increased lipid peroxidation in tissues of nickel chloride-treated rat. Ann Clin Lab Sci 15:229–236

Szefer P, Szefer K (1985) Occurrence of ten metals in *(Mytilus edulis)* L. and *(Cardium glaucum)* from Gdansk Bay. Mar Poll Bull 16:446–450

Szefer P, Wolowcz M (1993) Occurrence of metal in the cockle (*Cerastoderma glaucum*) from different geographical regions in view of principal component analysis. Mar Poll 64:253–246

Szefer P, Wolowcz M, Kusak A, Deslous Poli JM, Czarmowski W, Frelek K, Berlzunce M (1999) Distribution of mercury and other trace metals in the cockle (*Cerastoderma glaucum*) from the Mediterranean Lagoon Etang de Thau. Arch Environ Contam Toxicol 36:56–63

Viarengo A, Ponzano E, Dondero F, Fabbari R (1997) A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic molluscs. Mar Environ Reas 44:69–84