Heavy metals in the sea star *Asterias rubens* (echinodermata): basis for the construction of an efficient biomonitoring program

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Introduction

The heavy metals that enter marine trophic chains are naturally directed towards the benthos in a multiple-step sequence. Physico-chemical processes such as precipitation, adsorption, and complexation, incorporate them into the seston. Through grazing and predation, zooplankton organisms ingest the heavy metals present in the seston (Bremer *et al.*, 1990). Only a small proportion of these metals is absorbed by the zooplankton, the major part is eliminated with the fecal pellets. Being denser than seawater, the fecal pellets sink. A large proportion of these pellets is swallowed (up to 40%, Joiris *et al.*, 1982) with dead plankton by benthic (micro)heterotrophic organisms. Similarly, these benthic organisms absorb only a small fraction of the metals present in their diet. Their excrements thus have a high content heavy metals as well. Therefore, both abiotic and biotic processes direct metals towards the sediments. Heavy metals associated with the sediments are bioavailable to benthic organisms through exchanges between the water column and the interstitial water, ingestion and through sediment resuspension. As resuspension is stimulated by activity of the in fauna, by human activity (e.g. dredging), and by hydrodynamics, the sediments become a secondary source of contamination. Such a source can sometimes be detected much later than the primary contamination (Skei, 1981). Because of this secondary source of contamination, the benthic zone of littoral ecosystems is particularly exposed to metal contaminants. As an example, the loads of Cd, Pb, and Hg accumulated in the macrobenthos of the North Sea exceed 8 tons (T) Cd, 4T Pb, and 1T Hg, mainly from anthropogenic origin (Karbe *et al.,* 1994).

Heavy metal pollution is a major problem in several sites of the NE Atlantic, particularly in coastal zones and in zones of sedimentary deposits (NSTF, 1993). These zones are generally ecologically sensitive and are economically important because they include fisheries and recreational areas. According to Kröncke & Rachor (1992), the macrobenthic communities of the NE Atlantic are affected by the presence of multiple pollutants including heavy metals. The disrupted communities typically have larger numbers of species that are of smaller size and shorter lifespan than the healthy communities. According to NSTF, (1993), four metals constitute a major threat to the animal communities in the NE Atlantic: Sn (as tributyltin) affects bivalves and gastropods through endocrine disruption, Cd and Hg constitute a major threat to the top-predators, and Pb is a major threat to mollusc predators.

Bioindicators and complementarity

It is no longer accepted that marine systems can receive an unregulated load of heavy metals, and programs monitoring the health of ecosystems are required. It is generally recognised that biological parameters should be studied in such programs in conjunction with both chemical and physical measurements. Nowadays, ecotoxicological risk assessment has become a tool in several regions of the world and is used by decision-makers in chemical regulation. Environmental decision making can be a multi-million dollar/euros issue and efficient biomonitoring programs must therefore be based on carefully calibrated bioindicators.

Two types of organisms can be selected for biomonitoring programs. If the aim of the program is to protect human health, edible species should be considered. Alternatively, if the aim of the program is to determine temporal or geographical trends of a contamination, ubiquitous species should be considered. Mussels *Mytilus* spp have a double advantage from the standpoint of ecotoxicology: they are both edible and have a wide distribution. This is why they are so often used by ecotoxicologists. But mussels cannot be considered as the ultimate bioindicator because they are not ubiquitous [e.g.: limited bathymetric distribution, absence from some biotopes, such as seagrass meadows (Hayward & Ryland, 1990)], and because they do not always indicate environmental conditions. Indeed, according to Coleman *et al.* (1986), there is no simple relationship between the Cd concentrations in *Mytilus edulis* and the concentrations in the seawater. Similarly, Cd, Pb, Cu, and Zn concentrations in the mussels of Sørfjord (Norway) were not correlated to contamination gradients along the fjord (NSTF, 1993). If mussels are not the ideal bioindicator, it is likely that no organism is. Indeed, in each organism, the body concentrations indicate the specific local bioavailability of the heavy metals rather than the global environmental conditions. A sub-optimal bioindicator should not be replaced by another one. However, in Sørfjord, the asteroid *Asterias rubens* better indicated the environmental conditions than any other species (including *M. edulis*); the measured body concentrations being highly correlated to metal concentrations in the sediments, which represent the major source of contamination in the area (Temara *et al.*, 1998c). Even so, analyses of metal concentrations in mussels have been of importance to public health: the detection of highly elevated levels all along the fjord led the regional authorities to ban their consumption (Skei, 1995). According to Gray (1989), several species should thus be included in biomonitoring programs. Phillips (1990) reviewed the bioindicators widely used at the time of his writing; bioindicators were gathered in three groups: macrophyte algae, crustaceans, and molluscs. Each of these groups had both advantages and disadvantages.

To select an adequate taxon, it has been proposed to focus on the species that qualitatively or quantitatively structure the biocenosis (key-species) (Gray, 1989). This approach could also assist in assessing the impact of contamination on the whole ecosystem (if one or several key-species are affected, the whole ecosystem is affected). However, according to Hurlbert (1997), the concept of keystoneness has hardly been demonstrated for most organisms and in the ecotoxicological perspective, the concept of complementarity might be more appropriate as far as selection of bioindicators is concerned. To be complementary, species should be chosen among biologically distanced taxa. In this view, *M. edulis* and *A. rubens* are complementary. The first one is a filtrating protostome with an exoskeleton that keeps the other tissues out of contact with the sediments [mussels settle on hard bottoms as well as on soft bottoms as aggregates (Hayward & Ryland, 1990)]; the second one is a predatory deuterostome with an endoskeleton; the respiratory surfaces, the podia, are in close contact with the sediments. Contact with heavy metals, metal bioaccumulation, and sensitivity to metals will thus be dramatically different for these two species. The joint study of such species would thus be more representative of the biodiversity in marine ecosystems. Macrophyte algae would favourably complement these two species as these organisms mainly bioaccumulate metals from the dissolved phase (e.g. Phillips, 1990; Warnau *et al.*, 1996a).

The ecotoxicology of *M. edulis* has extensively been reviewed (see *e.g.* references above) and the present paper describes the ecotoxicological information available for *A. rubens*. It is a top-predator feeding mainly on molluscs and is regarded as a keystone predator in several communities (see hereafter). Therefore, the effects of Cd, Pb, and Hg on this species are likely to have an impact on whole communities (see above). The present paper reviews the modes of bioaccumulation and loss/detoxification of such metals in *A. rubens* in order to ascertain the value of the species as a bioindicator of metal contamination.

Asterias rubens, a key-species in NE Atlantic macrobenthic communities

Asterias rubens is euryhaline while most other echinoderms are generally stenohaline. Thus, it commonly settles in low salinity zones such as the Baltic Sea or estuaries. It is precisely such low salinity zones that are likely to be exposed to human activities and where the most dramatic heavy metal-related problems are found in the marine environment.

In some NE Atlantic littoral ecosystems, *A. rubens* can represent the most significant fraction (up to 40 %) of the mobile epifauna biomass (Hostens & Hammerlynk, 1994). Other species of the same genus (e.g. *A. amurensis*) that would presumably share some ecotoxicological characteristics together with *A. rubens*, are dominant benthic predators in the other oceans of the North hemisphere and are invasive organisms in the Southern Pacific (Byrne *et al.*, 1997). The position of the species in the benthic trophic webs of these ecosystems is strategic (Menge, 1982). *A. rubens* is a major predator (it feeds on bivalves, filter-feeders that are known to accumulate metals) and is also an opportunistic species. The ecological pressure due to the presence of the predator (in addition to the predation by itself) affects the fitness of its prey (Reimer *et al.*, 1995). Eventually, it is an important intermediate link in several trophic webs: (1) within its own community, through predation by other echinoderms (Menge, 1982); (2) to the endofauna, through *post-mortem* decomposition; (3) to other benthic and pelagic communities, through predation by flat fishes (Keats, 1990) and, due to fishery activities, indirectly to man; and (4) to the terrestrial communities, through predation by sea birds such as the laridae (sea gulls) or the eider *Somateria mollissima* (Bustnes & Erikstad, 1983).

All of this data indicates that predation by and on *A. rubens* is a major selective factor on benthic communities; this asteroid has therefore been identified as a key-species (Menge, 1982).

Heavy metal bioaccumulation in *A. rubens*

Baseline studies on heavy metal contamination of *A. rubens* have been conducted in the field by several authors (e.g. Everaarts & Fisher, 1989; Everaarts *et al.*, 1990; Vyncke *et al.*, 1991; Temara *et al.*, 1997a) and the asteroid is now recognised as one of the species that can bioindicate metal contamination of an ecosystem (NSTF, 1993).

A sampling of *A. rubens* from the coastline of the Netherlands to the Dogger Bank (central North Sea) showed that the populations in the Dogger Bank (a region with high sediment deposition rate, elevated organic content, and where metal bioavailability is elevated; Kersten & Kröncke, 1991) had significantly higher Cd concentrations than the other populations studied (Everaarts *et al.*, 1990), while Zn and Cu concentrations did not vary significantly along the

(1991), *A. rubens* would be the most suitable species to bioindicate heavy metal (Pb, Cr, Hg, Ni) contamination among the organisms studied (these organisms were the crustaceans *Pagurus berhnardus* and *Macropipus holsatus*, the ophiuroid *Ophiura texturata*, the bivalve *Spisula subtruncata*, and *A. rubens*). According to the latter authors, metal concentrations in asteroids collected close to the

The importance of the factors (body compartment, season, sampling site, sex) influencing heavy metal concentrations were investigated in adult asteroids in the southern bight of the North Sea. According to multi-way analyses of variance, the considered factors accounted for a significant proportion of total variability in Pb (93%), Cd (88%), and Hg (27%) concentrations. Body compartment appeared as the most critical factor (Pb: 88%, Cd: 40%, Hg: 10%) in background environments (Temara *et al.,* 1997a). Concentration ratios towards prey (invertebrates of various trophic categories) were lower than 1 for Pb, around 1 for Hg, and up to 7.8 for Cd, indicating that limited biomagnification may occur in the trophic web *A. rubens* belongs to (Temara *et al.,* 1997a). The same study showed that sex-related differences were significant for Cd concentrations (1.75 times higher in female than in male gonads). Significant allometric relationships were measured and statistically fitted models were positive (Cd concentrations in the body wall and the digestive system) or negative (Pb concentrations in the digestive system) power functions.

According to Temara *et al.* (1997a), Pb is particularly concentrated in the skeleton while its concentration in the other tissues is rather low in background environments. Pb is known as a calcic skeletal-seeking element [calcic skeletons represent the vast majority of types of mineralised skeletons in the metazoans], regardless of the nature of the calcic skeleton [e.g. phosphate skeletons of vertebrates, carbonate skeletons of molluscs]. Among the carbonate skeletons, the crystallographic structure seems surprisingly inconclusive for Pb bioaccumulation that occurs in aragonitic skeletons as well as in calcitic skeletons (Kröncke, 1987; Temara *et al.,* 1995; Warnau *et al.,* 1995a). According to Sorensen (1991), the accumulation of Pb in calcitic skeletons is facilitated by the similarity between the ionic radius of Pb and Ca.

In *A. rubens,* Cu, Zn, and Fe, which are cofactors of several enzymes, were preferentially concentrated in body compartments that are characterised by high metabolic activity. Concentrations were significantly higher in the pyloric caeca (Temara *et al.,* 1997a), nutrient storage organs that have high metabolic activity (Oudejans *et al.,* 1979). Cu and Zn, as well as other Ib and IIb elements (Cd, Hg, Ag), generally have a high affinity for the -SH peptidic groups of the tripeptide glutathione and of metallothioneins (MTs) (Roesijadi, 1992). In *A. rubens*, the quantification of MTs in the pyloric caeca (Temara *et al.*, 1997b) confirmed the presence of this ligand in sufficient quantity to fix accumulated Cd. In contrast, MTs are absent from the gonads (den Besten *et al.*, 1990), accounting for the low Cd concentrations in this compartment. However, Hg concentrations were high in the gonads (Temara *et al.*, 1997a). As opposed to the MTs of most other organisms studied so far, the MTs in the pyloric caeca of *A. rubens* showed a low affinity for Hg (Sorensen & Bjerregaard, 1991), which could account for its rapid transfer to the gonads. Such transfer could take place via the haemal system (Rouleau *et al.*, 1993) which is part of the circulatory system in echinoderms (Ruppert & Barnes, 1994).

Metal sources for the organism can vary according to the element and are debatable. According to Voogt *et al.* (1987), the principal source of Cd for *A. rubens* was the water. In contrast, den Besten *et al.* (1990) showed that Cd seemed to be accumulated by *A. rubens* mainly from its diet. According to experimental exposures (Temara *et al.*, 1996a), Cd accumulated in the pyloric caeca was mainly of dietary origin (Figure 1). However, the importance of diet as a source of Cd is apparently not a rule in echinoderms. Indeed, the contribution of diet to the Cd load of the echinoid *Paracentrotus lividus* (grazing sea urchin) is comparatively much lower (Warnau *et al.*, 1995b; 1996b).

The sources of Pb can be ions dissolved in seawater, Pb present in the diet, and Pb adsorbed on particules in suspension. Such particules enter the body *via* the microphagic activity of *A. rubens* (Jangoux, 1982). The relative importance of such sources has not yet been evaluated. In contrast to Cd that is taken up through the integument and the digestive tract (Temara *et al.*, 1996), recent studies (Temara *et al.*, 1998a) have shown that the main entry route of Pb in the organism would be the digestive wall (Figure 2), possibly through an antiporter system. Secondary entry routes could be the podia, the papulae, and the madreporite, viz. structures that control the equilibrium of internal fluids and that are involved in respiratory exchanges. The metal ions associated with dissolved organic matter could also enter the asteroid through the epidermis of the body wall, whose role in the absorption of dissolved organic molecules has been demonstrated by Fergusson (1982).

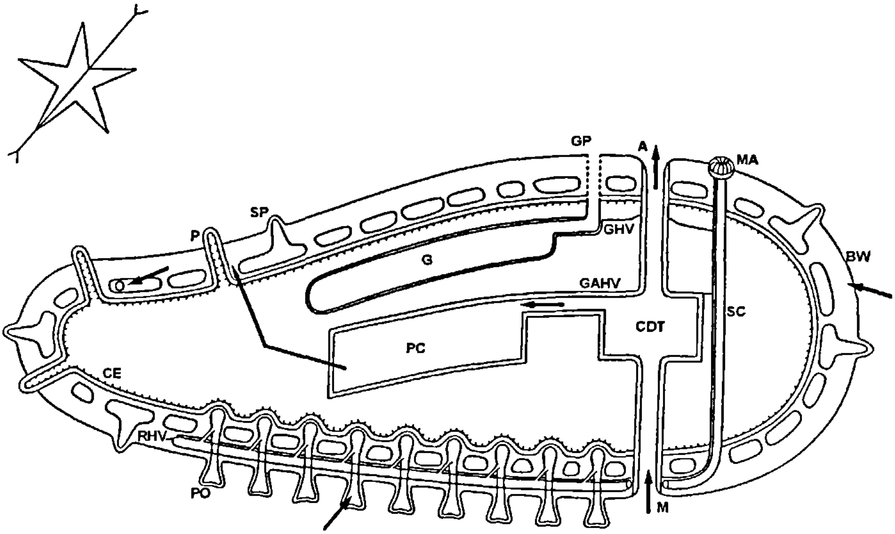


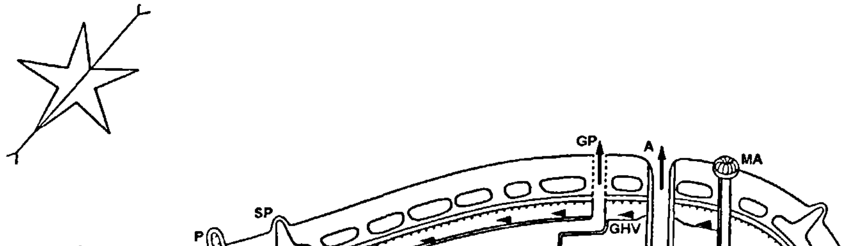
Figure 1
Sagital section through an asteroid arm showing known and supposed routes of in- and out-fluxes of Cd. Direction of section is shown on the small asteroid. A: anus. BW: body wall. CDT: central digestive tract. CE: coelomic epithelium. G: gonad. GAHV: gastric hemal vessel. GHV: genital hemal vessel. GP: gonopore. MA: madreporite. M: mouth. O: ossicle. P: papula. PC: pyloric caecum. PO: podion. RHV: radial hemal vessel. SC: stone canal. SP: spine.

Impact and detoxification of heavy metals in *Asterias rubens*

Preliminary studies on Mn assessed the survival of exposed asteroids. The lowest observed effective concentration (50 $mg.l^{-1}$.Mn) calculated in aquarium (Hansen & Bjerregaard, 1995) was well above the concentrations that can be observed in the most contaminated sites in the field (800 $\mu g.l^{-1}$.Mn in few heavily contaminated sites of the Baltic Sea; Kremling, 1983). Cu did affect the oxygen consumption in *A. rubens* at concentrations = 25 $\mu g.l^{-1}$ Cu (Gerets *et al.*, 1972). Cd disrupted steroid metabolism (Voogt *et al.*, 1987),

= 25 µg Cd l^{-1} (den Besten *et al.* 1989). Lower concentrations (1 µg.l^{-1} Cd) could disrupt Zn metabolism (Temara *et al.,* 1998b). Considering these relatively high effective concentrations, it can be proposed that detoxification systems (including constitutive MTs, synthesis of inducible MTs, incorporation of heavy metals into the skeleton) appear relatively efficient in *A. rubens* and that the species is particularly resistant to the heavy metals studied so far. This is confirmed by a study in the field, as *A. rubens* is one of the few macro-invertebrates that survive in the most polluted sites of Sørfjord where they can be found in dense populations around, and on, mussel beds (Temara, personal observations). However, sublethal effects have been detected (partial inhibition of alkaline phosphatase function and disruption of skeletogenesis, larval settlement, and/or growth, Temara *et al.,* 1997c; 1998c).

A route of Pb elimination in *A. rubens* has been proposed (Figure 2): the transfer of the metal from the digestive system (the main route of entry in the organism) to the gonads and its probable expulsion



during spawning (Temara *et al.*, 1998a). The toxicity of Pb accumulated in the gonads towards the maturing gametes depends on its chemical speciation and on the type of tissues (somatic or germinal) in which it is stored. It is noteworthy that Pb presented low toxic effects to mature echinoid gametes (Dinnel *et al.*, 1989.

The use of biomarkers to detect the biological impact of pollutants in asteroids has received little attention (Everaarts *et al.*, 1998). According to Everaarts (1995), assessment of DNA integrity is a valuable method as a biomarker of polycyclic aromatic hydrocar- [illegible]

of *Asterias rubens*	References
covers the Northern al zone down to -650 asive *A. amurensis* in	Hayward & Ryland, 1990 Byrne *et al.*, 1997
ears	Guillou & Guillaumin, 1985
	Hostens & Hammerlynck, 1994
nds of individuals)	Hostens & Hammerlynck, 1994
g, diving	
otoxicological interest	
ns about its biology century	
boratory conditions	
Se, Mn, Pb, Zn, Ag,	Binyon, 1978; Guary *et al.*, 1982; den Besten *et al.*, 1990; Sorensen & Bjerregaard, 1991; Rouleau *et al.*, 1993; Hansen & Bjerregaard, 1995; Temara *et al.*, 1996a; 1998c; Warnau *et al.*, 1998a; 1999.
ar concentrations by ectrometry	den Besten *et al.*, 1990; Sorensen & Bjerregaard, 1991; Everaarts & Fischer, 1989; Temara *et al.*, 1997.
and Cd	Gerets *et al.*, 1972; den Besten *et al.*, 1989; Temara *et al.*, 1997b; 1998c.
	Bjerregaard, 1988; Temara *et al.*, 1996a; 1998a,c.
ed sites, e.g. Sørfjord	Temara *et al.*, 1997b; 1998c.

1984).

ecies an ideal bioindicator of heavy metal

In *A. rubens*, Cd concentrations in the pyloric caeca were directly proportional to contaminating concentrations in the range of 0.025 to 2.5 $\mu g.l^{-1}$ Cd (Bjerregaard, 1988). The bioconcentration factor decreased only with higher environmental concentrations according to a threshold effect (Bjerregaard, 1988; Temara *et al.*, 1996a). Moreover, Cd concentrations in the skeleton of asteroids collected along a well-marked contamination gradient in the field (Sørfjord, Norway) were significantly correlated to concentrations in the sediment (Temara *et al.*, 1998c).

The study of elimination kinetics provides information on the temporal scale of a bioindicator. While the pyloric caeca rapidly eliminated a significant proportion of the accumulated Cd (65% within a few days), the skeleton kept Cd for a longer time (elimination of 30% over a period of 6 weeks) (Temara *et al.*, 1996a). However, a study of the asteroids from the Sørfjord showed that the skeleton was not able to indicate variations in Cd concentrations over a period of several years. Therefore, according to observations by Temara *et al.* (1996a; 1998c), the Cd retention time in the skeleton is rather a few months. Further study on the retention capacity of Cd by the skeleton over a long period of time is needed in order to ascertain the half-time of the metal in this compartment. It is worthwhile noting that in the echinoid *Paracentrotus lividus*, the biological halftime has been estimated to a few months (Warnau *et al.*, 1995b). The pyloric caeca of *A. rubens* should thus be considered as a short-term bioindicator of Cd contamination, while the skeleton should be used as a mid-term bioindicator.

Because of the wide distribution of Asterias spp. (see Table 1), it is tempting to propose them as valuable bioindicators over the whole Northern hemisphere, including arctic regions where asteroids represent a significant proportion of the benthic biomass and

which are increasingly polluted. However, biokinetics of Cd and Pb in *A. rubens* have been studied at temperatures characteristic of temperate regions only (i.e., 5-20 °C). According to Hutchins *et al.* (1996), elimination kinetics of radionuclides from *A. forbesi* were much slower at temperatures characteristic of northern habitats and extrapolation of conclusions drawn from observations in warmer ecosystems might be invalid in such extreme environments.

The Pb accumulation ratios in the asteroid body compartments were directly proportional to the environmental contaminating concentrations (Temara *et al.*, 1998a). Study of the contamination of asteroids from the Sørfjord showed that after long-term exposures, the body concentrations were in good agreement with environmental conditions at steady-state (Temara *et al.*, 1998c). The digestive organs accumulated and eliminated Pb rapidly; in contrast, the skeleton integrated the variations over the lifespan of the asteroid (*i.e.* several years, Temara *et al.*, 1998a, c).

Based on the information available so far, a sampling strategy of *A. rubens* may be proposed for future biomonitoring programs.

Sampling strategy

The variations in heavy metal concentrations in the body compartments of asteroids living in sites located far from any point sources of contamination have clearly shown that some precautions have to be taken in a biomonitoring program based upon that species.
(1) It is not advised to use *A. rubens* as an homogeneous compartment since the distribution of metals within the organism is selective.
(2) The gonads should not be included in biomonitoring programs: they are not present during part of the reproductive cycle, which limits extemporised samples. Furthermore, gametogenesis in *A. rubens* depends closely on water temperature and on food availability, which means that asteroids sampled at the same time but at different latitudes could present different metal concentrations in the gonads due to differences in gametogenic stages.
(3) The central digestive system is of limited use because of its small mass that does not allow various analyses on any one organism. Its analysis could, however, be useful in the frame of a biomonitoring program focused on Pb contamination due to its rapid elimination from that compartment.
(4) Heavy metal concentrations do vary with the size of the asteroid. Therefore, it is advised to sample asteroids of the same size-class. The amplitude of the allometric variations being minimal in the lar-

gest size-class, asteroids should be collected among individuals of this size-class in each population.

(5) As a rule, the advised body compartments are the pyloric caeca and the skeleton (prepared as described in Temara *et al.,* 1996a); they should be sampled on asteroids belonging to the largest size-class in the same gametogenic stage.

The results obtained so far during our study of the natural variations in metal concentrations in *A. rubens* populations in the NE Atlantic (deposited in the database of the International Council for the Exploration of the Sea, and that of the Management Unit of the North Scheldt and North Sea Mathematical Model, Belgium; Reference MUMM 94 CF. BE) provided a set of data corresponding to a range of concentration variations in relatively non-contaminated environments. The 95th percentile of such distributions has been chosen (for each metal) as a critical value beyond which any mean concentration can be considered as an indication of contamination. According to the variations between compartments, it is necessary to define different critical values for each of the compartments. These values are shown in Table 2 for the body compartments selected previously and for two of the metals whose concentrations are known to vary with environmental conditions (Cd and Pb).

	Skeleton	Pyloric caeca	Central digestive system
Pb	10	1.1	1.5
Cd	1.2	5.1	-

Table 2
Critical values (µg.g^{-1} dw) of heavy metals in the body compartments of adult *A. rubens.* (The central digestive system is necessary for Pb analyses).

Taking into account the critical values of Table 2 and uptake and elimination kinetics of Pb, concentrations in the digestive system and in the skeleton may thus indicate different situations.

(1) Pb concentrations in the central digestive system and in the skeleton are lower than the critical values: the ecosystem has not been sub-

jected to a major contamination for years before the sampling (up to 7 years, *i.e.* the lifespan of asteroids). It is noteworthy that a limited or a minor past contamination might not be detectable due to the dilution effect of skeletal growth.

(2) Pb concentrations in the central digestive system are higher than the critical values while concentrations in the skeleton are lower than the critical values: the ecosystem has been submitted to a recent contamination (early signal).

(3) Pb concentrations in both compartments are higher than their respective critical values: there is current long-lasting contamination.

(4) Pb concentrations in the central digestive system are lower than the critical values while concentrations in the skeleton are higher than the critical values: the ecosystem had been contaminated but the source has disappeared.

Taking into account the size/age relationship observed in asteroids and the long retention time of Pb in the skeleton, an allometric study of Pb concentration in the asteroid skeleton can provide further information on the history of the contamination of the site studied, as it has been undertaken in Sørfjord (Temara *et al.*, 1998c). Similarly, further investigations might determine the validity of assessing the remote history of contamination by studying the metal content of echinoderm fossils as proposed by Ferrara *et al.* (1997). The biokinetics in the various body compartments were quite different for Cd and a similarly precise approach to biomonitoring cannot be applied for this metal.

Conclusions

The main route of Cd and Pb uptake in *A. rubens* would be the digestive wall. Transfers to other body compartments including the body wall and the gonads have been observed. At steady state, the distribution of metals in the asteroid depends mainly on the type of body compartment. The pyloric caeca are the main target of classes Ib and IIb elements. The skeleton is the main target of

Pb. For the two elements that have been studied in detail (Pb and Cd), there is a simple relationship between the body concentrations and the concentrations in the environment (Bjerregaard, 1988; Temara *et al.,* 1996a; 1998a, c). Uptake and elimination of Pb and Cd by the pyloric caeca are rapid processes. In contrast, the retention periods are longer in the skeleton (Cd: several months, Pb: up to several years). Because of the satisfactory relationship between the ecotoxicological parameters of metals in *A. rubens* and the selection criteria of bioindicators, this species should be included in biomonitoring programs (such programs are currently underway in the Southern Bight of the North Sea by our own team as well as teams in the Netherlands, Everaarts *et al.,* 1998). Analysis of concentration variability in the populations located far from any point sources of contamination allowed definition of a sampling strategy as well as critical values. Mean concentrations exceeding such values indicate a contamination in the ecosystem at the 95% confidence level. The body compartments "pyloric caeca" and "skeleton" are proposed as complementary bioindicators of Cd and Pb contamination: the pyloric caeca as a short-term bioindicator, the skeleton as a mid- to long-term bioindicator. Concerning Pb, an allometric study of the skeleton can indicate temporal variations in environmental contamination on the scale of the decade.

Detoxification systems of Cd and Pb are relatively efficient in *A. rubens*, which is therefore fairly resistant to these metals. The major detoxification system of Cd would be its complexation to inducible proteins, the metallothioneins. Incorporation into the skeleton seems to be the major detoxification route for Pb.

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