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**THE ACCUMULATION OF HEAVY METALS
IN MARINE ORGANISMS**

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The understanding of the heavy metal contamination of aquatic organisms is rather difficult because it depends not only on the nature and concentration of the pollutant but also on many physico-chemical and biological characteristics of the ecosystem. Moreover a high concentration of metal present in an organism is not necessarily correlated with a high degree of pollution of the medium. Indeed, in contrast to the non-essential trace metals such as mercury and cadmium, the essential ones such as copper and zinc have important biological functions in the organisms and can therefore be found in important amounts in some of their organs in unpolluted as well as in polluted media.

On another hand, several species are able to develop physiological mechanisms of storage, under a detoxified form, of heavy metals present in excess in the organism.

So, from an ecotoxicological point of view, the occurrence of high heavy metal concentrations in marine organisms is to be carefully studied considering at one time environmental factors influencing their accumulation and toxicity, and at another time the physiology as well as the adaptative ability of the organism to stand unusual concentrations of trace metals in their environment.

1.- Occurrence of high heavy metal concentrations in marine organisms.

Table 1 presents the exceptionally high heavy metal concentrations we could observe in some tissues from marine organisms together with some relevant bibliographic data. These high values are compared in this table with the concentration of the same metal in other samples from the same area. Animals were collected in natural environments with relatively little or no pollution except for samples coming from the Bristol Channel where Cd concentration in water lies around 5 ppb (Abdullah et al, 1972 ;

Table 1

	Locality	n	Mean metal concentration of the tissue compared with that of other organs or organisms from the same locality (ppm wet weight/ppm dry weight)
CADMIUM	Roscoff	8	99 ppm w.wt/ 502 ppm dr.wt versus 0.5 ppm w.wt/ 2.5 ppm dr.wt in muscle
	Monaco	1	61 ppm w.wt/ 315 ppm dr.wt versus 0.5 ppm w.wt/ 3.7 ppm dr.wt in gills
	Corsica	1	52 ppm w.wt/ 114 ppm dr.wt versus 2 ppm w.wt/ 12 ppm dr.wt in digestive gland
	Bristol. Channel	40	54 ppm w.wt/ 351 ppm dr.wt versus 0.7 ppm w.wt/ in <i>Actinia equina</i>
	Bristol. Channel	22	93 ppm w.wt/ versus 8 ppm w.wt/ in <i>Littorina littorea</i>
	Bristol. Channel	1	43 ppm w.wt/ versus 0.2 ppm w.wt/ in muscles and skin
	Roscoff	4	1995 ppm w.wt/11602 ppm dr.wt versus 8 ppm w.wt/ 57 ppm dr.wt in mantle
	Corsica	2	825 ppm w.wt/ 4040 ppm dr.wt versus 104 ppm w.wt/683 ppm dr.wt in mantle
	Monaco	1	540 ppm w.wt/ 2769 ppm dr.wt versus 17 ppm w.wt/ 73 ppm dr.wt in muscles
	Bristol. Channel		9860-35120 ppm dr.wt BOYDEN & ROMERIL (1974)
ZINC	Bristol. Channel	22	719 ppm w.wt/ versus 35 ppm w.wt/ in <i>Littorina littorea</i>
	Bristol. Channel	1	897 ppm w.wt/ versus 14 ppm w.wt/ in muscles
	Cardigan. Bay	1770-3438	ppm w.wt/ WALKER et al. (1975)
	<i>Pecten maximus</i> kidneys		
	<i>Pinna nobilis</i> digestive gland		
	<i>Spondylus gaederopus</i> kidneys		
	<i>Crassostrea gigas</i> soft parts		
<i>Musella lapillus</i> soft parts			
<i>Sepia officinalis</i> digestive gland			
<i>Balanus balanoides</i> soft parts			

	Locality	n	Mean metal concentration of the tissue compared with that of other organs or organisms from the same locality (ppm wet weight/ppm dry weight)
COPPER	Bristol Channel*	22	131,6 ppm w.wt/ versus 5 ppm w.wt/ in <i>Arenicola marina</i>
	Bristol Channel*		/1760-6480 ppm dr.wt BOYDEN & ROMERIL (1974)
	Bristol Channel*	1	1175 ppm w.wt/ versus 1.6 ppm w.wt/ in muscles
IRON	Irish Sea		/104-3800 ppm dr.wt PRESTON et al. (1972)
	Monaco	1	425 ppm w.wt/1446 ppm dr.wt versus 0.4 ppm w.wt/ 3.2 ppm dr.wt in mantle
	Roscoff	8	327 ppm w.wt/1319 ppm dr.wt versus 7 ppm w.wt/ 30 ppm dr.wt in mantle
	New-Zealand		/2915 ppm dr.wt BROOKS & RUMSBY (1965)
	New-Zealand		/1960 ppm dr.wt BROOKS & RUMSBY (1965)
	Irish Sea		/5400 ppm dr.wt SEGAR et al. (1971)
	Irish Sea		/2060 ppm dr.wt PRESTON et al. (1972)
MANGANESE	Roscoff	4	2516 ppm w.wt/14620 ppm dr.wt versus 0.2 ppm w.wt/ 1 ppm dr.wt in muscles
	Coraisca	1	13730 ppm w.wt/30227 ppm dr.wt versus 4 ppm w.wt/ 20 ppm dr.wt in digestive gland
	Polynesia	3	247 ppm w.wt/ 1130 ppm dr.wt versus 3 ppm w.wt/ in digestive gland
LEAD	Coraisca	1	432 ppm w.wt/ 951 ppm dr.wt versus < 3 ppm w.wt/ in gills

* Polluted area.

Peden et al, 1973). After dissection, tissues were directly frozen. Wet or dry samples were analysed by atomic absorption spectrophotometry (Perkin-Elmer, model 370A) after mineralization for 8 hrs at 80°C in HNO₃ 65% (2.5 ml g⁻¹ fresh tissue) and twenty-fold dilution.

It is of importance to note that, except for the case of samples coming from the Bristol Channel (Noël-Lambot et al, 1978, 1980b) and Cardigan Bay (Walker et al, 1975), the high metal concentrations observed in the tissues are not associated with environments known as polluted and thus may correspond to normal values. It thus appears that some tissues are able to specifically accumulate very high amounts of some metals occurring in trace concentration in their environment. This property is very widespread in molluscs particularly in the kidneys and digestive gland as shown in table 1. It should be observed that, in vertebrates, kidneys and liver are also the principal organs involved in heavy metal storage.

Metal distribution in the organs is thus very heterogeneous. This distribution is quite different from a species to another and it varies depending on the metal considered. Moreover individual variations are important. In some cases, as in *Patella vulgata* and *Nucella lapillus*, a correlation between age and the Cd, Zn and Cu concentration could be established (Noël-Lambot et al, 1980a & b) but this observation cannot be generalized.

It is well known that molluscs have a particular accumulation capacity for heavy metals ; though some of the results presented in table 1 are probably the highest values ever reported.

Heavy metals tend to accumulate in living matter because of their high affinity for cellular components. Binding sites are provided by practically all normal cell constituents but there also exist more specific storage mechanisms which contributes largely to the existence of high heavy metal loads in some organisms.

2.- Factors controlling the accumulation of heavy metals by aquatic organisms and importance of storage mechanisms.

As far as the pollutant concentration and the physico-chemical characteristics of the surrounding medium remain constant, the direct uptake of metals from water can often be described by the following equation (see for example Bouquegneau et al, 1979) :

$$C_t = C_{ss} (1 - e^{-Kt})$$

where C_t is the concentration of the metal at time t ; C_{ss} the steady-state concentration;

$$K = \frac{0.693}{t_{b/2}},$$

$t_{b/2}$ being the biological half-time.

The shape of the curve depends both on the initial rate of uptake of the pollutant and on its rate of elimination (biological half-time).

The rate of uptake of metals may depend on several physiological mechanisms such as passive diffusion, facilitated diffusion, active transport and, in the case of colloidal metal species, endocytosis (for a review, see Coombs, 1980).

Working with the teleost *Serranus cabrilla*, we have shown that the effect of mercury concentration on the metal uptake by the gills revealed the existence of a mercury carrier at the gill epithelium level (Bouquegneau et al, 1982).

On the other hand, the biological half-time should be theoretically equal to the half-time of elimination of the pollutant when the animal is put back in clean water. Sometimes it is effectively the case, but in many species such as *Serranus cabrilla*, we have found that the steady-state could be reached very quickly even when the half-times of elimination of the pollutant were very long (Radoux & Bouquegneau, 1979).

Thus, it appears that other factors are able to modify the uptake kinetics of the pollutants.

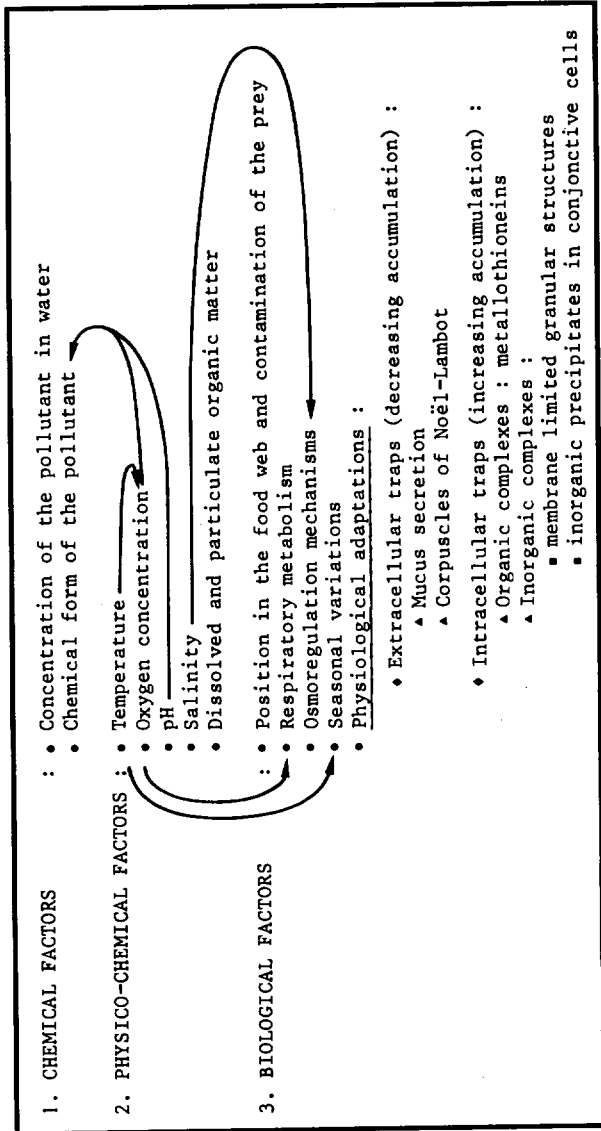
Table 2 shows the different factors affecting the heavy metal load of an organism exposed to a polluted environment. Those factors are numerous and can influence each other as shown by the arrows in the table. The concentration of the pollutant in water or in sediment of course but also its chemical form can affect the uptake significantly.

For example, it is now well known that the more hydrophobic alkyl and aryl mercury compounds are more readily taken up and are more toxic to phytoplankton or to fish than inorganic mercuric chloride. Moreover, mercury as sulphide in sediments is made unavailable and become immobilized. Many environmental factors are able to affect too the uptake of heavy metals. The main factors are salinity and temperature, and they influence both the chemical form of the pollutant and the physiology of exposed organism (see table 2).

Reduction in salinity generally results in an increase in heavy metal uptake. For example, the rate of mercury accumulation of the chinese crab *Eriocheir sinensis* is much more important in

Table 2

Factors affecting the heavy metal load of an organism exposed to a polluted marine environment.
 Arrows show some interactions between these factors.
 (Adapted from Bouquegneau & Verthé, 1981)



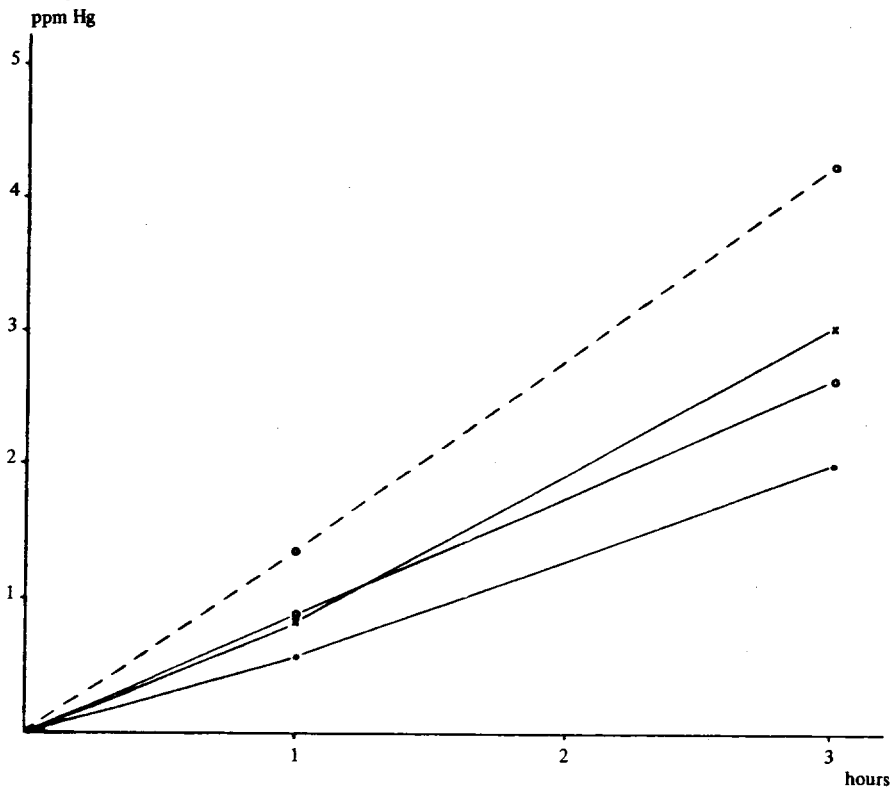


fig. 1.

Accumulation of mercury by *Leptomysis linguura* in filtered sea-water (Millipore 0.22 μ - - -) and in sea-water containing complexing agents as: cystein (—●—), citrate (—x—) and EDTA (—○—). Each point is the mean of the results obtained for 10 specimens (from Verthé et al., 1982).

fresh water than in sea water (Bouquegneau et al, 1979). Generally, increase in temperature results in an increase in uptake until lethal effects begin to take effect.

A possible important environmental factor which is often neglected is the presence in water of more or less dissolved and particulate organic matter.

Fig.1 (Verthé et al, 1982) shows that the rate of uptake of mercury by *Leptomysis linguura* is decreased by the presence in the medium of some highly concentrated organic complexes such as cystein, citrate and EDTA. However, it seems that such an inhibitory effect cannot be generalized to all natural organic

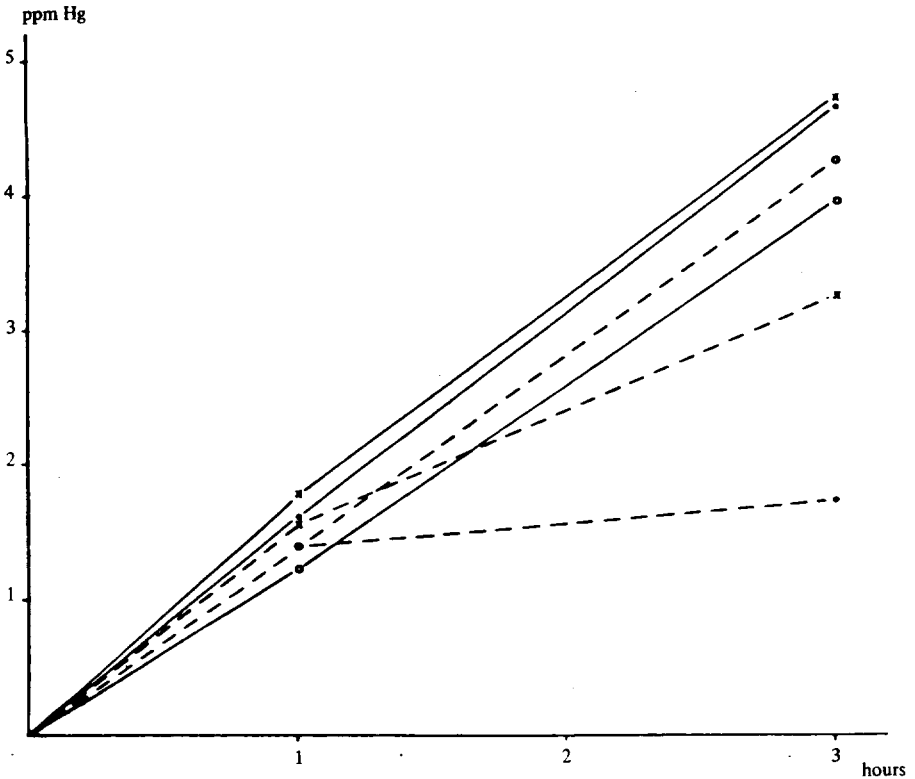


fig. 2.

Mercury accumulation by *Leptomysis linguura* in sea-water filtered on a 0.22μ Millipore filter (---o---), in unfiltered water (---o---), in sea-water containing $2 \times$ (—●—), $5 \times$ (---x---) and $10 \times$ (—o—) times the normal amount of organic matter with molecular weight higher than 10 000 daltons and in ultra-filtered sea-water (---x---) [organic matter molecular weight < 10 000 daltons]. Each point is the mean of the concentrations observed in 10 animals (from Verthé et al., 1982).

complexes since no effect on the uptake has been detected when natural high molecular weight dissolved organic matter is added to the medium (fig. 2, Verthé et al., 1982).

Moreover, when considering cadmium complexation, different effects can be observed depending on the highly concentrated organic complex used : EDTA totally inhibits the entry of cadmium in *Leptomysis linguura* and in *Patella caerulea* ; cystein largely increases the rate of entry of Cd in *Leptomysis linguura* but has no short-term effect in *Patella caerulea* ; citrate has no effect in both species (fig.3 & 4, Noël-Lambot et al, 1982).

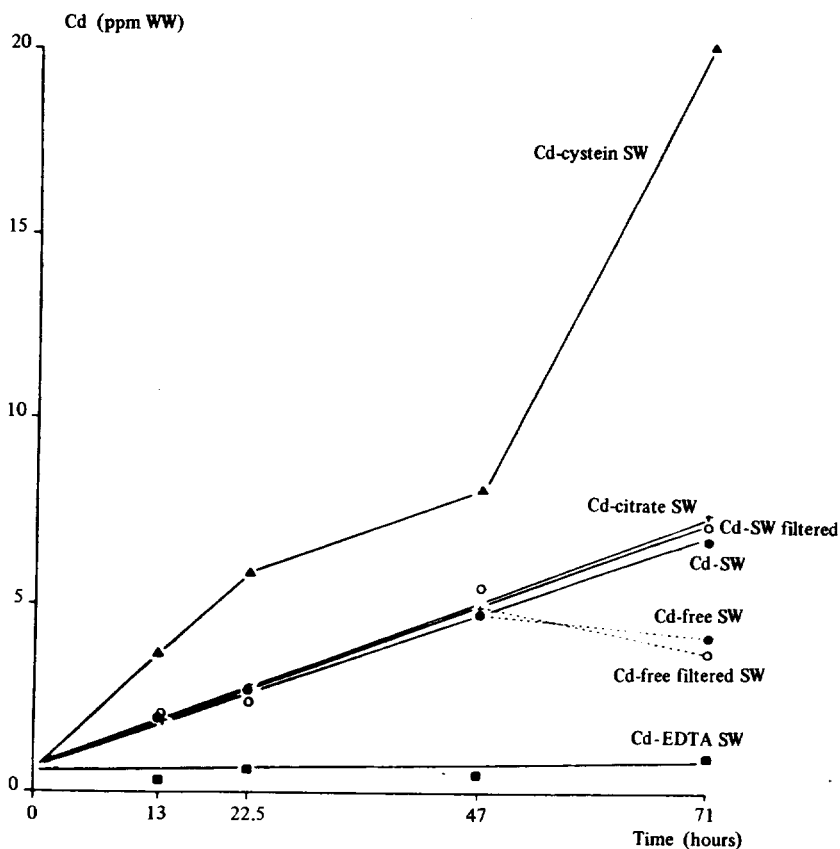


fig. 3.

Effect of different complexing substances on the Cd accumulation kinetics in *Mysidacea* exposed to 0.1 ppm Cd. Each point corresponds to a sample containing about 15 animals (from Noël-Lambot et al., 1982).

So it appears that metal complexation by dissolved organic matter has a variable effect on the rate of accumulation depending on the nature of the organic complex and of the metal, and on the studied species.

On the other hand, particulate organic matter has an important inhibitory effect on the uptake of metals by macroorganisms (Verthé et al., 1982).

Among biological factors responsible for the accumulation of heavy metals, the position of the species in the food web is worth to be discussed.

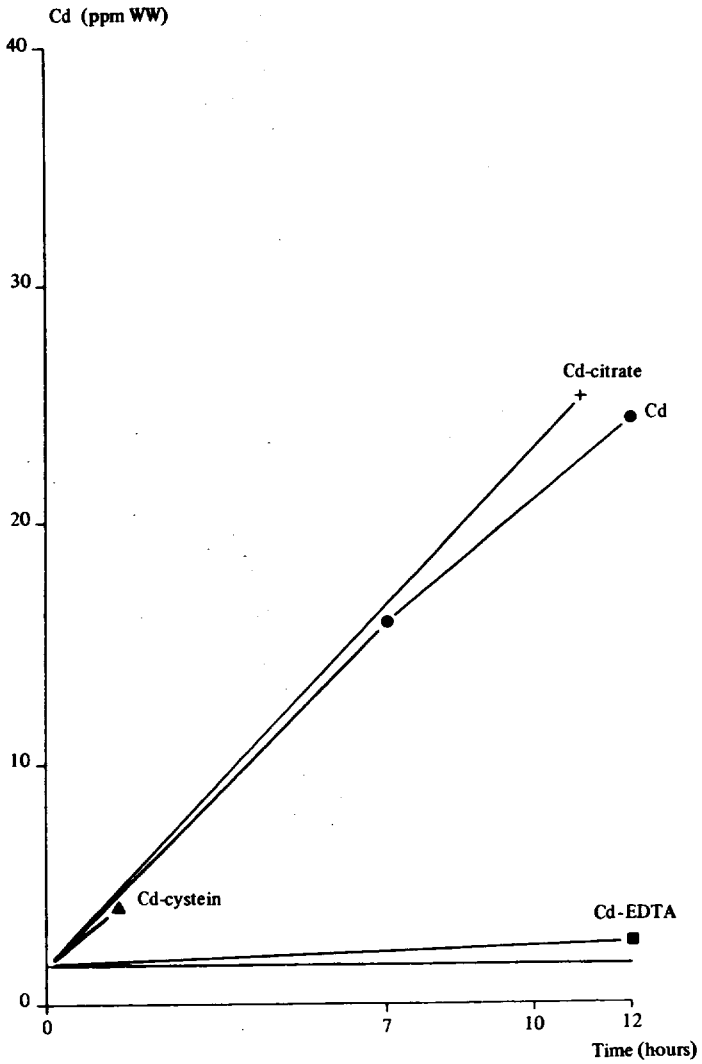


fig. 4.

Effect of different complexing agents on the Cd accumulation kinetics in *Patella caerulea* (Cd water concentration : 0.5 ppm). Each point corresponds to an average of data obtained from 2 to 9 specimens (from Noël-Lambot et al., 1982).

Most of the in situ measurements do not reveal any increase in the metal loads along the marine food chains (for a review, see Bouquegneau et al, 1976 ; Bouquegneau & Noël-Lambot, 1977). On the contrary, the following increasing sequence of heavy metal concentrations is often shown :

water < fish < zooplankton < sediments and
macroinvertebrates

suggesting that the main route of entry of metal pollutants is a direct contamination from water and that the percentage of metal assimilated from food must be small.

Some experiments have been carried out to test this hypothesis (see Bouquegneau et al, 1979). The results show that the percentages of ingested heavy metals assimilated from food are generally low (0.1 - 10.0 %) except in the case of methylmercury intoxicated preys (30 - 50 %), so that probably a difference can be defined between liposoluble and non-liposoluble pollutants in this regard (see also Bouquegneau, 1980).

The presence of heavy metals in abnormal concentrations in water sometimes can be considered as a stress to which the animal may be able to response by developing one or several physiological mechanisms which may lead to either a decrease of the rate of entry of the pollutant by the production of extracellular traps or an increase which is generally accompanied by a tolerance to the pollutant due to a storage under a detoxified form (intracellular traps).

One of the physiological adaptation mechanisms which decreases the rate of accumulation of heavy metals is a high production of mucus.

Radoux & Bouquegneau (1979) and Bouquegneau et al (1979) have shown that $HgCl_2$ intoxication induced an increase of mucus production by gills of *Serranus cabrilla*. This phenomenon and the subsequent mucus delamination limits the rate of entry of the pollutant in the animal.

In several species of sea water fish, other kinds of extracellular traps have been described by Noël-Lambot (1980, 1981). They are white mucous corpuscles and have been observed in the intestinal lumen of unfed teleosts and are regularly evacuated by the anus. 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 presence of 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 intestine wall, since they decrease the pollutant concentration of the intestinal liquid and thus protect fish against these pollutants.

On the contrary to the mechanisms described above, two other ones lead to important concentrations of heavy metals in animals.

The fact that many aquatic organisms can concentrate metals to very high levels and apparently survive and reproduce normally indicates that they have evolved some forms of tolerance.

The two mechanisms of tolerance described till now consist in a storage of metals inside the cells, either in membrane limited granular structure or bound to metallothioneins.

The capacity of all studied phyla of marine animals to synthesize metallothioneins when exposed to heavy metals such as Cd and Hg accounts for the high heavy metal load of some organs, the long half-time of elimination of those pollutants and the good tolerance of some species against such contaminations. Detailed study on the heavy metal storage as metallothionein by marine animals is described in the next chapter of this volume. It is worth noticing that metallothioneins are quickly degraded after the death of the organisms (fig.5) so that metals stored in that way can be recycled again in the food web.

On another hand, many phyla of marine and terrestrial invertebrates are able to store heavy metals in intracellular granules or vesicles (Coombs, 1980).

Metallothioneins may also be associated with particulate structures within the cell and not be freely available within the cytoplasm (Jeantet et al, 1980 ; Ballan-Dufrançais, Jeantet & Bouquegneau, to be published).

Numerous inorganic precipitates have been now identified. For example, Martoja & Berry (1980) have shown in mediterranean cetaceans the biosynthesis and the storage of pure thiammanite in the liver. Cetaceans are unable to excrete the thiammanite particles. Such a detoxification process leads to the fossilization of mercury under the form of a non-biodegradable component.

A similar study has been performed in our laboratory (in collaboration with M. Martoja, Institut Océanographique, Paris, France) about the storage of copper as copper sulphide by Gastropods (Bouquegneau & Martoja, 1982).

The copper, cadmium and zinc content of four species of Gastropods (*Monodonta crassa*, Archeogastropod ; *Littorina littorea*, Mesogastropod ; *Thaïs lapillus* and *Murex brandaris*, Neogastropods) collected in non-polluted areas has been investigated.

When considering copper, a mineralization technique has been used which allows to separate the most strongly bound copper identified as CuS from copper complexed to organic matter. There is no correlation between soft tissue weight and copper concentration in *Monodonta crassa* and young *Thaïs lapillus*. In these animals, no copper sulphide was found either.

On the contrary, in *Littorina littorea*, *Murex brandaris* and aged *Thaïs lapillus*, there is a significant correlation between copper content and age. Moreover, copper sulphide has been both chemically and histologically detected in their tissues. However, copper sulphide has been detected only at the visceral mass level and is responsible for the increase of the copper content of oldest animals.

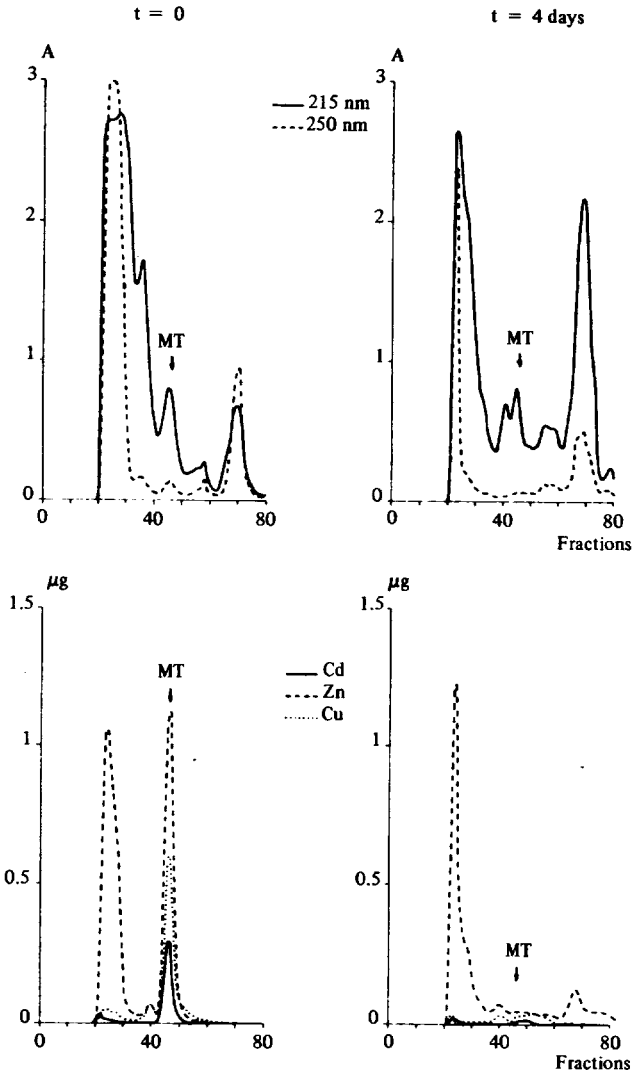


fig. 5.

After death degradation of hepatic metallothioneins in eels.

Eels have been used after decerebration, having been submitted to 50 days intoxication in water containing 1.3 ppm of Cd. Some animals have been used immediately for analysis, others have been kept four days in non contaminated sea-water, aerated and replaced each day. Chromatographic extracts from two livers: ACA 54 (2.6×83 cm). Volume of fractions: 7 ml. The metal content of the eluted fractions are given in μg per fraction corresponding to 1.5 g of liver.

From those results and from literature data, it appears that copper metabolism in Archaeogastropods is quite different from that in Meso- and Neogastropods. In the two latter, copper from hemocyanin catabolism is stored as copper sulphide in conjunctive cells which are situated in the visceral mass of the animal, and the number of these cells increases with age. In Archaeogastropods, copper from hemocyanin catabolism is either used again or excreted out of the soft tissues.

The described phenomenons have two important ecotoxicological implications : 1° Meso- and Neogastropods are to be considered bad copper pollution biological indicators because most of the copper load results of a natural metabolic process ; 2° the ability of predators to assimilate CuS should be studied in order to evaluate the importance of such a storage for the food web contamination. Probably copper sulphide is a non-biodegradable compound which cannot therefore be metabolized by any organism of marine food chains. The final result of such phenomenons is therefore a fossilization of heavy metals and an enrichment of their concentrations in the sediments.

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