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# Some mechanisms promoting or limiting bioaccumulation in marine organisms

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# Some mechanisms promoting or limiting bioaccumulation in marine organisms

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## Introduction

Two approaches can be followed separately or simultaneously to study the effect of heavy metals on marine organisms. Either one does toxicity tests and eventually tries to understand the physiological perturbations involved, or one is more interested in bioaccumulation.

Heavy metals tend to accumulate in living matter, simply because of their high affinity for numerous organic substances, especially proteins. Their binding to enzymes, or other cellular components, often explains their toxicity but inert traps - or at least apparently so - may exist. Heavily loaded animals then become a potential danger for predators.

The understanding of the fate of heavy metals released by natural sources or by man in the sea depends to a large extent on our knowledge about bioaccumulative processes without neglecting speciation and complexation with dissolved or suspended organic matter. To try and predict contamination levels in marine organisms becomes a very complicated matter furthermore since uptake and elimination depend on environmental circumstances, physiological conditions, and differ from species to species.

We will describe in this paper some mechanisms controlling the bioaccumulation of heavy metals, mainly Hg and Cd, by marine organisms.

## Uptake

Heavy metals are taken up by marine animals either from water or from food. The relative importance of both routes varies from metal to metal and from animal to animal, but direct uptake from water is often much more important as we have shown earlier [Bouquegneau *et al.* (1976, 1979)].

Most of the metals in food are therefore found back in faeces at high concentrations, thus favouring vertical transport of heavy metals in the sea [Boothe and Knauer (1972) ; Benayoun *et al.* (1974)].

In most aquatic animals large surfaces are in direct contact with the surrounding water and hence with dissolved metals : gills, digestive tract and skin. Moreover, the digestive tract of teleosts and gills are submitted to a large continuous flow of water likely to increase adsorption probability.

The factors that control the direct entry are many : type of metal, its speciation, relative permeability of different organs, size of surface of contact, environmental factors (salinity, temperature, etc.), physiological conditions (age, etc.). For a recent review of the matter one should consult the papers from Bryan (1979) and Coombs (1980). To take an example from our own work, the gills of teleosts reveal to be less permeable to inorganic $Hg^{++}$ than to $(CH_3Hg)^+$ and their permeability for $Cd^{++}$ is even smaller [Bouquegneau (1975) ; Noël-Lambot (1980)].

Several physiological mechanisms can be implied in the metal uptake by marine organisms. For instance, in most plants [see Coombs (1980)] simple passive diffusion phenomena are involved and, in that case, there exists a linear relationship between initial rate of intake and external concentration, but there are some examples in animals where the former increases more slowly than the latter, suggesting that heavy metal uptake might then

involve mechanisms that imply facilitated diffusion, i.e. carrier assisted transport, or even active transport as in the case with Na, K and Ca. This was shown in our laboratory working on *Serranus cabrilla* (Bouquegneau and Radoux, to be published) and also at the level of cadmium uptake by mussels [Coombs and George (1978)].

When considering metals bound to particulate or colloïdal matter, there are moreover examples that the metal can be taken in by a process of endocytosis, as shown for the common mussel *Mytilus edulis*, able in that way to absorb iron and lead, present in sea water as colloïdal hydrous oxides [George *et al.* (1976) ; Schulz-Baldes (1978)].

## Release

The metal taken up by the animal is released at a rate varying considerably from case to case.

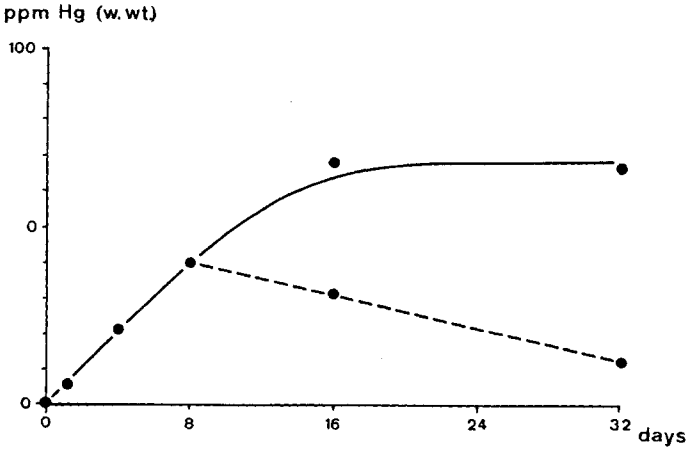


fig. 1.

Kinetics of accumulation and release of Hg in a tissue displaying a high rate of elimination : *Anguilla anguilla* gills.

——— : intoxication in sea water containing 100 ppb $Hg^{++}$ as $HgCl_2$
------ : intoxicated fish put back in clean water (from Bouquegneau, 1975)

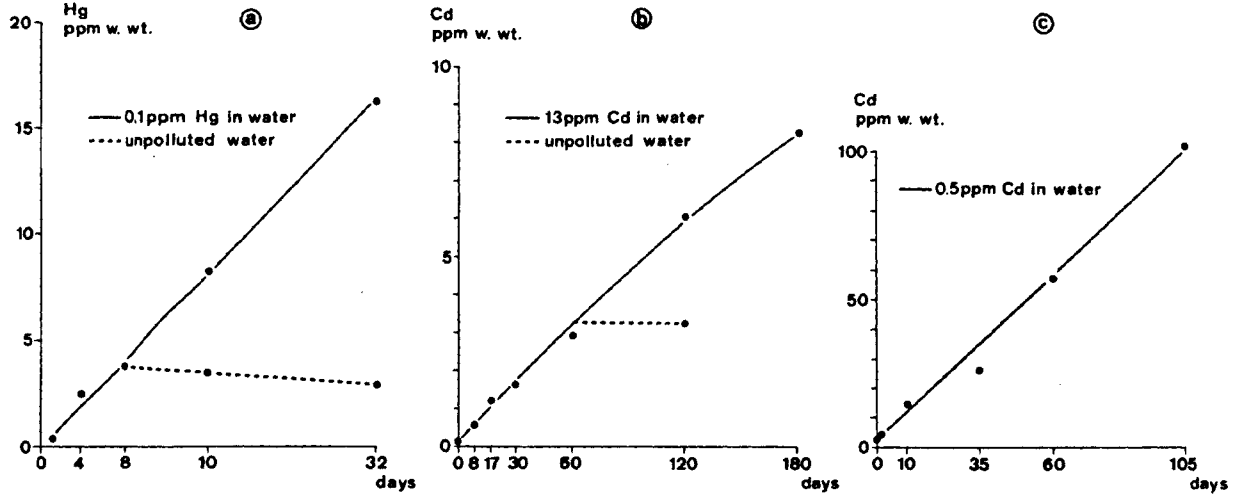


fig. 2.

Kinetics of accumulation of $Hg^{++}$ or $Cd^{++}$ in animals with slow elimination rate

a) Accumulation (———) and release (------) of $Hg^{++}$ ($HgCl_2$) in the whole body of *Anguilla anguilla* (from Bouquegneau, 1975).

b) Accumulation (———) and release (-------) of $Cd^{++}$ in the whole body of *Anguilla anguilla* (from Noël-Lambot, 1980).

c) Accumulation of $Cd^{++}$ in the whole body of *Patella caerulea* (from Noël-Lambot, 1979).

If elimination is fast, the metal output will quickly balance the input and the metal concentration in the tissues will reach a plateau. Fig.1 describes such a case for *Anguilla anguilla* gills.

The same type of curve can of course also be observed in the absence of fast elimination, when for instance the rate of uptake is slowed down for some reason like for example the formation of a protective mucus layer [Bouquegneau *et al.* (1979) ; Radoux and Bouquegneau (1979)].

If the elimination is very slow, the kinetic of accumulation becomes linear. Fig.2 shows three examples for *Anguilla anguilla* and *Patella vulgata* intoxicated with $Cd^{++}$ or $Hg^{++}$.

## Accumulation — Storage mechanisms

If, as in the case in fig.2, the elimination of the heavy metal is extremely slow, why is it that uptake continues whilst the metal concentration in most of the tissues greatly exceeds that of the contaminated water ?

Accumulation implies strong binding between metals and cellular components. Heavy metals, as Cd or Hg, have a strong affinity for -SH group for instance. When bound to organic constituents such metals do not obey the rules governed by electrochemical gradients in relation with the transport of charged ions across living membranes. Binding sites are provided by practically all normal cell constituents but there also exist more specific storage mechanisms. Examples follow at the intra- and extracellular level.

### 1.- INTRACELLULAR TRAPS

#### 1.1.- *Metallothioneins*

The properties of metallothioneins — low molecular weight proteins (6000 — 7000) with high cystein content (about 30 % of total amino acids) and a metal load corresponding to one atom for two or three cysteins — extracted from marine animals either Hg

or Cd intoxicated have been described in earlier papers [Noël-Lambot *et al.* (1978a,b) ; Bouquegneau *et al.* (1979)] and recent reviews are available on this subject [Bouquegneau and Noël-Lambot (1978) ; Kägi and Nordberg (1979)]. Metallothioneins have first been considered to explain the resistance of animals when intoxicated with heavy metals [Piscator (1964) ; Nordberg (1971) ; Bouquegneau *et al.* (1975) ; Bouquegneau (1979)]. Actually, more attention is drawn on their role in bioaccumulation and we will here essentially deal with the case of Cd.

Cd induces the biosynthesis of metallothioneins in tissues where, in absence of exposure, no or very small amounts of these proteins can be detected. The total amount of metallothioneins grows steadily during intoxication and one is faced with a storage system, the size of which increases the more Cd there is to be trapped, either because of longer exposure or a larger Cd concentration.

One example of this phenomenon is given in fig.3. It shows the change with time of total Cd in the animal and that found at subcellular level in *Patella caerulea* tissues. The curve showing the kinetic of accumulation in the whole body is identical with the one presented in fig.2c.

The amount of Cd found in the MT fraction (see fig.3) - that is bound to metallothioneins - can be considered as a reliable estimate of the abundance of these proteins [Noël-Lambot *et al.* (1978b),(1980)]. Their concentration increases with time.

These laboratory results are quite comparable with observations made on a population of limpets (*Patella vulgata*) naturally exposed to Cd in the Bristol Channel.

Fig.4 shows a linear relationship between Cd concentration and body size [good indicator of age, see Noël-Lambot *et al.* (1980)], whereas in the case of Zn and Cu an inverse relationship is observed.

Most of the Cd in heavily loaded limpets is bound to metallothioneins, but this is not the case for Zn and Cu. Fig.5 shows a sharp correlation between total accumulated Cd and the Cd attached to thioneins.

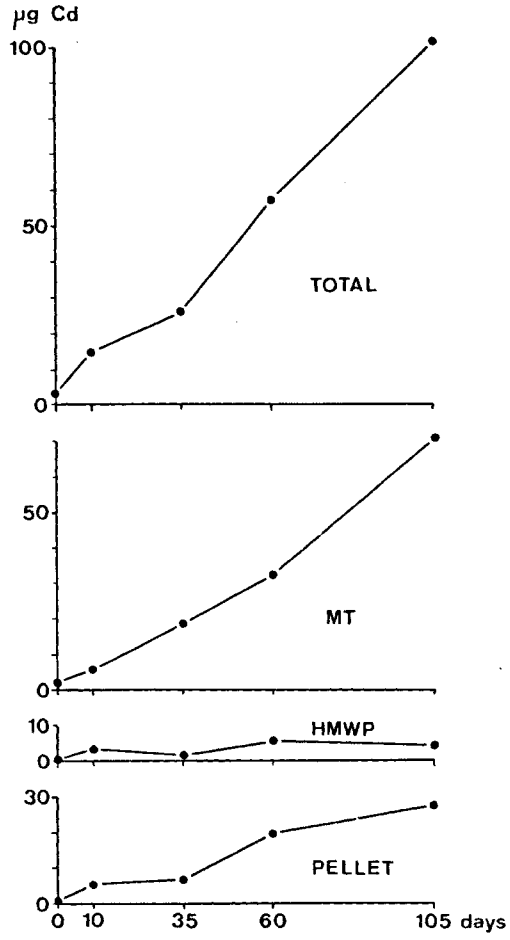


fig. 3.

Cd accumulation in whole limpets (*Patella caerulea*) and in their various subcellular fractions during an intoxication in sea water containing 0,5 ppm Cd. For each time of intoxication, the soft parts of three specimens were pooled and homogenized. After centrifugation, Cd was measured in the pellet and in the supernatant fractions separated by gel chromatography. All concentrations are expressed in µg metal/g whole tissue.
MT = metallothioneins ; HMWP = soluble proteins of high molecular weight.
(From Noël-Lambot, 1979.)

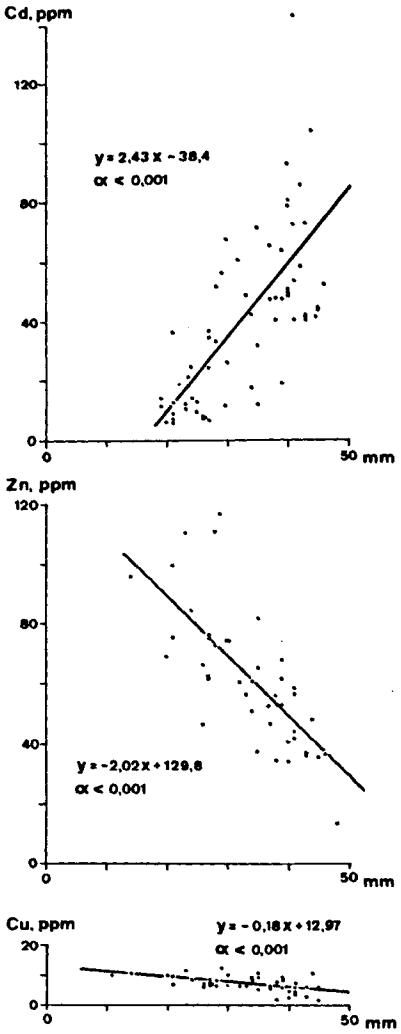


fig. 4.

Relation between metal concentration in soft tissue (ppm w.wt.) and shell length for Cd, Zn and Cu in limpets (*Patella vulgata*) collected from Weston-super-Mare. (From Noël-Lambot *et al.*, 1980.)

In young limpets with minimum Cd load (Cd concentration < 13 ppm) Cd-thioneins are not found.

Fig.5 also shows that a significant part of the total Cd is always present in the centrifugation pellet. Cd concentration in this fraction also increases with the total Cd load. On the other hand, the amount of Cd bound to soluble proteins of high molecular weight increases very slightly as compared to cadmium bound to metallothioneins. Intracellular distribution of Cd is, however, quite different in small individuals, thus having a low Cd concentration. In small limpets, Cd is almost exclusively stored at the level of pellet and soluble proteins of high molecular weight ; in large ones, Cd bound to metallothioneins represents about 85 % of soluble Cd which corresponds to approximately 50 % of total Cd.

Thus metallothioneins appear in the limpets at a critical level of Cd load in the tissues. When this critical Cd concentration for metallothionein induction is reached, Cd bound to high-molecular-weight proteins hardly increases any more as the total Cd concentration rises. It thus may be considered that metallothioneins only appear when the high-molecular-weight soluble proteins reach a certain level of saturation by Cd and that, from then on, Cd accumulation in the cytosol almost exclusively occurs at the level of metallothioneins.

Similar results as those described in fig.4 between metal content and body size were reported previously by Boyden (1974) for *Patella vulgata* collected from Portishead, Bristol Channel. This work, like ours, indicates that Cd displays a relationship opposite to Zn and Cu (fig.4), suggesting quite different metabolic pathways of these elements.

Concerning the increase of Cd concentration with body weight, Boyden (1974) concluded : "... this relationship can best be explained as being due to removal of this element from body circulation and accumulation within specific tissue, possibly as a result of some exceptional affinity".

Our results with Cd-thioneins confirm and allow to better understand this interpretation. Metallothioneins may be considered

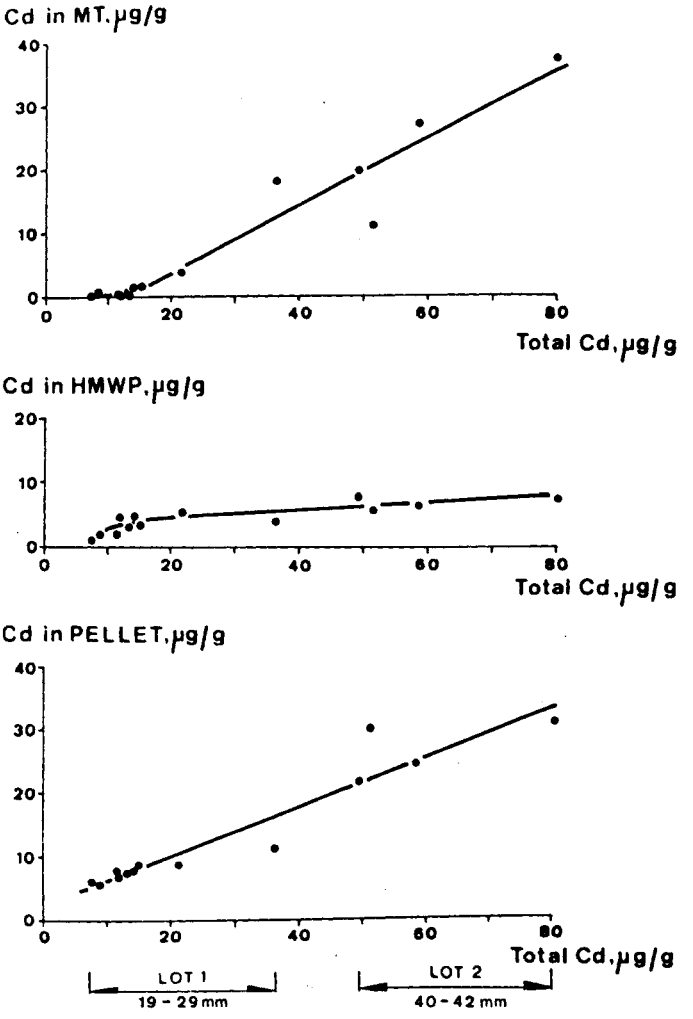


fig. 5.

Relation between Cd concentration in whole soft parts of limpets (*Patella vulgata*) collected from Weston-super-Mare and concentrations of Cd associated with
(1) metallothioneins (MT);
(2) high-molecular-weight proteins (HMWP) – both isolated by gel filtration of the supernatant of the homogenate;
(3) the centrifugation pellet of this homogenate.
All concentrations expressed in µg Cd/g wet tissue.
Based on shell length, limpets were classed into two lots.
(From Noël-Lambot *et al.*, 1980.)

as the specific Cd-binding compound responsible for the unusually high levels of Cd in old limpets. It is evident that increase in the amounts of metallothioneins with time corresponds to an equivalent increase of Cd-binding sites and thus to a more and more extensive capacity of Cd storage.

Moreover, as we previously pointed out [Bouquegneau *et al.* (1979)], binding of Cd to metallothioneins may explain how limpets from the Bristol Channel can tolerate such high Cd levels in their tissues : Cd complexed to thioneins may be considered as toxically inert. But can this mechanism be really considered as a protective system ? In other words, do metallothioneins protect limpets against Cd injury during a long term exposure ? The problem is not as simple as one would believe at first thought. There is no doubt that the synthesis of metallothioneins favours Cd bioaccumulation. But this means that in the eventual absence of synthesis of these proteins, one might expect that Cd concentration in the limpets or other animals either from the Bristol Channel or intoxicated in the laboratory would not reach such high values.

The question of the so called "protective effect" of metallothioneins thus consists in determining whether the presence of such proteins really reduces to a significant extent the amounts of Cd available to interact with normal cellular functions. The study of the uptake and intracellular location of Cd in relation to the synthesis of metallothioneins might help solve this question as well as further work on the general physiology of these animals, contaminated or not.

Results presented in fig.5 concern whole soft parts of limpets. Viscera have twice higher Cd concentrations than foot muscles but metallothioneins can be detected in both tissues where they bind about 80 % of soluble Cd.

It is surprising to observe relatively high levels of Cd bound to metallothioneins in limpet muscular tissues. This is quite different from observations made in vertebrates where muscles never reach concentrations higher than 1 or 2 ppm wet weight, even in the case of drastic Cd intoxication [Noël-Lambot and Bouquegneau

(1977)]. Moreover, muscle is to our knowledge the only tissue in which the existence of metallothioneins has so far not been reported. Once again, high Cd concentrations in tissue and abundance of metallothioneins seem to be closely linked.

When limpets from the Bristol Channel are exposed for 80 days to unpolluted sea water, the Cd level of the animals does not decrease and as previously observed for other species under laboratory conditions [Bouquegneau *et al.* (1979)], the largest part of the metal persists as Cd-thionein, although the cause of the formation of these proteins has disappeared [Noël-Lambot *et al.* (1980)]. Cadmium is thus really trapped by the metallothioneins.

The observations on limpets have been confirmed and complemented by studies on other invertebrates collected in the Bristol Channel, in the British Channel or at Cape Gris-Nez.

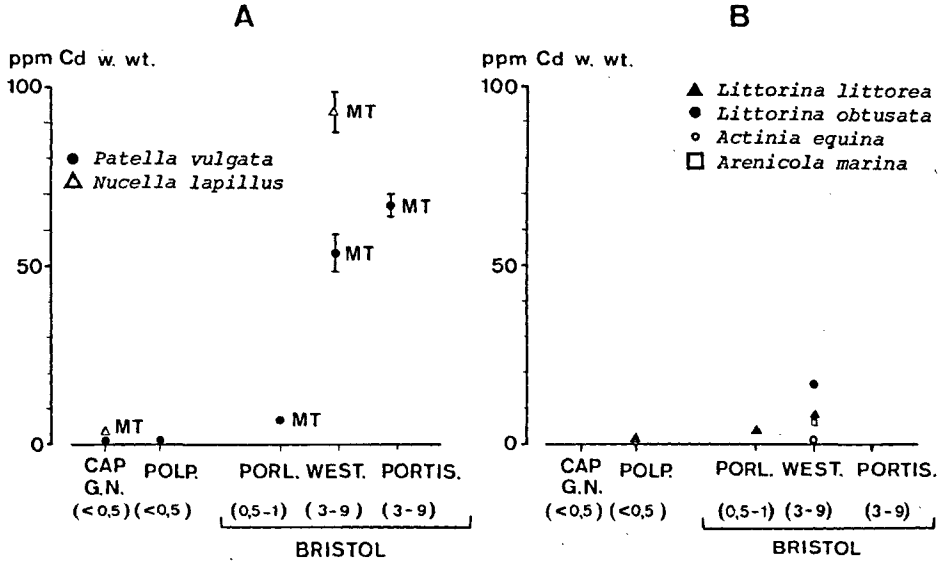


fig. 6.

Cd concentration (m ± standard error) and occurrence of metallothioneins in some invertebrates collected from various localities of the Bristol Channel or from unpolluted areas (see figure 7). For species presented in graph A, metallothioneins were identified in the population living in the Bristol Channel. This is indicated by the mention "MT". For species of graph B, metallothioneins were undetectable in all populations. Approximate Cd concentration in water (in ppb) is given under the name of the stations.

Fig.6 (see fig.7 for the location of the explored sites) shows clearly that in all the studied species high Cd concentration is always related to high metallothionein content.

Cd content increases with age only in animals with metallothioneins.

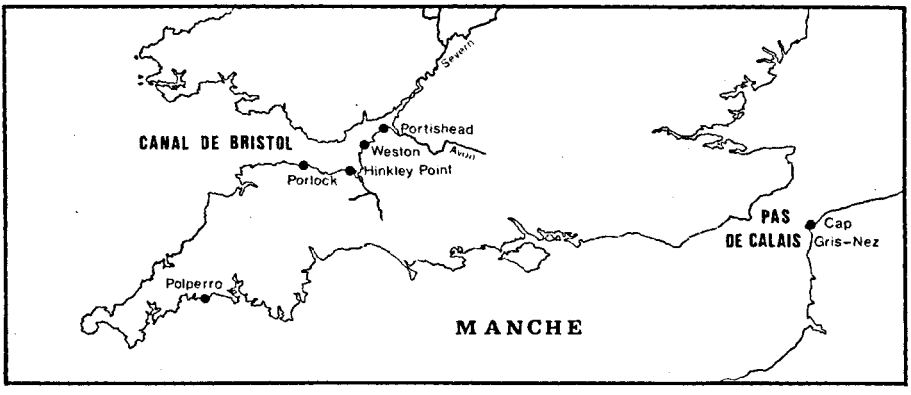


fig. 7.
Location of stations referred to in fig. 6

When the Cd level required to induce metallothionein formation is low (as is the case for dog whelk, *Nucella lapillus*), there is a direct relationship between Cd concentration and size, even in populations exposed to very low Cd concentrations (Cape Gris-Nez, fig.8).

No such correlation is observed in limpets living in non polluted water [Boyden (1974)] and no metallothioneins are formed [Noël-Lambot *et al.* (1978a)].

In eels and flets (*Anguilla anguilla* and *Platichtys flesus*) collected in the Bristol Channel, similar observations can be made. Table 1 gives the Cd, Zn and Cu levels in various organs. As was observed in laboratory experiments (Noël-Lambot (1980)) on fish exposed to large concentrations of Cd, the tissues displaying the highest Cd level are also those that contain thio-

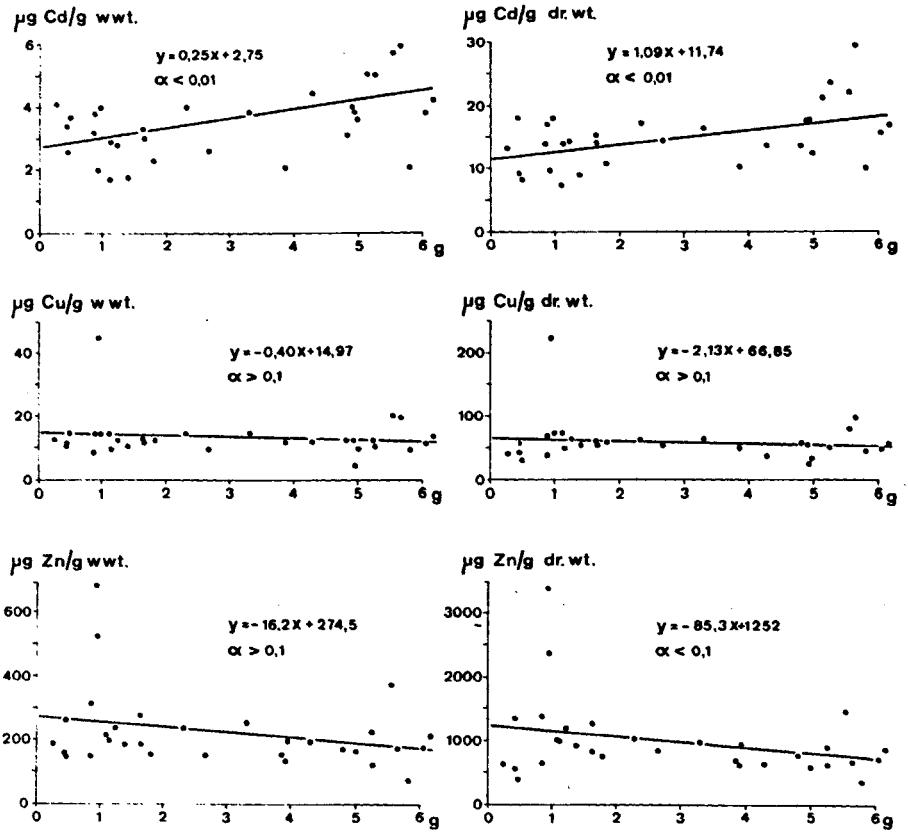


fig. 8.

Relationship between Cd, Cu or Zn concentrations in soft tissue (ppm wet or dry weight) and total wet weight of the animals (with shell) in dog whelks, *Nucella lapillus*, collected from Cape Gris-Nez, Pas-de-Calais, France.

neins. In liver and kidney for instance, it is possible to detect an increase of Cd with age (Mears and Eisler (1977), Müller and Prosi (1978)), but this has never been shown for muscle (Lovett *et al.* (1972) ; Stevens and Brown (1974) ; Tong (1974)).

To conclude briefly the capacity of marine animals to synthetize metallothioneins when exposed to Cd explains the well known process of cumulative absorption, the long half-time of the bound

Table 1

Concentration of Cd, Zn and Cu in various organs of two teleosts captured at Hinkley Point (Bristol Channel) in connection with the presence or not of metallothioneins (from Noël-Lambot, 1980).

| | *Anguilla anguilla* (49 cm) | | | | *Platichtys flesus* (31 cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration, µg/g | | | Presence of MT | Concentration, µg/g | | | Presence of MT |
| | Cd | Zn | Cu | | Cd | Zn | Cu | |
| Muscles | 0.3 | 11.5 | 0.5 | - | 0.3 | 13.5 | 0.6 | - |
| Skin | 1.1 | 18.2 | 3.6 | | 0.2 | 138.3 | 13.1 | |
| Stomach | 0.3 | 22.0 | 1.5 | | - | - | - | |
| Intestine | 0.5 | 24.0 | 2.0 | +? | 0.5 | 37.3 | 2.4 | +? |
| Liver | 2.9 | 42.5 | 20.7 | + | 0.4 | 53.2 | 25.1 | + |
| Bile | 0.4 | 5.8 | 4.9 | | - | - | - | |
| Kidney | 9.5 | 68.3 | 3.1 | + | 0.5 | 87.9 | 4.0 | +? |
| Gills | 0.4 | 20.1 | 1.3 | - | 0.2 | 20.2 | 1.2 | - |
| Heart | <0.2 | 23.8 | 2.1 | | - | - | - | |
| Brain | 0.2 | 18.0 | 2.2 | | - | - | - | |
| Air-bladder | 0.2 | 15.2 | 10.5 | | - | - | - | |

+ : clear evidence of MT
+?: MT near the detection limit
- : MT below the detection limit

metal and how some animals can tolerate extremely high contamination levels when chronically exposed.

The existence of these proteins to which Hg, Cu and Zn also bind cannot be ignored in ecotoxicological research applied to marine systems. They were so far only studied in mammals and their existence in other phyla was only shown recently. Reviews written by Kägi and Nordberg (1979), Noël-Lambot *et al.* (1980) tend to show that these proteins might well be widely distributed in the biosphere.

It is interesting to note that properties of metallothioneins extracted from limpets living in the Bristol Channel (see tables 2 and 3) are very similar to those from other molluscs and from vertebrates (Kägi and Nordberg (1979) ; George *et al.* (1979) ; Frankenne *et al.* (1980)). Limpets have at least two metallothioneins carrying different charges, they probably as in mammals correspond to isoproteins (Kojima and Kägi (1978)).

Table 2

Amino acid composition of metallothioneins from *Patella vulgata* (from Noël-Lambot *et al.*, 1980)

| Amino acid | Number of residues (%) □ | |
|---|---|---|
| | MTa | MTb |
| Lysine | 8.8 | 8.6 |
| Histidine | 1.0 | 1.4 |
| Arginine | 0.8 | 0.9 |
| Aspartic acid | 11.2 | 12.0 |
| Threonine | 7.2 | 6.8 |
| Serine | 8.8 | 8.5 |
| Glutamic acid | 7.7 | 8.5 |
| Proline | 3.8 | 4.1 |
| Glycine | 11.0 | 10.6 |
| Alanine | 8.7 | 8.9 |
| △ Cysteine (1/2) | 21.0 | 20.0 |
| Valine | 3.2 | 2.7 |
| ○ Methioneine | 0.5 | 0.5 |
| Isoleucine | 1.8 | 1.5 |
| Leucine | 2.6 | 2.7 |
| Tyrosine | 1.8 | 1.8 |
| Phenylalanine | 1.0 | 1.0 |
| ▶ Tryptophan | - | - |

□ 24 h hydrolysis
△ Determined as cysteic acid
○ Determined as methionine sulfone
▶ Not determined

It is clear that the fate of Cd discharged in the environment depends greatly on the presence or absence of metallothioneins in living matter and what happens after death. A knowledge of threshold Cd concentrations triggering the biosynthesis of these proteins and of the rate at which they are formed appears to be essential to investigate the impact of Cd pollution.

Table 3

Metal content (number of metallic ions per 30 cysteinyl residues) of metallothioneins from *Patella vulgata* (from Noël-Lambot *et al.*, 1980)

| | Cd | Zn | Cu | Total | Cysteine metal |
|---|---|---|---|---|---|
| MTa | 12.4 | 0 | 0.9 | 13.3 | 2.3 |
| MTb | 12.0 | 0 | 1.0 | 13.0 | 2.3 |

1.2.- *Membrane limited granular structures*

Besides being capable of storing heavy metals on thioneins, many animals can accumulate these toxics in intracellular granules or vesicles. This phenomenon of metal storage in particulate structures is very widespread in marine and terrestrial invertebrates and its occurence has been shown in numerous phyla [Ballan-Dufrançais *et al.* (1979) ; George and Pirie (1979) ; Janssen and Scholz (1979) ; Georges *et al.* (1980) ; for a review, see Coombs (1980)].
Some recent results suggest that metallothioneins may also be associated with particulate structures within the cell and not be freely available within the cytoplasm [George and Pirie (1979); C. Ballan-Dufrançais and A.Y. Jeantet, personal communication (1979)].

2.- EXTRACELLULAR TRAPS

The mucus layer or the cuticle of water exposed tissues (skin, intestine, gills) of marine animals and microorganisms through which metals penetrate the body may be considered as extracellular traps because of their high affinity for many heavy metals [Martin (1970) ; Cossa (1976) ; Wright (1977) ; Kremling *et al.* (1978)]. These external storage sites generally act as a limiting factor to the entry of the metals and in some way control internal bioaccumulation.

2.1.- *Branchial mucus of teleosts*

The mucus layer on fish gills has a very high affinity for Hg as shown in table 4.
In eels adapted to sea water the gills are the main route of entry of dissolved Hg (Bouquegneau (1975)). Fixation of Hg by branchial mucus is an important step, since it means that the metal is first heavily concentrated in mucus before it reaches the branchial tissue where it is found at a much lower concentration, to be finally transported to the different organs by the blood circulation.

Table 4

Time evolution of the mercury concentration in the mucus of the gills, the gill tissue and the whole body of eels (*Anguilla anguilla*) intoxicated in sea water containing 50 ppb Hg ($HgCl_2$)

| Time of exposure | Hg concentration (ppm w.wt.) | | | |
|---|---|---|---|---|
| | sea water | Branchial mucus | Branchial tissue | Total body |
| 1 day | 0.05 | 31 | 3 | 0.5 |
| 2 days | 0.05 | 14 | 4 | 1 |
| 4 days | 0.05 | 60 | 1 | 2 |

Some of the heavy metals are known to stimulate mucus production by fish [Eisler (1974) ; Varanasi and Markey (1978)]. They also can induce increased mucus shedding [Baker (1969) ; Coombs *et al.* (1972)].

Working with *Serranus cabrilla* we have observed an increase of mucus production by gills during $HgCl_2$ intoxication. It results both from a stimulation of secretion and from an increase of the number of mucus producing cells in the branchial epithelium (Bouquegneau and Radoux, to be published). This increase of the mucus layer considerably limits the absorption of $Hg^{++}$, since the Hg loaded mucus is regularly shed. The accumulation kinetics in the whole body reflects this limiting process : although elimination of Hg stored inside the animal is very slow, the level reached in these animals quickly comes to a plateau [see Bouquegneau *et al.* (1979) ; Radoux and Bouquegneau (1979)]. This observation is clearly explained by a limitation of the rate of entry because of mucus formation and subsequent delamination.

In *Anguilla anguilla*, where the whole body accumulation of Hg is linear in time (fig.2), no effect of the metal has been found on the production of mucus (Bouquegneau *et al.*, to be published).

*2.2.- Intestinal corpuscles in teleosts*

In several species of sea water fish, white mucous corpuscles may be observed in the intestinal lumen of unfed animals (fig.9).

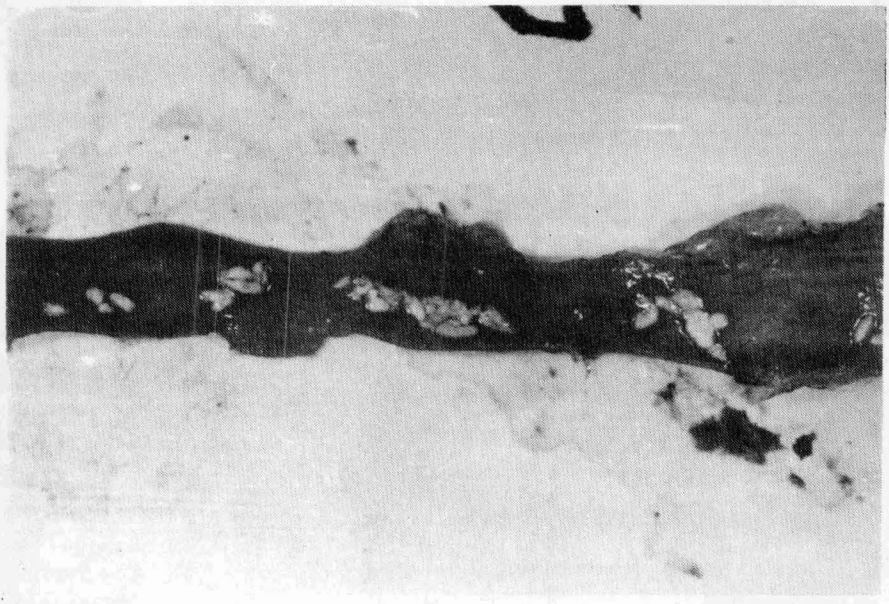

fig. 9.

Intestinal corpuscles in *Anguilla anguilla* in a longitudinal section of the intestine

This material, regularly evacuated by the anus was termed "intestinal corpuscles" [Distèche (1974) ; Noël-Lambot (1980)]. Its content in Ca and Mg is very high ; these metals are probably present in the form of carbonates precipitated from the sea water contained in the intestine at the level of an organic support made of mucus and cellular debris.

In fish intoxicated with $CdCl_2$, $ZnCl_2$ or $CuCl_2$ added to sea water, the corpuscles are found to contain enormous concentrations of these metals and although their weight is small, they carry a very large part of the total metals found in the animals. The data presented in fig.10 show the intestinal corpuscles retain more than 99 % of the Cd present in the intestine and this amount of trapped Cd is even greater than the total Cd accumulated by all the tissues of the animal during 6 hrs intoxication. The corpuscles are eliminated with the faeces. The presence of

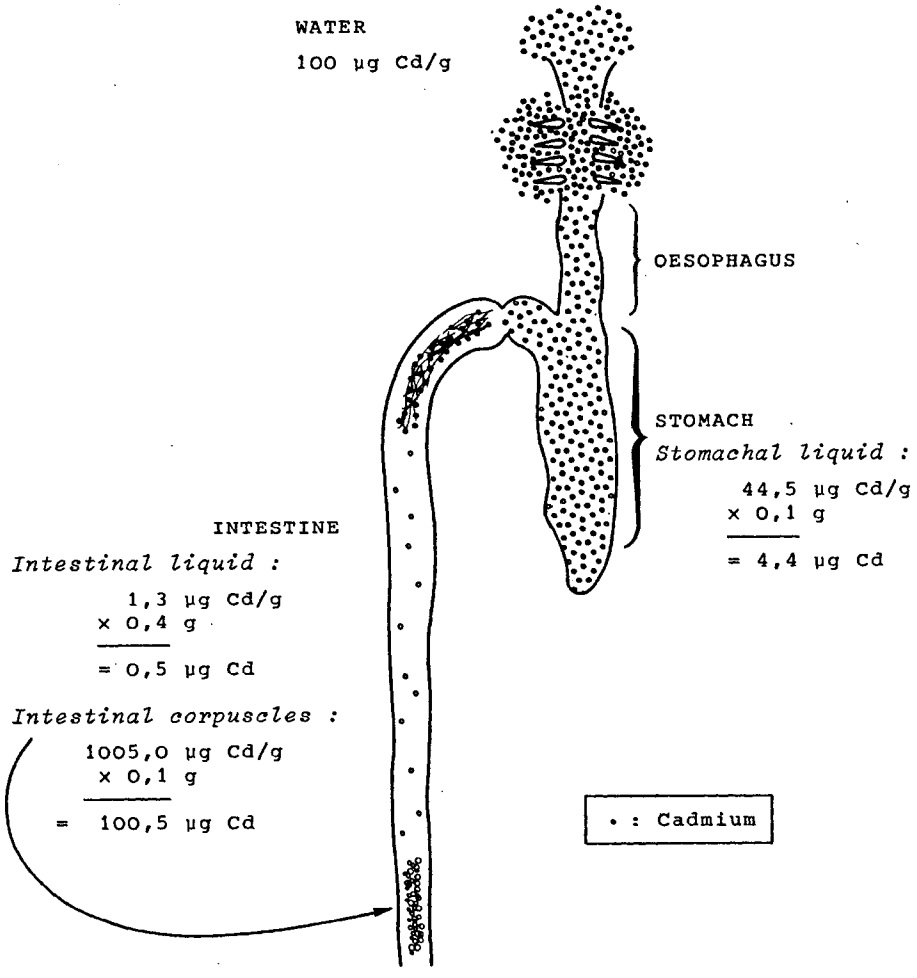


fig. 10.

Cd concentration inside the digestive tract of eels intoxicated during 6 hrs in sea water containing 100 ppm Cd. For each constituent the Cd concentration (ppm or µg/g) is given as well as the load (µg) equal to the product of the concentration and the weight of the constituent considered. The loads are calculated for eels the weight of which being adjusted to 100 g.

intestinal corpuscles, directly accumulating Cd or other metals from the sea water ingested by the animals, seems therefore to

greatly limit the entry of heavy metals through the intestinal wall and thus protect fish against these pollutants.

*2.3.- "Mucous pellets" in Tunicates*

The mucous secretions of the branchial pharynx and of the intestine of Tunicates can accumulate large quantities of Cd. Mucous filaments or pellets enriched in Cd are regularly ejected by the exhalant siphon. The mean Cd concentration of these secretions is 1700 ppm when the water concentration is kept at 0.5 ppm. As in fish the amount of Cd attached on these little mucous filaments is larger than the total Cd present in the whole animal (fig.11).

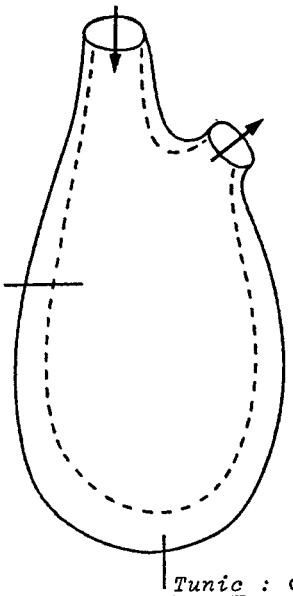


fig. 11.

Cd concentration and content in mucus pellets and in the tissues of *Halocynthia papillosa* after 10 days intoxication in sea water containing 0.5 ppm Cd ($CdCl_2$)

It is well known that in these animals the branchial pharynx, especially the endostyle, produces large amounts of mucus and protein secretions [Thorpe and Barrington (1965)]. Olsson (1963) has shown that some of the excreted proteins are rich in sulphur which might explain Cd fixation.

## Conclusions

While we don't know yet the exact details of the physico-chemical and physiological mechanisms implied in the translocation of heavy metals, we have to bear in mind a general picture of the fate of heavy metals discharged in a marine environment when dealing with ecotoxicology (and bioaccumulation).

Few or no metals remain present under their ionic form. Some, like iron and lead, are normally present as colloïdal hydrous oxides. Other ones form inorganic complexes, but most of heavy metals are bound to the dissolved and particulate organic matter.

In this regard, we suggest that plants and animals should be considered as part of the particulate organic matter. There is indeed a competition between the dead and alive particulate organic matter to adsorb heavy metals in solution.

When considering animals, the mucus layer which covers the whole body has a high affinity for heavy metals. First adsorbed at that level, they may be taken up into the tissues by physiological processes such as passive or facilitated diffusion and active transport. Those mechanisms, both with the high affinity of some intracellular compounds (such as proteins), may lead to a huge accumulation of toxic metals in the organisms.

How can organisms tolerate such high concentration since, in many cases, they remain able to survive and reproduce normally ?

Two storage mechanisms inside the cells, can account for such phenomena : either a binding to metallothioneins or a storage inside vesicles.

Another way to control high concentrations is to increase the rate of excretion of the pollutant or to decrease the rate of en-

try into the cells, for example by an increase of the mucus secretion by the external tissues.

Another mechanism responsible for the accumulation of heavy metals in organisms is an uptake via the food chain. In some cases (inorganic Hg, Cd), it has been shown that little metal could be taken up by that way. The consequence is then an important increase of the metal faeces concentrations which, after elimination and sedimentation, may lead to an enrichment in heavy metals of benthic ecosystems.

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