

Experimental cadmium contamination of the echinoid *Paracentrotus lividus*: influence of exposure mode and distribution of the metal in the organism

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ABSTRACT: Echinoids *Paracentrotus lividus* (Lamarck, 1816) were contaminated with Cd (as CdCl₂) in field or laboratory conditions through 3 modes of exposure: (1) exposure via the sea water, (2) exposure via the food chain (*Posidonia oceanica* leaves), and (3) 'mixed-source' exposure via both sea water and food. Results indicate that Cd accumulation by *P. lividus* varies with both the body compartment of the echinoid and the exposure mode to the metal. The digestive wall shows the highest Cd uptake rates regardless of exposure mode. Bioaccumulation from sea water is prevalent in all compartments. Cd accumulation in the digestive wall and body wall of echinoids directly exposed to Cd in sea water is probably a dose-dependent process. Echinoids take up Cd with a lower efficiency (up to a 65% decrease) when they are contaminated via both sea water and food than when they are contaminated via sea water only. We suggest that this phenomenon is due to an increase in both excretion and mucus production (impeding Cd absorption from sea water) triggered by high Cd levels in the food. Cd loss rates are low in comparison with uptake rates and are dependent upon the body compartment and exposure mode. The data indicate that Cd has a relatively long biological half-life (≥ 15 d) once incorporated in echinoid tissues. It is concluded that *P. lividus* — particularly its digestive wall — could serve as a good bioindicator of Cd contamination in the environment.

KEY WORDS: Cadmium · Exposure mode · Field contamination · *Paracentrotus lividus*

INTRODUCTION

The usefulness of biological indicator organisms in monitoring heavy metal contamination in the marine environment has been supported by numerous studies (see e.g. Haug et al. 1974, Phillips 1976, Bouqueneau & Joiris 1988, Dinnel et al. 1988). In particular, bioindicator species allow the estimation of environmental contamination while avoiding time-consuming and easily contaminated sea water analyses. Furthermore, from both the ecological and health-safety point of view, bioindicators provide data that are generally more relevant to the bioavailable fraction of metal contaminants than those obtained from physico-chemical studies.

Organisms must fulfill several criteria to achieve the status of bioindicator species (Phillips 1976). The most

relevant of these characteristics is a simple relationship between contaminant levels in the environment and in the organism tissues (i.e. dose-dependent bio-concentration). Mussel species are generally considered good indicators of heavy metal pollution (see e.g. Fowler & Oregioni 1976, Phillips 1976, Borchardt et al. 1989). However, mussels are not distributed in all ecosystems, and so additional indicator species are needed in communities where mussels are absent. The echinoid *Paracentrotus lividus* could be a valuable candidate for bioindication of heavy metal contaminations in the Mediterranean Sea. Indeed, it is a well distributed and abundant species throughout the Mediterranean (Hayward & Ryland 1990) and previous studies have indicated that it effectively concentrates Hg, Cd, Pb, Zn, Cu, Ni, and Mn (Sheppard & Bellamy 1974, Shiber 1979, Augier et al. 1989). Other

studies have shown that echinoids (including *P. lividus*) efficiently accumulate V, Zn, Co, Mn, and Cs (Miramand et al. 1982, Nakamura et al. 1986). However, there are no data available on dose-bioconcentration responses for any metal, including those of most concern, viz. Hg and Cd.

The present field and laboratory work aimed to investigate the route of Cd uptake in *Paracentrotus lividus* contaminated via sea water, food, or both, and to provide a first assessment of the relationship between Cd levels occurring in the environment and in the body compartments of the echinoid.

MATERIALS AND METHODS

Samplings of and experiments on *Paracentrotus lividus* (Lamarck, 1816) were performed in the *Posidonia oceanica* meadow off Lacco Ameno (Ischia Island, Bay of Naples, Italy) between 15 March and 15 May 1993.

Three exposure modes of *Paracentrotus lividus* to Cd (as CdCl_2 ; Merck, synthesis quality) were investigated: (1) exposure via sea water, (2) 'mixed-source' exposure via both sea water and food (i.e. *Posidonia oceanica* leaves), and (3) exposure via food. Some of these experiments were followed by decontamination periods in uncontaminated conditions.

Contamination from sea water. To study contamination of echinoids via sea water we performed field experiments under Plexiglass containers (capacity: 30 l) at 6 m depth (Fig. 1). The containers were anchored in sediment areas devoid of *Posidonia oceanica* shoots. Water in the containers was renewed daily, as well as their Cd content (CdCl_2 was injected through a membrane on the top of the container; after Cd addition water was homogenized for 30 min with a temporarily incorporated submersible water pump). Three sets of echinoids ($n = 20, 30$, and 20 , respectively; ambital diameter 38.6 ± 3.7 mm, mean \pm SD) were exposed for 18 d to 3 different Cd levels in sea water (final calculated levels: 5, 20, and $50 \mu\text{g Cd l}^{-1}$). Samples of 3 individuals each were collected at different time intervals. Throughout the experiments, echinoids were fed ad libitum with fresh *P. oceanica* leaves that were renewed daily. (*P. oceanica* is the echinoid's main food source.) All *P. oceanica* leaves used in the present experiments bore epiphytes.

'Mixed-source' contamination. The mixed-source contamination experiment was also performed in the field, using a similar Plexiglass container. The container was anchored in the meadow, enclosing ca 30 *Posidonia oceanica* shoots. *P. oceanica* shoots within the container were contaminated for 5 d with $20 \mu\text{g Cd l}^{-1}$ prior to introduction of echinoids (30 echinoids;

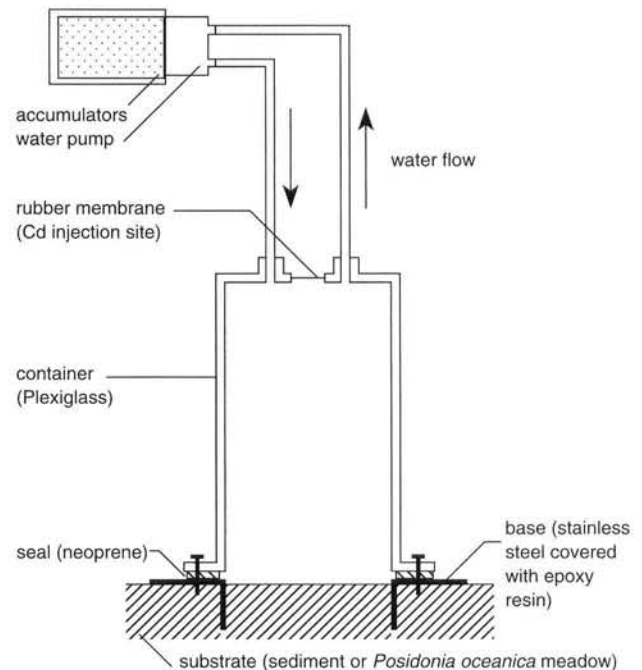


Fig. 1. Plexiglass container used for field Cd contaminations of *Paracentrotus lividus*

ambital diameter 38.4 ± 3.7 mm, mean \pm SD). Then, both the echinoids and the shoots were exposed to $20 \mu\text{g Cd l}^{-1}$ for 30 d. Throughout the contamination period, the water of the container was renewed daily, as well as its Cd content. Echinoids fed on the *P. oceanica* shoots enclosed in the container. Samples of 3 echinoids each were removed for analysis at different time intervals.

Contamination through food. One aquarium in the laboratory (capacity 45 l; open circuit constantly, aerated; water flux 20 l h^{-1}) was divided into 2 sections with a 1 cm mesh polyethylene net which allowed water to flow through both parts while echinoids were confined within their respective sections. One section constituted the experimental area while the other was a control area. The echinoids placed in the experimental area ($n = 40$; ambital diameter 38.9 ± 2.9 mm, mean \pm SD) were contaminated by ingestion of food for 30 d. Food consisted of *Posidonia oceanica* leaves previously contaminated for 5 d in aquarium by $20 \mu\text{g Cd l}^{-1}$. Contaminated leaves were replaced daily by freshly contaminated ones. Echinoids in the control area ($n = 15$; ambital diameter 35.3 ± 3.4 mm, mean \pm SD) were fed with noncontaminated leaves collected daily from the meadow. Samples of 3 echinoids each were collected at different time intervals from experimental and control areas.

Decontamination. Following the exposures to contaminated water ($20 \mu\text{g Cd l}^{-1}$), contaminated food, and

both contaminated sea water and food, the echinoids were placed in uncontaminated conditions for 10, 17, and 19 d, respectively. For the sea-water and mixed-source contamination experiment the Plexiglass containers were replaced by polyethylene meshed cages. For the food contamination experiment, the echinoids from the experimental area were fed with fresh *Posidonia oceanica* leaves collected daily from the meadow.

Controls. Parallel to each contamination experiment, echinoids were sampled in the field in order to assess fluctuations of Cd levels that might be independent of those in the experiments ('field controls'). In order to assess possible effects of the experimental device, 35 echinoids (ambital diameter 37.8 ± 3.5 mm, mean \pm SD) were placed for 18 d under a Plexiglass container and submitted to the same treatment as the experimental echinoids except for Cd exposure ('device controls'). These control echinoids were sampled at different time intervals (each sample consisted of 3 echinoids). In the food contamination experiment, echinoids were placed in the control aquarium area in order to assess possible contamination via Cd leached from contaminated leaves into the surrounding sea water ('leaching controls'). In addition, leaves were sampled before and after their use in the experimental area in order to quantify the release of Cd. Leaves provided as contaminated food in the food and mixed-source contamination experiments were regularly analyzed to monitor possible variations during the course of the experiments.

Sample preparation and metal analysis. Each sampled echinoid was dissected just after collection. The digestive wall (after removal of the gut contents), gonads, and body wall were separated, dried at 100°C for 48 h, and stored in clean, hermetically sealed polyethylene containers. Subsequently, to isolate the skeleton, a fraction of each body wall (ca 20%) was cleansed of non-calcified tissues using a 1‰ (w/v) proteinase N (Serva) solution according to the method of Dubois & Jangoux (1985). Ossicles were dried at 100°C for 24 h and stored in polyethylene containers. *Posidonia oceanica* shoots were also dissected in order to isolate the leaves (epiphytes were not removed from the leaves). The leaves were dried at 100°C for 48 h, and stored in polyethylene containers. The 4 echinoid body compartments and the *P. oceanica* leaves were digested with 65% HNO_3 (Merck, p.a. grade) (2 ml HNO_3 g⁻¹ dry wt of echinoid samples and 10 ml g⁻¹ dry wt of *P. oceanica* samples). These acid digestions were carried out successively at 20, 40, 60, and 80°C for 24, 24, 12, and 12 h, respectively. Digests were then diluted 12 times with milli-Q water (Millipore) and filtered on Whatman GF/A glass microfiber filters. The levels of Cd were measured by atomic emission spectrometry using a Jobin-Yvon 38+ ICPS.

Data analysis. Uptake and loss kinetics were fitted using simple linear regressions. Linearity was tested by the linearity test for regression with replication (Zar 1984). Significance of the slopes of the resulting regression equations was tested by 1-way analysis of variance (ANOVA). Comparisons between slopes of the regression equations were tested: (1) by the bilateral *t*-test (comparison between 2 regression slopes), for comparisons of experimental data with either field control or leaching control data; (2) by analysis of covariance (ANCOVA) followed by Tukey's multiple comparison test (comparison between more than 2 regression slopes), for comparisons between experimental data; (3) by ANCOVA followed by Dunnett's multiple comparison test, for comparisons between experimental data and the device control data (Zar 1984). Comparisons between the slopes of the regression equations of the different body compartments were performed by ANCOVA and Tukey's multiple comparison test after $\log(x+1)$ transformation of the data to fit the homoscedasticity requirement. Relevance of this transformation was tested by residual examination (Zar 1984). Comparisons of Cd levels between experimental and control treatments at the end of the contamination period were performed by ANOVA followed by Tukey's multiple comparison test (Zar 1984). Statistics were performed taking into account that numbers of compared data were unequal (Zar 1984). The level of significance was always set at $\alpha = 0.05$. The relationships between the final Cd levels in the different body compartments (direct contamination experiments) and the contaminating level in sea water were fitted by quadratic regression.

RESULTS

Cd uptake

Cd contamination of *Posidonia oceanica* leaves

For the food and mixed-source contamination experiments, *Posidonia oceanica* leaves were first contaminated in the laboratory and in the field, respectively, by exposure to CdCl_2 in sea water ($20 \mu\text{g Cd l}^{-1}$) for 5 d, as described in the 'Materials and methods'. Cd levels in the leaves were systematically measured at the end of these initial contaminations. Tests showed that Cd levels did not significantly differ ($p_{\text{ANOVA}} = 0.25$, $n = 30$) between leaves contaminated in the field ($45.7 \pm 13.7 \mu\text{g g}^{-1}$ dry wt, mean \pm SD) and those contaminated in the laboratory ($57.5 \pm 13.5 \mu\text{g g}^{-1}$ dry wt). Furthermore, Cd levels did not significantly differ between the successive leaf batches used in the food contamination experiment ($p_{\text{ANOVA}} = 0.51$, $n = 15$).

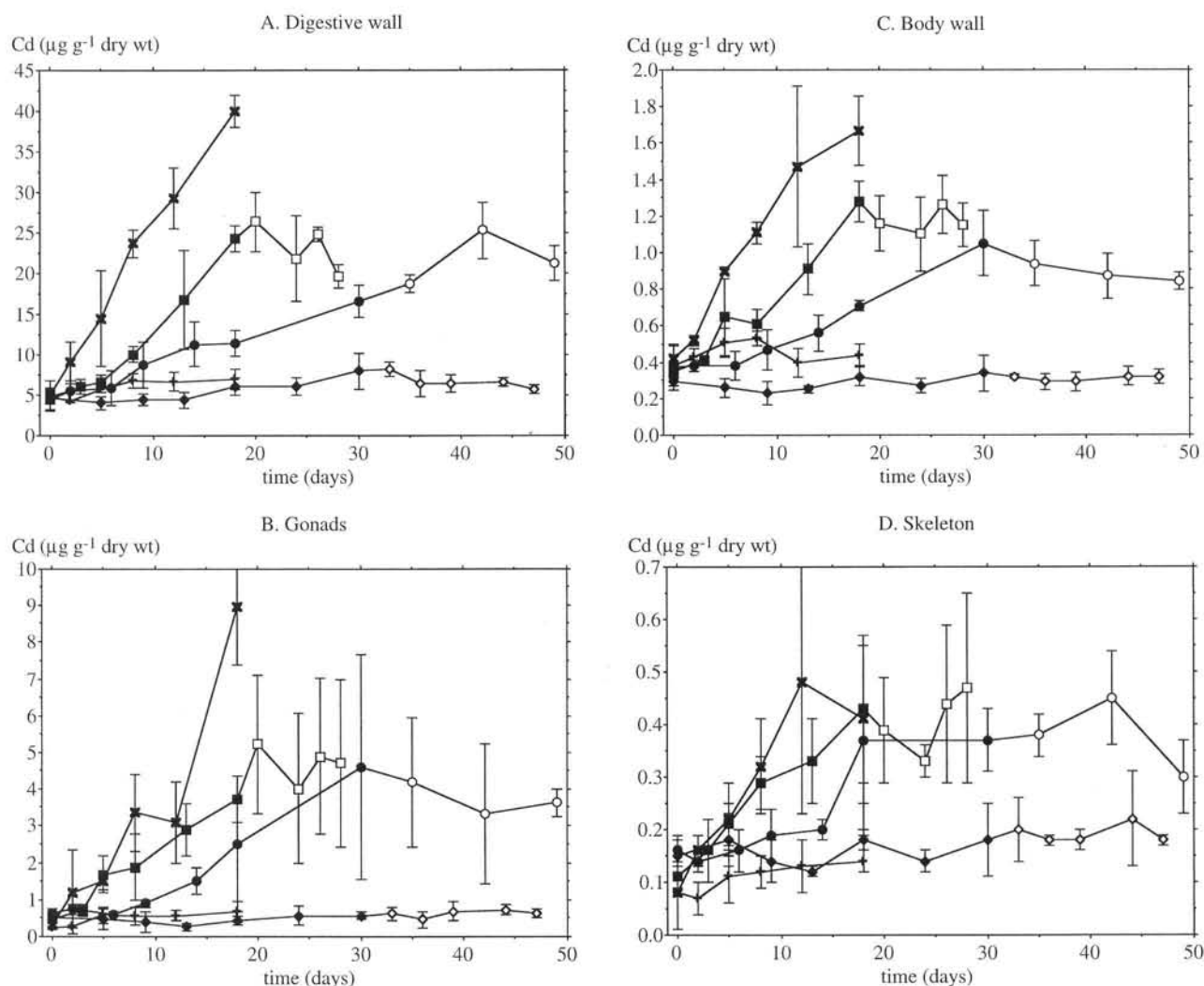


Fig. 2. *Paracentrotus lividus*. Uptake and loss kinetics of Cd (means \pm SD, $n = 3$) in 4 body compartments (digestive wall, gonads, body wall, and skeleton) of echinoids exposed to Cd through different contamination modes: via sea water with 5 (+), 20 (■, □) and 50 (×) $\mu\text{g Cd l}^{-1}$; via both sea water and food (●, ○); and via food (◆, ◇). Crosses and solid symbols: uptake kinetics; open symbols: loss kinetics

Once the echinoid contamination began in the mixed-source contamination experiment, previously contaminated *Posidonia oceanica* leaves were further submitted to dissolved Cd during the period of echinoid contamination (30 d). Cd levels in these leaves did not increase significantly above the level reached during the initial 5 d contamination ($p_{\text{ANOVA}} = 0.35$, $n = 15$). Contaminated *P. oceanica* leaves used in the food contamination experiment remained in the experimental aquarium for 24 h. The Cd level in these leaves did not significantly decrease during this period ($p_{\text{ANOVA}} = 0.99$, $n = 6$).

These results indicate (1) that food contaminating conditions may be considered to have been constant throughout both the food and the mixed-source con-

tamination experiments, and (2) that the results from the sea-water, food and mixed-source contamination experiments (at 20 $\mu\text{g Cd l}^{-1}$) may be validly compared.

Cd contamination of echinoids

The kinetics of Cd uptake were followed in the digestive wall, the gonads, the body wall, and the skeleton of echinoids exposed to Cd via the sea water, the food, or both (Fig. 2A to D). These kinetics are accurately described by linear regressions except in 2 cases, one of them being nonsignificant (Table 1).

Different controls were performed to test possible fluctuations of Cd levels in the field, possible effects of

Table 1. *Paracentrotus lividus*. Parameters of the simple linear regressions ($Y = a + bX$) describing Cd uptake kinetics in the body compartments of echinoids exposed to Cd (via sea water; both sea water and food; and food). Y: Cd level in $\mu\text{g g}^{-1}$ dry wt; X: exposure time in days; n: number of analyzed individuals; t: duration of the experiment (d); R^2 : determination coefficient; p: probability of the regression slope; p(linearity): probability of the linearity test performed on the simple linear regressions

	n	t	Regression parameters:					p(linearity)
			a	b	R^2	p		
Digestive wall								
Water								
5 $\mu\text{g Cd l}^{-1}$	18	18	4.756	0.146 ^a	0.50	0.001		0.21
20 $\mu\text{g Cd l}^{-1}$	18	18	2.571	1.130 ^b	0.88	≤ 0.0001		0.39
50 $\mu\text{g Cd l}^{-1}$	18	18	5.484	1.968 ^b	0.95	≤ 0.0001		0.69
Water and food	21	30	4.654	0.403 ^b	0.81	≤ 0.0001		0.86
Food	21	30	3.689	0.119 ^b	0.50	0.0004		0.51
Gonads								
Water								
5 $\mu\text{g Cd l}^{-1}$	18	18	0.305	0.023 ^c	0.41	0.0045		0.48
20 $\mu\text{g Cd l}^{-1}$	18	18	0.444	0.186 ^b	0.84	≤ 0.0001		0.79
50 $\mu\text{g Cd l}^{-1}$	18	18	-0.162	0.432 ^b	0.80	≤ 0.0001		0.02
Water and food	21	30	0.119	0.135 ^b	0.55	≤ 0.0001		0.86
Food	21	30	-	-	-	0.21		0.51
Body wall								
Water								
5 $\mu\text{g Cd l}^{-1}$	18	18	-	-	-	0.77		0.04
20 $\mu\text{g Cd l}^{-1}$	18	18	0.305	0.051 ^b	0.86	≤ 0.0001		0.24
50 $\mu\text{g Cd l}^{-1}$	18	18	0.469	0.073 ^b	0.85	≤ 0.0001		0.50
Water and food	21	30	0.297	0.023 ^b	0.83	≤ 0.0001		0.42
Food	21	30	-	-	-	0.33		0.45
Skeleton								
Water								
5 $\mu\text{g Cd l}^{-1}$	18	18	0.080	0.004 ^c	0.25	0.0349		0.92
20 $\mu\text{g Cd l}^{-1}$	18	18	0.120	0.017 ^b	0.75	≤ 0.0001		0.93
50 $\mu\text{g Cd l}^{-1}$	18	18	0.121	0.021 ^b	0.51	0.0008		0.49
Water and food	21	30	0.130	0.009 ^a	0.53	0.0002		0.37
Food	21	30	-	-	-	0.68		0.42

–: Slope not significantly different from 0 ($\alpha = 0.05$)
^aSlope not significantly different from that of field and/or device controls (ANCOVA) but end levels significantly different from both controls (ANOVA) ($\alpha = 0.05$)
^bSlope significantly different from that of both field control (bilateral *t*-test) and device control (Dunnett test) or, for food exposure, leaching control (bilateral *t*-test) ($\alpha = 0.05$)
^cSlope not significantly different from that of field and device controls ($\alpha = 0.05$)

the experimental device, or, during the food experiment, possible recycled contamination of the echinoids by Cd leached from the contaminated food. None of these controls revealed significant Cd uptake in any of the echinoid compartments (slopes of the regression lines not significantly different from 0, $\alpha = 0.05$; data not shown). Mean Cd levels in these different controls are given in Table 2.

Sea-water contaminations of *Paracentrotus lividus* by exposure to 20 and 50 $\mu\text{g Cd l}^{-1}$ revealed significant uptake kinetics in all compartments. These kinetics were always significantly different ($\alpha = 0.05$) from field and device controls. Contamination by 5 $\mu\text{g Cd l}^{-1}$ did not result in uptake kinetics significantly different from field

and device controls in any compartment. However, ANOVA showed that the Cd levels in the digestive wall following contamination via sea water were significantly different from the levels measured in echinoids at the beginning of the experiments and from those measured in the field and device controls at the end of the experiments ($p = 0.021$). Although a steady state was not reached in these experiments, final Cd levels measured in both the digestive wall and the body wall showed a highly significant and continuous increase with the contaminant level in sea water (Table 3).

The mixed-source contamination experiment (20 $\mu\text{g Cd l}^{-1}$ in sea water and $45.7 \pm 13.7 \mu\text{g g}^{-1}$ dry wt in *Posidonia oceanica* leaves) showed significant uptake

Table 2. *Paracentrotus lividus*. Cd levels ($\mu\text{g g}^{-1}$ dry wt; means \pm SD) in the body compartments of echinoids in the different controls. n: number of measurements

	n	Digestive wall	Gonads	Body wall	Skeleton
Field controls	39	4.65 \pm 0.95	0.45 \pm 0.18	0.34 \pm 0.07	0.13 \pm 0.04
Device controls	18	4.34 \pm 0.48	0.36 \pm 0.12	0.25 \pm 0.05	0.14 \pm 0.04
Leaching controls	15	3.68 \pm 1.21	0.57 \pm 0.36	0.27 \pm 0.06	0.14 \pm 0.06

Table 3. *Paracentrotus lividus*. Parameters of the quadratic regressions ($Y = a + bX + cX^2$) between Cd levels in the body compartments of echinoids (Y , $\mu\text{g g}^{-1}$ dry wt) after 18 d of contamination via sea water and contaminant levels in sea water (X , $\mu\text{g l}^{-1}$). Regressions were computed taking into account 4 contaminant levels (0, 5, 20, and 50 $\mu\text{g Cd l}^{-1}$). n: number of measurements; R^2 : determination coefficient; p: probability of the regression

	n	a	b	c	R^2	p
Digestive wall	12	2.822	1.237	-0.0098	0.99	≤ 0.0001
Gonads	12	0.264	-0.081	0.0004	0.58	0.020
Body wall	12	0.258	0.063	-0.0007	0.96	≤ 0.0001
Skeleton	12	0.138	0.018	-0.0002	0.57	0.023

kinetics in all compartments. These kinetics were significantly different from field and device controls ($\alpha = 0.05$) except for the skeleton, which was found to be not significantly different from the device control. However, in the latter case, Cd levels at the end of the exposure period were shown to be significantly different from those measured in the field and device controls ($p = 0.031$).

Contamination via the food ($57.5 \pm 13.5 \mu\text{g g}^{-1}$ dry wt of *Posidonia oceanica* leaves) resulted in significant uptake kinetics only in the digestive wall; these significantly differed from field and leaching controls ($\alpha = 0.05$).

Comparisons between slopes of significant Cd uptake kinetics according to exposure mode (exposure to $20 \mu\text{g Cd l}^{-1}$ via sea water, via the food, and via both food and sea water) show that the Cd uptake rate (i.e. slope of the regression lines) (1) was significantly lower in all compartments ($\alpha = 0.05$) during the mixed-source contamination experiment than when the uptake was only from sea water, and (2) was significantly lower ($\alpha = 0.05$) during the food contamination experiment than during the sea-water and mixed-source contamination experiments.

Cd uptake rates in the different body compartments were compared when they were significantly different from controls (i.e. for sea-water contaminations with 20 and $50 \mu\text{g Cd l}^{-1}$ and for mixed-source contamination). These comparisons show that the Cd uptake rate significantly differs from one compartment to another ($\alpha = 0.05$). The compartment ranking in order of decreasing Cd uptake rate was the same whatever the experiment, namely: digestive wall > gonads > body wall > skeleton (see Table 1).

Loss of Cd by echinoids

At the end of the contamination periods, echinoids submitted to each of the exposure modes were maintained in uncontaminated conditions to investigate the possible loss of Cd (Fig. 2A to D).

Most loss kinetics were not significant (slopes not significantly different from 0, $\alpha = 0.05$) during the periods considered (10 to 19 d, according to the experiment). In 2 cases, however, post-exposure loss of Cd was found to be significant, namely that in the body wall after the mixed-source contamination experiment [$Y = 1.337 - 0.011X$, $R^2 = 0.34$, $p = 0.046$, $n = 12$ (symbols as in Table 1); decontamination duration 19 d] and that in the digestive wall after the food contamination experiment ($Y = 11.72 - 0.126X$, $R^2 = 0.29$, $p = 0.020$, $n = 18$; decontamination duration 17 d). These equations indicate that the echinoids lost $0.011 \mu\text{g Cd g}^{-1}$ dry wt d^{-1} from their body wall after the mixed-source contamination experiment and $0.126 \mu\text{g Cd g}^{-1}$ dry wt d^{-1} from their digestive wall after the food contamination experiment. Comparing these data with those for Cd uptake rate indicates that the biological half-life of Cd averages 30 d in the body wall after 30 d mixed-source contamination, and 15 d in the digestive wall after 30 d of contamination through the food.

DISCUSSION

The present field study indicates that *Paracentrotus lividus* effectively accumulates Cd from its environment but that the accumulation process varies with

both the body compartment of the echinoid and the exposure mode to the metal.

The digestive wall and the gonads showed the highest Cd uptake rates regardless of exposure mode. This has already been reported in other marine invertebrates and vertebrates exposed to different metals and may be related to the high metabolic activity of these organs (Miramand et al. 1982, Nakamura et al. 1986, Bouqueneau & Joiris 1988, Sorensen 1991). The uptake kinetics observed in the echinoid body wall and skeleton indicate that these compartments also significantly bioaccumulate Cd, but at a much lower rate than the digestive wall (factor of 20 to 100 depending upon the exposure mode).

Cd appears to be much more efficiently accumulated from sea water than from food. The prevalence of this direct bioaccumulation has also been shown in *Paracentrotus lividus* for V, and in other echinoderm species (asteroids and holothuroids) for Cd, Pu, and V (Guary et al. 1982, Miramand et al. 1982, Besten et al. 1991). However, the relative prevalence of bioaccumulation from water and from food is metal dependent. For example, Nakamura et al. (1986) showed that Zn, Mn, Co, and, to a lesser extent, Cs are accumulated mainly from food by the echinoid *Strongylocentrotus nudus*.

Results of contaminations with 20 $\mu\text{g Cd l}^{-1}$ via sea water and via both food and sea water show that the uptake rate of Cd during mixed-source exposure is always significantly lower than the uptake rate from water. These results were confirmed by laboratory experiments using radioisotopic methods (in these experiments, the echinoids were exposed to the 2 modes of contamination in the same sea water) (Warnau & Fowler unpubl.). The echinoids thus take up Cd with a lower efficiency (25 to 65% less depending upon the body compartment) when contamination occurs through both sea water and food than when it occurs only from sea water. This was noted for all 4 investigated body compartments (including those that did not significantly accumulate Cd during exposure through food). Thus the presence of a high Cd level in the food of *Paracentrotus lividus* seemingly results in the reduction (up to 65%) of the net uptake of Cd from sea water. This process is probably induced in the digestive wall (no significant Cd accumulation through contaminated food was recorded in other compartments). It might consist either in a protective process which would decrease the metal bioavailability (e.g. via increasing mucus secretions) or in an excretory process which would increase metal elimination by the organism.

Experiments by Guary et al. (1982) and Nakamura et al. (1986) on 4 echinoderm species (including 1 echinoid) support the excretory hypothesis. These authors

pointed out that in each studied species, the loss rates of all the metals investigated were clearly higher in animals which had been contaminated through food than in those contaminated from sea water. Nakamura et al. (1986) reported that the metabolism of metals in these species is not dependent on their uptake pathway (i.e. sea water vs food). Consequently, the increased loss of metals absorbed through the food is not due to a higher susceptibility of these metals to elimination but to an actually increased excretion. As for the protective hypothesis, it has been demonstrated in various marine teleosts that their exposure to metals results in an increase of mucus secretion, which leads to a net decrease in accumulation of the contaminating metal (e.g. Bouqueneau et al. 1979, Radoux & Bouqueneau 1979, Lock & van Overbeeke 1981). This could also be the case in echinoderms, as mucus secretion is among the first responses of these animals to external stress factors (Nance & Braithwaite 1979, Lawrence 1987, Astley & Ratcliffe 1989). Thus both processes — protective and excretory — could be involved in the decrease of Cd uptake from water which is linked to the presence of high Cd levels in the food of *Paracentrotus lividus*.

The process responsible for the decrease of Cd uptake induced in the digestive wall has to 'propagate' towards the body wall. This propagation could be ensured by the echinoid nervous system. Indeed, in echinoderms, locally applied stimuli (e.g. photic, chemical or mechanical stimuli) induce stress responses at the systemic level through neurosecretory pathways (Moore & Cobb 1985a, b). Moreover, it has been shown that epithelial mucocytes in echinoderms are always intimately associated with the basi-epithelial nerve plexus (Buchanan 1963, Cobb 1987).

Loss of Cd was generally found to be nonsignificant, and quantification of loss rates was possible only in the digestive wall and body wall after food and mixed-source exposures, respectively. Loss rates were always low in comparison with uptake rates and varied depending upon the body compartment and exposure mode. Results also indicate that Cd has a relatively long biological half-life (≥ 15 d) once it is incorporated in the echinoid tissues.

Cd levels in the digestive wall and body wall of echinoids exposed to Cd via sea water have highly significant quadratic relationships with the contaminant levels. As steady states were not reached during these experiments, these relationships may not be considered as 'calibration curves' of the responses of these 2 body compartments to Cd exposure via sea water. However, this suggests that a simple relationship exists between the environmental Cd levels and those occurring in both the digestive wall and body wall of *Paracentrotus lividus*.

The sensitivity of the echinoid digestive wall to Cd contamination (whatever the exposure mode) indicates that this organ could rapidly reveal increases in environmental levels of this metal. (The digestive wall of *Paracentrotus lividus* could thus be used as a tool for assessing Cd pollution as prescribed by Council Decision 85/613/EEC of the European Union.) Furthermore, the results obtained to date indicate that Cd accumulation from sea water by this compartment is dose-dependent. Finally, the rather long biological half-life of Cd in the echinoid digestive wall makes this a recorder of Cd contamination on weekly or monthly time-scales. The present work thus supports the use of *P. lividus* — particularly its digestive wall — as a bioindicator of Cd contamination.

Acknowledgements. We thank Prof. S. W. Fowler (International Atomic Energy Agency, Monaco) for critical reading of the manuscript and valuable suggestions. We thank Prof. L. Mazzella (Stazione Zoologica di Napoli, Italy) for providing laboratory and diving facilities, and Prof. M. Hecq (Université de Mons-Hainaut, Belgium) for providing access to facilities at the ICPS. We gratefully thank Captain A. Rando for his skilled navigation assistance. This research was supported by an IRSIA grant to M. Warnau (ref. 910528) and by an ECC STEP Program (ref. STEP-0063-C). Ph. Dubois is a Research Associate of the National Fund for Scientific Research (Belgium). Contribution of the 'Centre Interuniversitaire de Biologie Marine' (CIBIM).

LITERATURE CITED

- Astley, M. R., Ratcliffe, N. A. (1989). Marine invertebrates mucus — agglutinating and antibacterial activity, with emphasis on *Metridium senile*. Symp. Soc. exp. Biol. 43: 367–377
- Augier, H., Ramonda, G., Rolland, J., Santimone, M. (1989). Teneurs en métaux lourds des oursins comestibles *Paracentrotus lividus* (Lamarck) prélevés dans quatre secteurs tests du littoral de Marseille (Méditerranée, France). Vie mar. 10: 226–239
- Besten, P. J. den, Bosma, P. T., Herwig, H. J., Zandee, D. I., Voogt, P. A. (1991). Effects of cadmium on metal composition and adenylate energy charge in the sea star *Asterias rubens* L. Archs environ. Contam. Toxicol. 21: 112–117
- Borchardt, T., Burchert, S., Karbe, L., Zeitniet, R. (1989). Enhanced heavy metal concentrations in *Mytilus edulis* from the central North Sea. Topics mar. Biol. 53: 725–728
- Bouqueneau, J. M., Joiris, C. (1988). The fate of stable pollutants — heavy metals and organochlorines — in marine organisms. In: Gilles, R. (ed.) Advances in comparative and environmental physiology, Vol. 2. Springer-Verlag, Berlin, p. 219–247
- Bouqueneau, J. M., Noel-Lambot, F., Distèche, A. (1979). Fate of heavy metals in experimental aquatic food chains: uptake and release of mercury and cadmium by some marine organisms; role of metallothioneins. ICES-CM 1979/E:58 (Mar. Environ. Qual. Comm.)
- Buchanan, J. B. (1963). Mucus secretion within the spines of ophiuroid echinoderms. Proc. Zool. Soc. Lond. 141: 251–259
- Cobb, J. L. S. (1987). Neurobiology of the Echinodermata. In: Ali, M. A. (ed.) Nervous systems in invertebrates. NATO ASI Series, Plenum Press, New York, p. 483–525
- Dinnel, P. A., Pagano, G., Oshida, P. S. (1988). A sea urchin test system for marine environmental monitoring. In: Burke, R. D., Mladenov, P. V., Lambert, P., Parsley, R. L. (eds.) Echinoderm biology. Balkema, Rotterdam, p. 611–619
- Dubois, P., Jangoux, M. (1985). The microstructure of the asteroid skeleton (*Asterias rubens*). In: Keegan, B. F., O'Connor, B. D. S. (eds.) Echinodermata. Balkema, Rotterdam, p. 507–512
- Fowler, S. W., Oregioni, B. (1976). Trace metals in mussels from the N.W. Mediterranean. Mar. Pollut. Bull. 7: 26–29
- Guary, J. C., Fowler, S. W., Beasley, T. M. (1982). Routes of plutonium uptake and their relation to biomagnification in starfish. Mar. Pollut. Bull. 13: 99–102
- Haug, A., Melsom, S., Omang, S. (1974). Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga *Ascophyllum nodosum*. Environ. Pollut. 7: 173–193
- Hayward, P. J., Ryland, J. S. (1990). The marine fauna of the British Isles and North-West Europe, Vol. 2. Clarendon Press, Oxford
- Lawrence, J. M. (1987). A functional biology of echinoderms. Croom Helm Ltd, London
- Lock, R. A. C., van Overbeeke, A. P. (1981). Effects of mercuric chloride and methyl mercuric chloride on mucus secretion in rainbow trout, *Salmo gairdneri* Richardson. Comp. Biochem. Physiol. 69C: 67–73
- Miramand, P., Fowler, S. W., Guary, J. C. (1982). Comparative study of vanadium biokinetics in three species of echinoderms. Mar. Biol. 67: 127–134
- Moore, A., Cobb, J. L. S. (1985a). Neurophysiological studies on photic responses in *Ophiura ophiura*. Comp. Biochem. Physiol. 80A: 11–16
- Moore, A., Cobb, J. L. S. (1985b). Neurophysiological studies on the detection of amino acids by *Ophiura ophiura*. Comp. Biochem. Physiol. 82A: 395–399
- Nakamura, R., Nakahara, M., Suzuki, Y., Ueda, T. (1986). Relative importance of food and water in the accumulation of radionuclides by sea urchin *Strongylocentrotus nudus*. Bull. Jap. Soc. scient. Fish. 52: 703–710
- Nance, J. M., Braithwaite, L. F. (1979). The function of mucous secretions in the cushion star *Pteraster tesselatus* Ives. J. exp. mar. Biol. Ecol. 40: 259–266
- Phillips, D. J. H. (1976). The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. 38: 59–69
- Radoux, D., Bouqueneau, J. M. (1979). The uptake of mercuric chloride from sea water by *Serranus cabrilla*. Bull. environ. Contam. Toxicol. 22: 771–778
- Sheppard, C. R. C., Bellamy, D. J. (1974). Pollution of the Mediterranean around Naples. Mar. Pollut. Bull. 5: 42–44
- Shiber, J. G. (1979). Heavy metal in Ras Beirut prawns and sea urchin eggs. J. environ. Sci. Health 14B: 73–95
- Sorensen, E. M. (1991). Metal poisoning in fish. CRC Press, Boca Raton, FL
- Zar, J. H. (1984). Biostatistical analysis, 2nd edn. Prentice-Hall, London