Chapter VI

Physiological effects of some pollutants

by

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(Based on work by G. PERSOONE, F. BENIJTS, C. CLAUS, P. SORGELOOS, L. VANHECKE-SARLET, Gent University; J.P. VANDEN BOSSCHE, Ch. PERPEET, M. VLOEBERGH, Brussels ULB University; A. DISTECHE, J.M. BOUQUEGNEAU, F. LAMBOT, Ch. GERDAY, Liège University)

1.- Phytoplankton

1.1.- Effect Hg^{++}, Pb^{++}, Cu^{++}, Zn^{++}, Cd^{++} on the growth of the phytoflagellate *Dunaliella viridis* and the diatom *Phaeodactylum tricornutum* [Benijts *et al.* (1974c)]

The organisms are grown under laboratory controlled conditions (light, aeration, CO_2, temperature) in artificial sea water (150 cm^3) prepared following Dietrich and Kalle (1963). Salinity is 30 ‰, the water is filtered through a 45 µm membrane filter; it is used either with or without addition of Vlasblom growth stimulating medium [Walne (1956)] containing $FeSO_4$, NaH_2PO_4, $NaNO_3$, $MnCl_2$, glycine.

The heavy metals are added as chlorides and the growth of the algae during 120 h is compared to the growth in absence of pollutants. The growth curves are drawn and integrated using Bode's formula. The ratio $\frac{\text{integrated growth curve test experiment}}{\text{integrated growth curve blank experiment}}$ allows to express the %

inhibition due to the addition of the heavy metal ions, either in presence or absence of culture medium.

The results are shown in figures 1 to 4.

It is obvious that the two test organisms react differently and that the toxicity scales are as follows : *Dunaliella viridis* :

$$Pb^{++} \gg Cu^{++} \gg Zn^{++} , \quad Hg^{++} , \quad Cd^{++}$$

in absence of added culture medium,

$$Pb^{++} \geq Hg^{++} \gg Zn^{++} , \quad Cu^{++} \gg Cd^{++}$$

in presence of added culture medium; *Phaeodactylum tricornutum :*

$$Hg^{++} , \quad Cu^{++} \gg Zn^{++} , \quad Cd^{++} , \quad Cu^{++}$$

in absence of added culture medium,

$$Hg^{++} \gg Pb^{++} , \quad Zn^{++} , \quad Cu^{++}$$

in presence of added culture medium.

It should be noticed that the addition of growth stimulating medium considerably decreases the toxic effect of the heavy metals.

These results show how difficult it is to rely on "test" organisms to evaluate water quality[1]. Different phytoplanktonic organisms react differently and the observed effects are sensitive to the presence of phosphates, nitrates, iron, amino-acids, substances favouring eutrophication, but found in a very wide range of concentrations in natural conditions.

1.2.- Kinetics of adsorption and intake of Zn^{++} by *Dunaliella viridis* and *Phaeodactylum tricornutum* [Benijts *et al.* (1974b)]

The phytoplankton cells are grown as described above in artificial sea water enriched with Vlasblom medium (plus 0.03 mg/ℓ Na_2SiO_3 when diatoms are grown). The growth is followed with a Coulter counter and samples are retrieved to analyse the total Zn content using atomic

1. Marine Algal assay Procedure Bottle test : Eutrophication and Lake Resteration Branch National Environmental Research Center, Corvallis, december 1974.

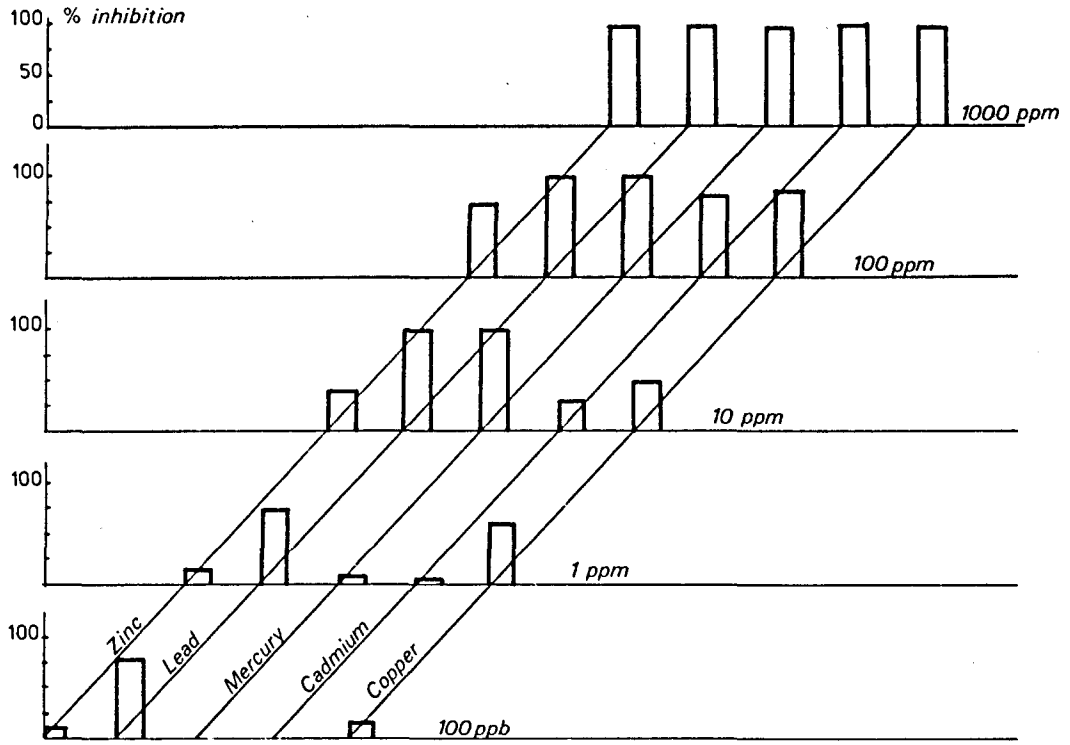


fig. 1.
Dunaliella viridis without addition of culture medium.

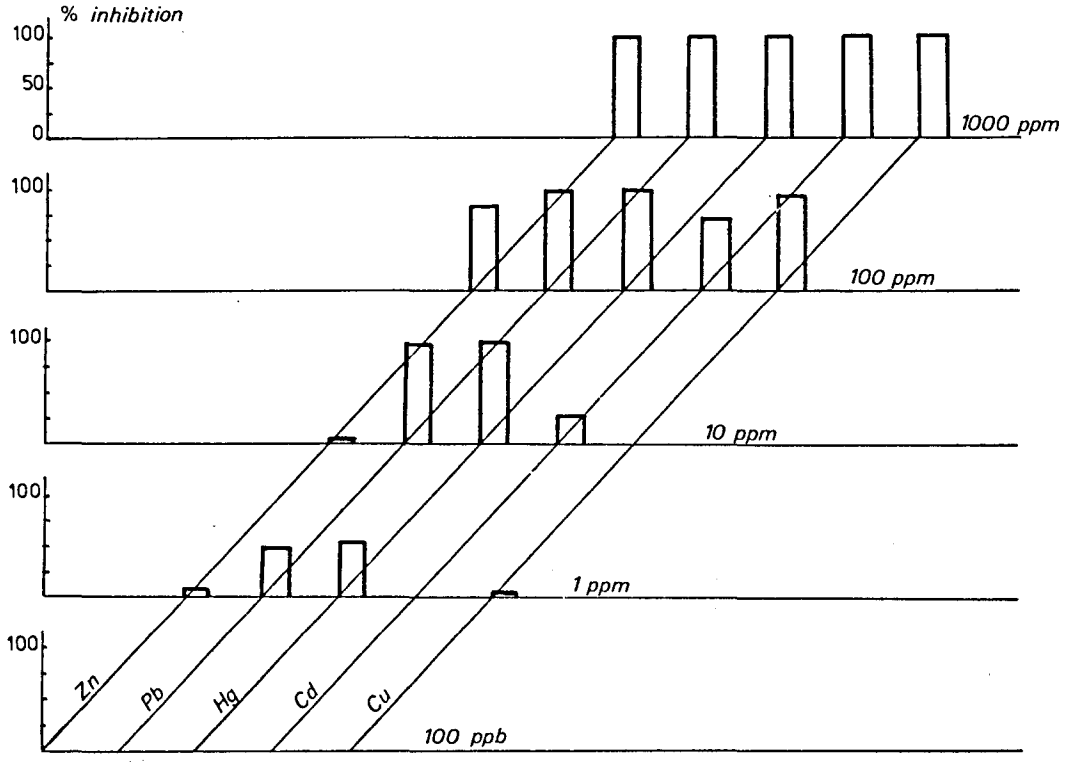


fig. 2.
Dunaliella viridis with addition of culture medium.

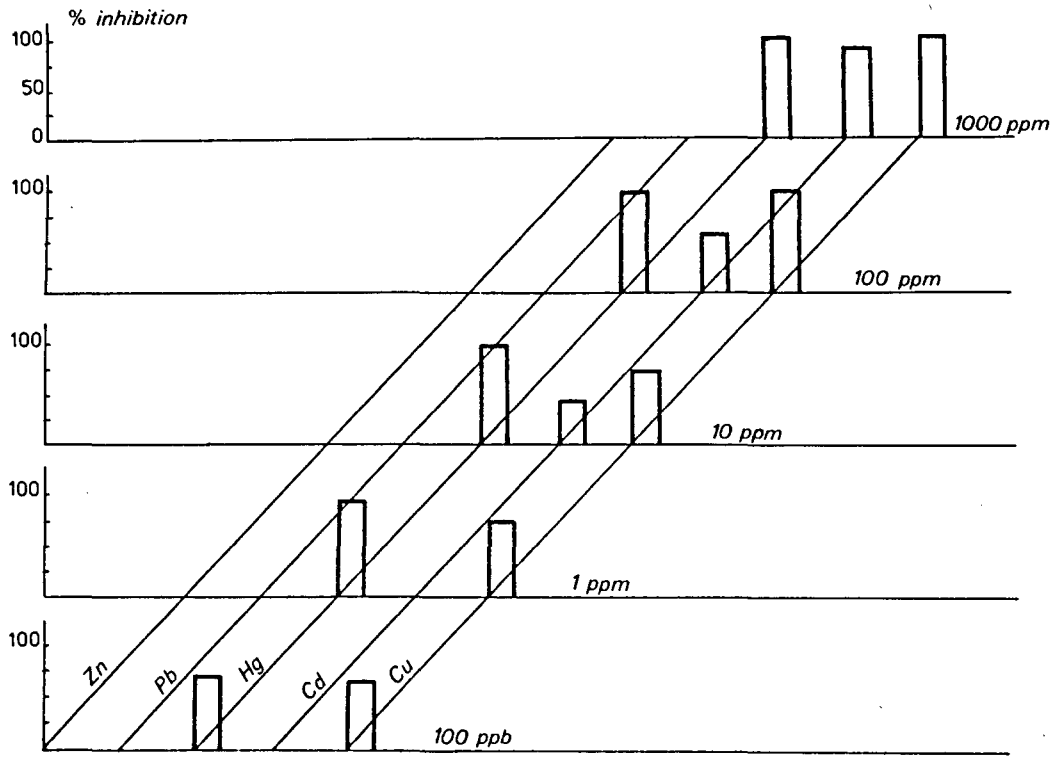


fig. 3.
Phaeodactylum tricornutum without addition of culture medium.

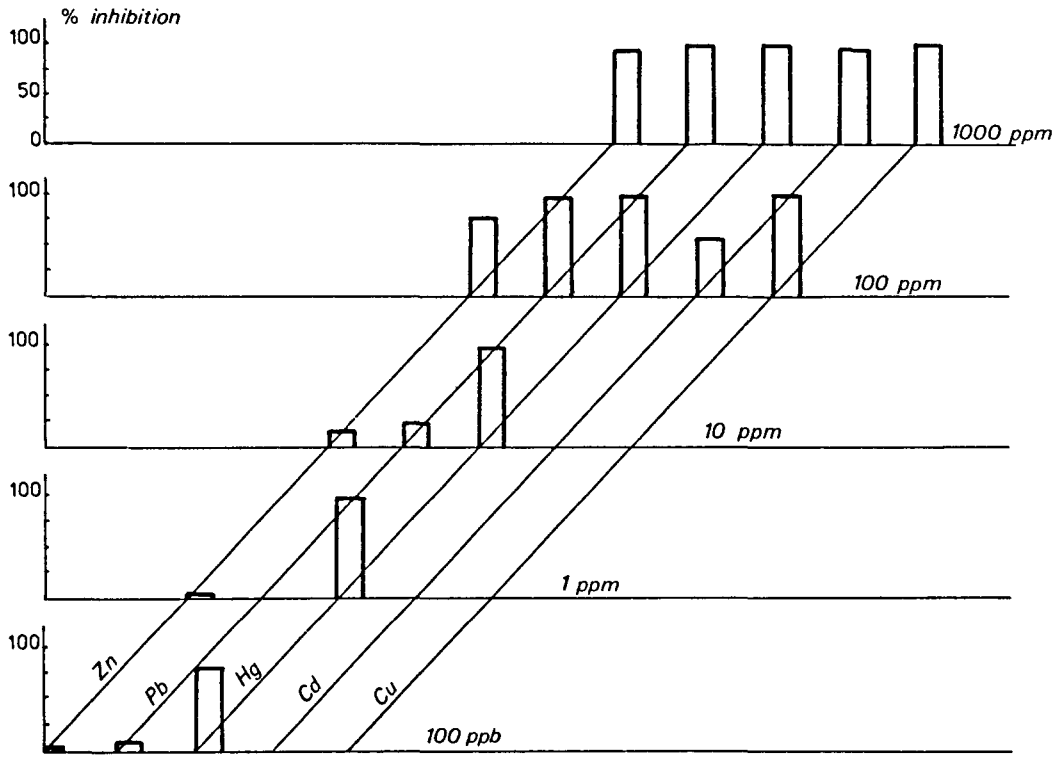


fig. 4.
Phaeodactylum tricornutum with addition of culture medium.

absorption [the centrifuged cells are mineralized in HNO_3 (65 % vol. conc.) + $HClO_4$ (70 % vol. conc.), 1/1].

Figures 5 and 6 show the effect of increasing Zn concentration on the growth curve of the two test organisms, figures 7 and 8a,b give the total amount of Zn, either adsorbed on the cell walls or entered in the protoplasm, as a function of time, expressed in 10^{-9} mg Zn per cell.

Without going into a detailed examination of the curves it is clear that the results are again quite different depending on the test organism :
a) in *Dunaliella* cultures the total Zn content per cell measured after one hour is almost the same whatever the amount of Zn^{++} added to the sea water; in *Phaeodactylum* cultures the Zn content varies enormously after one hour depending on the Zn^{++} concentration (with the exception of the 100 ppm case).
b) *Dunaliella* cells accumulate more Zn in function of time when the Zn^{++} concentration increases (not taking into account the exception at 100 ppm) and the curves show that higher accumulation can be correlated with a slowing down of the population growth.

Phaeodactylum cells which show high Zn contents at the early stages, lose Zn during the active phase of their growth curve (with the exception of what happens at 80 ppm , for which no explanation is given).

Table 1 shows the total Zn content of the cells after 7 days.

Table 1

Zn^{++} added (ppm)	Zn (10^{-9} mg) per cell	
	Dunaliella	Phaeodactylum
1	0.12	0.09
10	0.47	0.19
30	0.97	0.70
50	2.23	1.82
80	2.22	5.84
100	0.80	0.22

The amount of Zn per cell after 7 days increases although there is a large difference in the shape of the accumulation curves versus time,

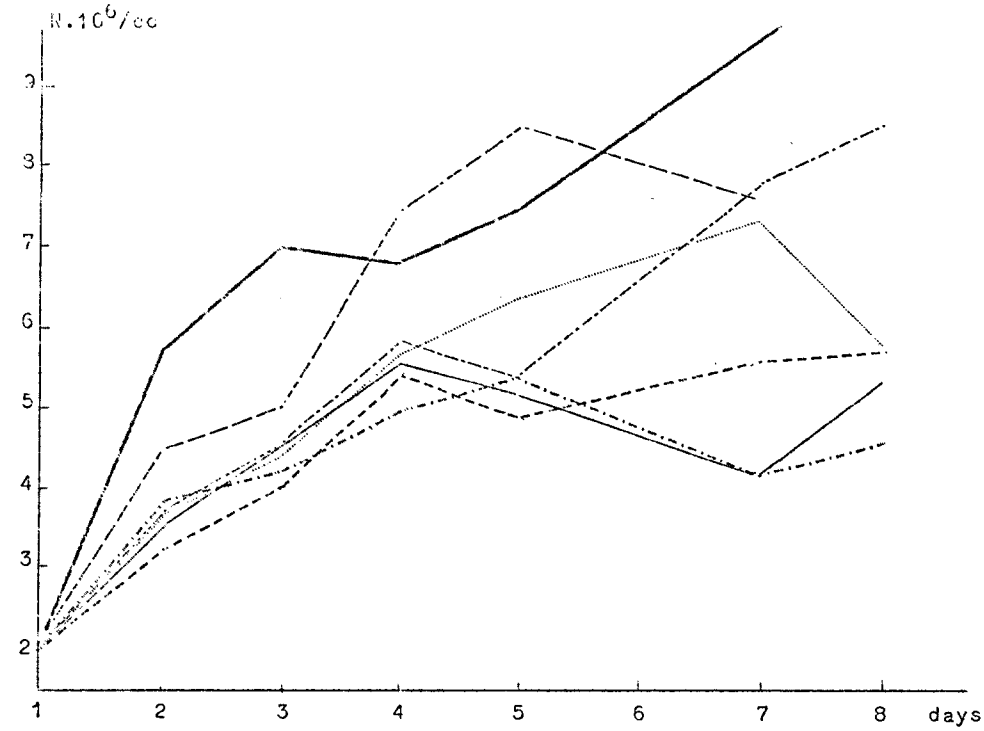
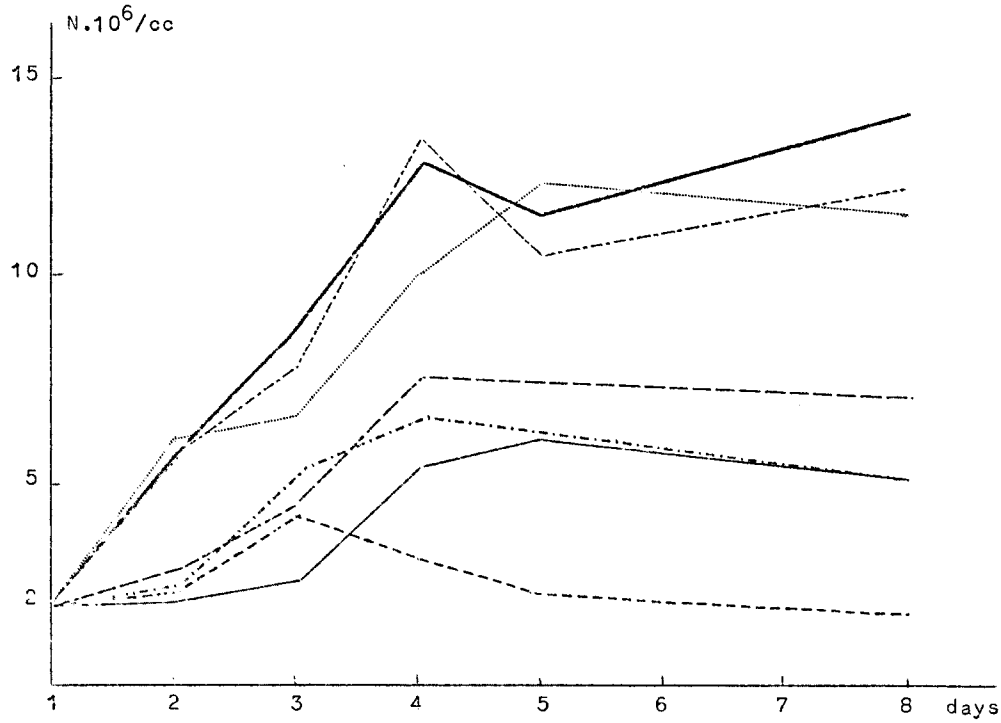


fig. 5.
Dunaliella viridis growth curve.



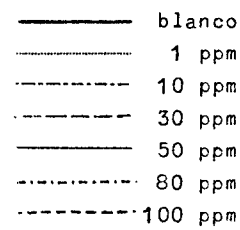


fig. 6.
Phaeodactylum tricornutum growth curve.

fig. 7.
Zn ad-absorption/cell unit by Dunaliella viridis.

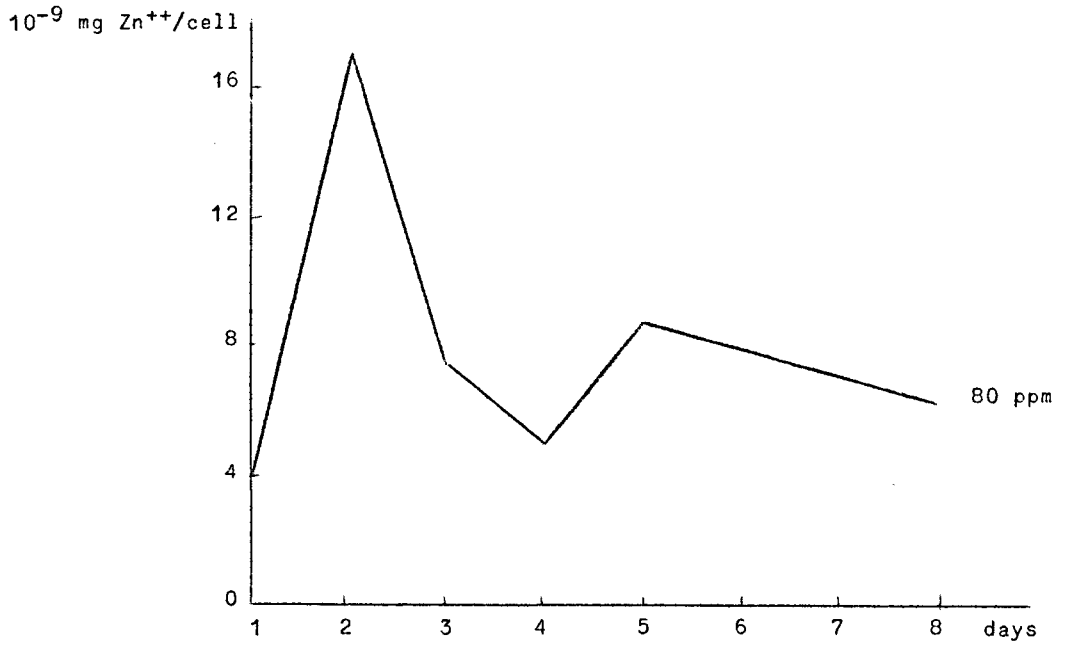


fig. 8a.

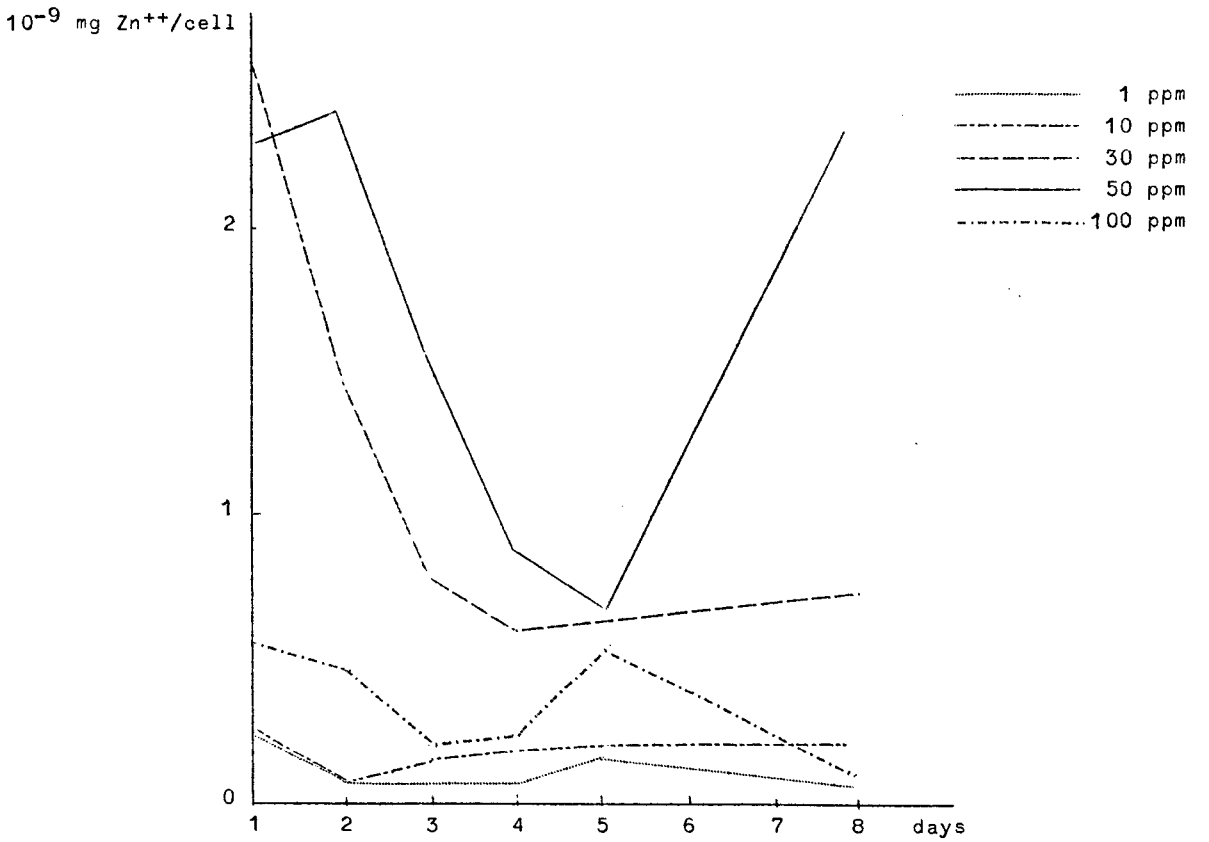


fig. 8b.
Zn ad-absorption/cell unit by Phaedactylum tricornutum.

with increasing Zn^{++} initial concentration in the sea water. A maximum is attained at 80 ppm . At 100 ppm the growth is at least for *Phaeodactylum* strongly inhibited and seems to be correlated to a marked decrease of the Zn content; the effect is less obvious for *Dunaliella* since the growth curves flatten at 50 , 80 and 100 ppm .

So it looks as if during active growth a decrease in the growth rate could be related to an increase in Zn content, but that strong growth inhibition has the opposite result and very much decreases the apparent Zn uptake.

The interpretation of these results is not easy since no distinction can be made between the Zn adsorbed on the cell walls or incorporated in the protoplasm without the use of radio-isotopes, besides the authors have made no attempt to evaluate mortality. Dead cells might behave differently compared to living ones.

The work however shows that the burden per cell can reach rather large values, and that there is a marked difference between naked cells and those having a silica shell.

If the amount of Zn adsorbed on the cell walls is large compared to what enters the cell and varies in time, toxicity experiments become difficult to interpret, although the input to the food chain can still be assessed.

A further complication arises from the fact that the amount found on and in the cells becomes in dense cultures large enough to alter the initial Zn^{++} concentration in the sea water. At 1 ppm , 88 % of the Zn is found bound to the cells after 7 days in *Dunaliella* cultures, dropping to 11.7 % at 80 ppm and 4.5 at 110 ppm . Similar effects are found in *Phaeodactylum* suspensions.

Strict control of the amount of toxic substances to which organisms are exposed, although at first sight a perequisite, seems to have been overlooked by many people. The above findings rightly draw attention to this important problem.

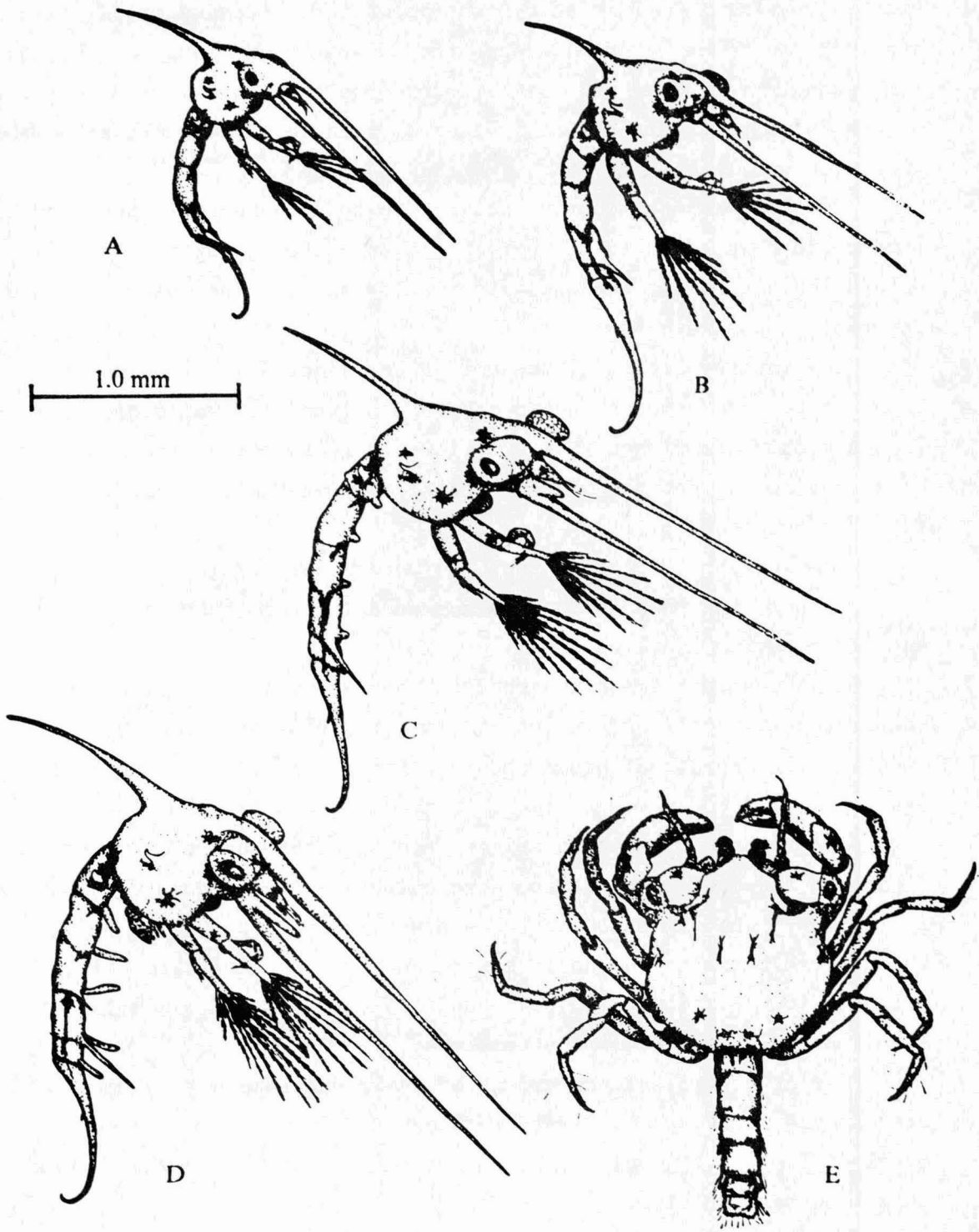


fig. 9.
Zoeal stages one through four (A, B, C, D) and megalops (E) of the R. harrisii.

2.- Invertebrates

2.1.- Combined effects of Zn^{++} and Pb^{++} on the larval development of the mudcrab *Rithropanopeus harrisii* [Benijts *et al.* (1974a)]

Costlow *et al.* (1971) have shown that the little mudcrab *Rithropanopeus harrisii* can rather easily be reared in laboratory conditions and that high survival conditions can be obtained.

The hatched larvae are kept in 20 % sea water in small glass scales; they are fed with Artemia-larvae; the temperature is kept at 23.5 °C , the animals are reared 2 h in the dark and 12 h under controlled light conditions. The larvae go through 3 zoeal stages (fig. 9); at the megalops stage each is kept in isolation to avoid cannibalism. The development is followed until the first crab-stage. Zn^{++} and Pb^{++} are added as chlorides and a series of concentrations pairs is tested (0 , 25 , 50 ppb) . Per concentrations pair 5 parallel experiments with 10 larvae are carried out. The average development duration is calculated, the combination 0 ppb Zn^{++} and 0 ppb Pb^{++} being the blank experiment for which a development duration of 14.35 days is found.

The results after statistical treatment are displayed in figure 10 showing the contours of equal mean time of development as a function of both the Zn^{++} and Pb^{++} concentration.

The graph shows a significative optimum combination at about 30 ppb Zn^{++} and 25 ppb Pb^{++} leading to a shortening of the larval development to 13.95 days.

It can further be shown that an increase of the Pb^{++} concentration from 0 to 50 ppb increases the development duration following a quadratic law. At 50 ppb Pb^{++} and 0 ppb Zn^{++} the duration is 14.35 days. An increase of the Zn^{++} concentration from 0 to 50 ppb has no significative effect. Lead is more toxic than zinc.

The "dramatic" synergism described for Zn^{++}, Pb^{++} and Hg^{++} in the growth of the marine ciliate *Cristigera* by Gray and Ventilla (1973) in the range 0-100 ppb Zn^{++} and 0-188 ppb Pb^{++} , the effects of both these metal ions being more important than that of Hg^{++}, is not observed in the present case, on the contrary.

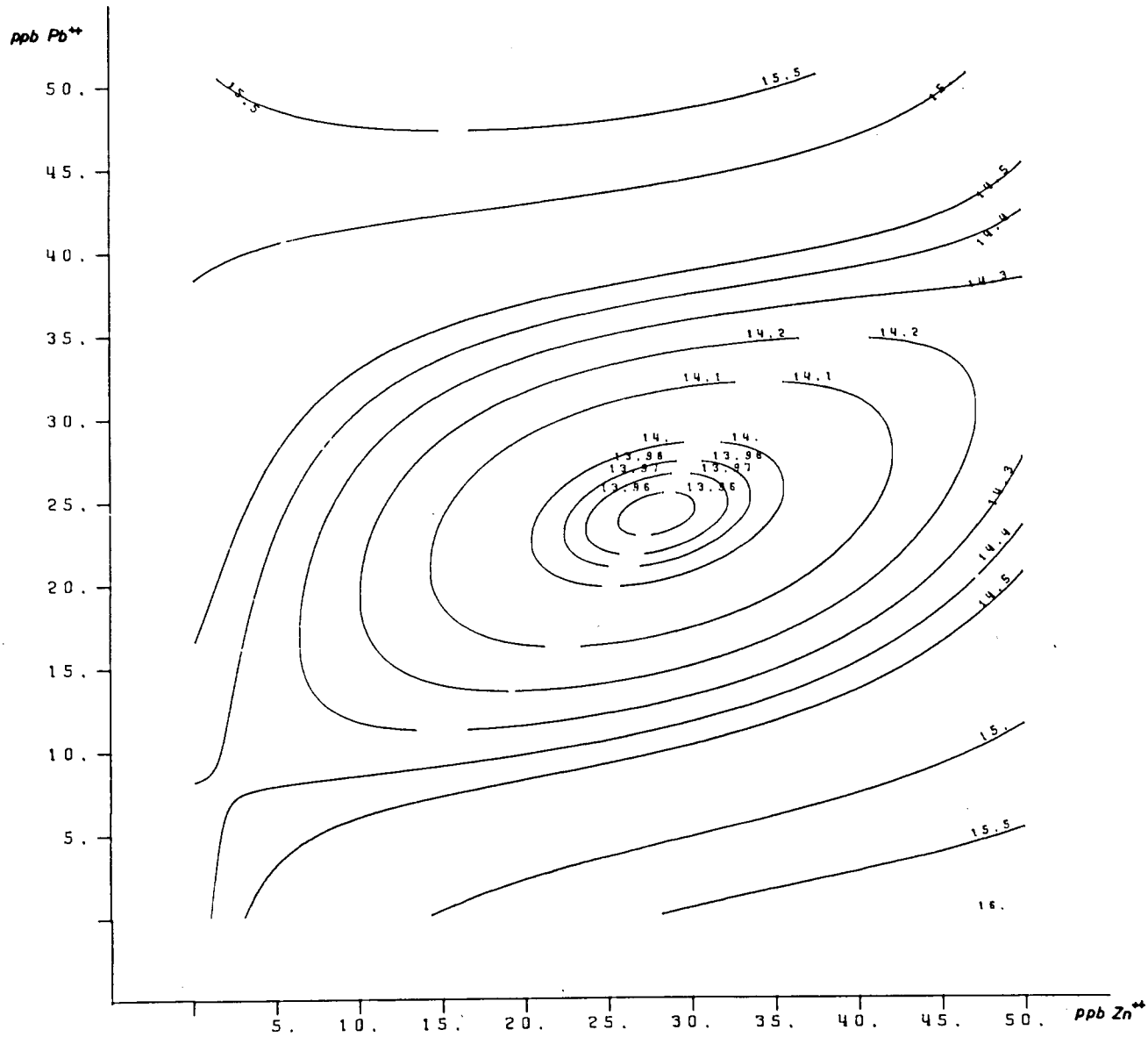


fig. 10.
Mean time of development of Rithropanopeus harrisii from hatch to megalops in days (5 replicates).

Again the choice of the test organisms is crucial and certainly there must exist species, especially at very low metal concentrations like in these experiments, where one might well be in the range where metal ions play an essential role in some metabolic pathways and therefore enhance biological processes instead of inhibiting them.

Whatever the result however the method used by Benijts *et al.* with its type of display, is an elegant tool to show synergisms either positive

or negative of importance either in normal physiological conditions or in acute intoxications.

2.2.- Effect of Hg^{++} on *Mytilus edulis* and *Asterias rubens* [Perpeet, Vloebergh (1974)]

2.2.1.- *Mytilus edulis*

a) Distribution of ^{203}Hg in the organs

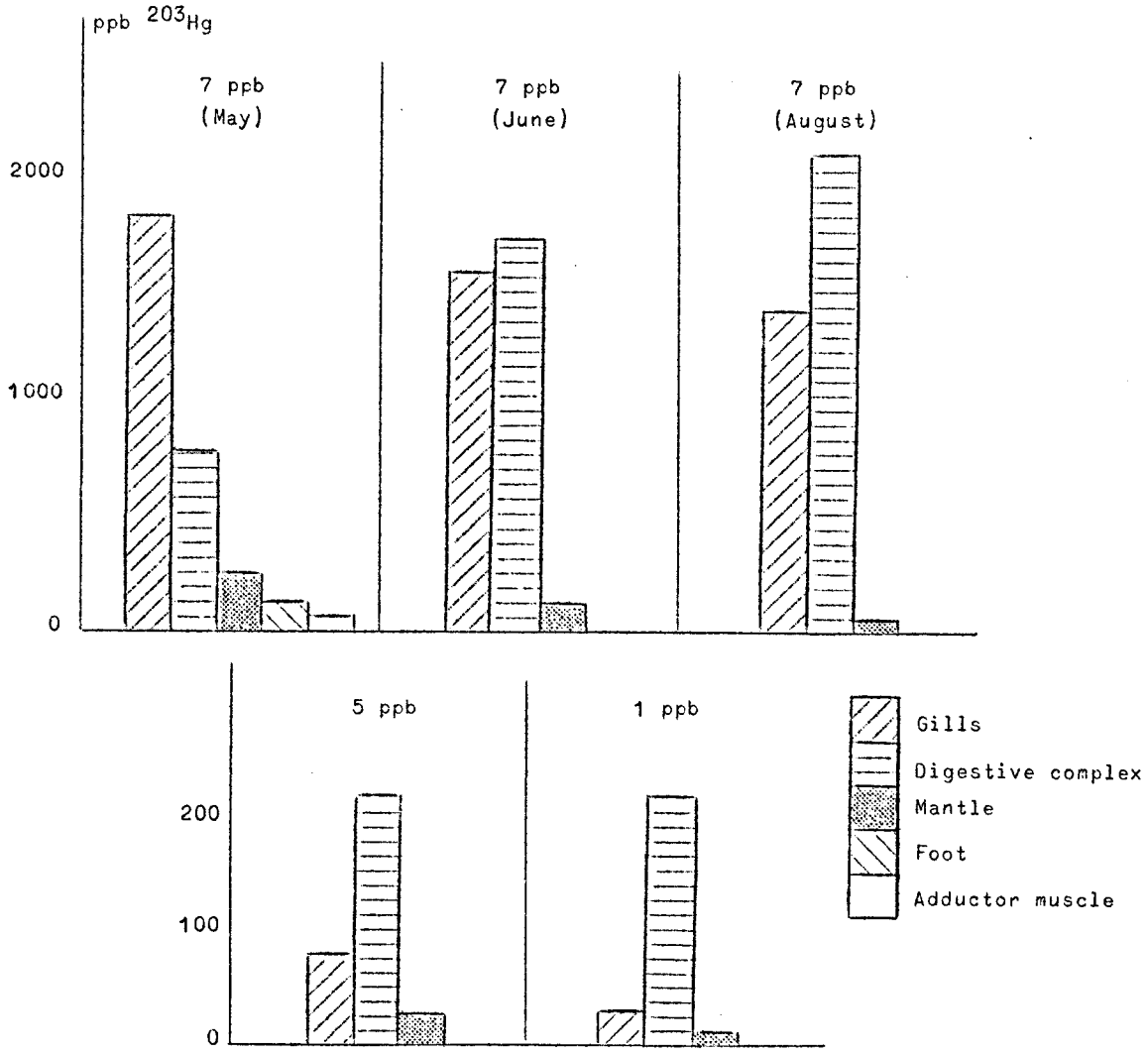


fig. 11.

Hg content of the organs of Mytilus edulis exposed to sea water containing 7, 5 and 1 ppb Hg.

Mussels (20 specimens) are placed during 24 h in 51 artificial (32 ‰) sea water contaminated with 7 ppb ^{203}Hg (0.005 µCie)($HgCl_2$), the radioactivity in the gills, the digestive tract and related organs, the mantle, the adductor muscle and the foot is measured using a liquid scintillation technique (Packard Tri-Carb 3375). The tissues are dissolved in 1 cm^3 Soluene 350 (50 °C, 2 h); 10 cm^3 Dimilume are then added. The experiments are carried out in May, June and August. In May the mussels are sexually mature and have used most of their stored glucids and lipids. Figure 11 shows that in May accumulation is highest in the gills, it remains important in June and August, but becomes even higher in the digestive tract. The adductor muscle contains 30 times less Hg, the mantle and the foot are a little more contaminated.

At 1 ppb and 7 ppb, during sexual resting period, the distribution follows the same pattern, but the amounts of Hg found are much reduced. It seems that there is a threshold concentration between 5 and 7 ppb where accumulation increases sharply.

b) Kinetics of the ^{203}Hg accumulation in the organs at 5 and 1 ppb

The sea water is replaced every day during 7 days. Figure 12 shows the rate of intake of ^{203}Hg in the various organs. Accumulation is faster the higher the Hg concentration.

Figure 13 indicates the effect of salinity : the gills accumulate at a lower initial rate in 16 ‰ sea water containing 5 ppb ^{203}Hg.

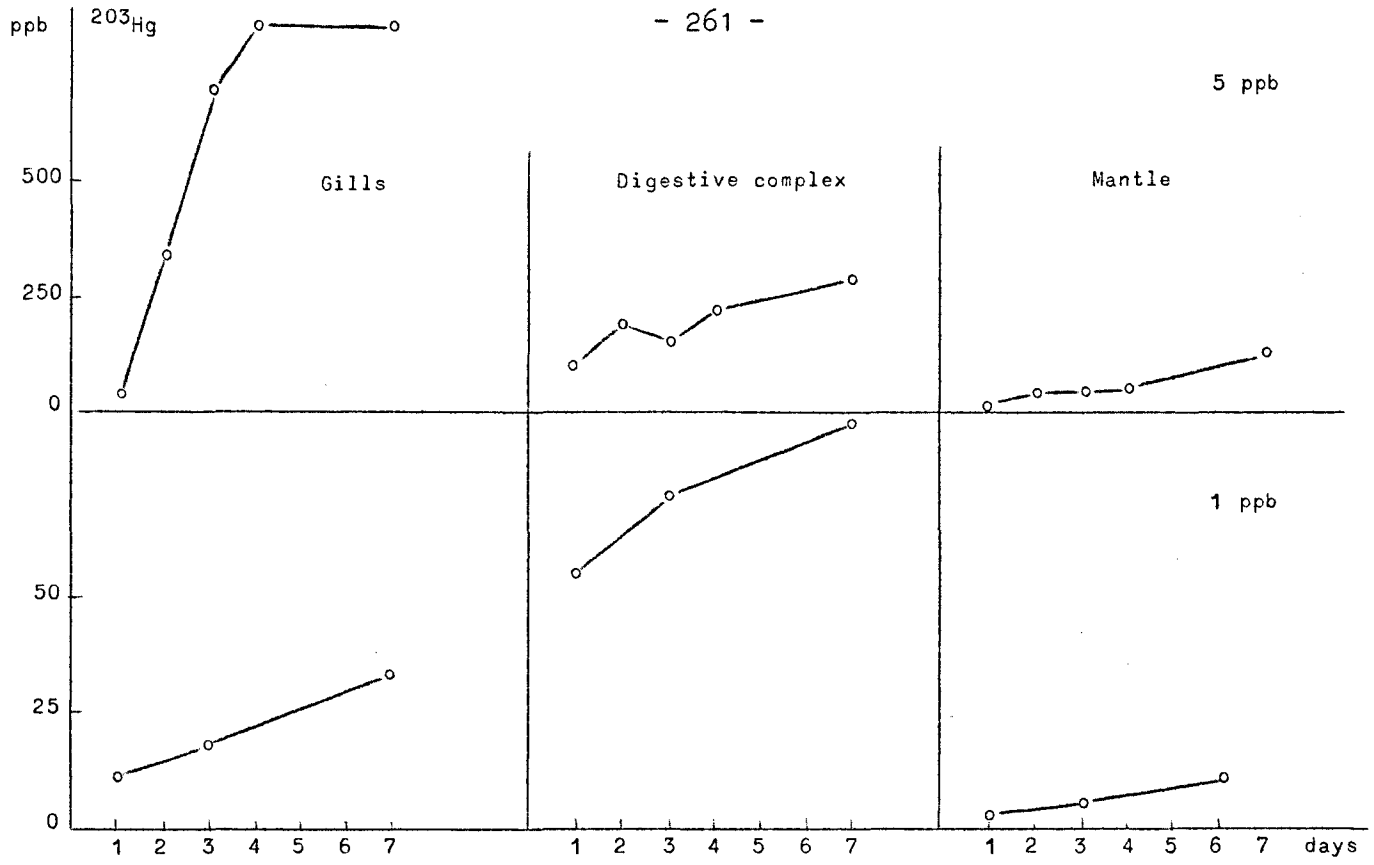


fig. 12.
Kinetics of accumulation of Hg in Mytilus edulis exposed to sea water containing 5 and 1 ppb Hg.

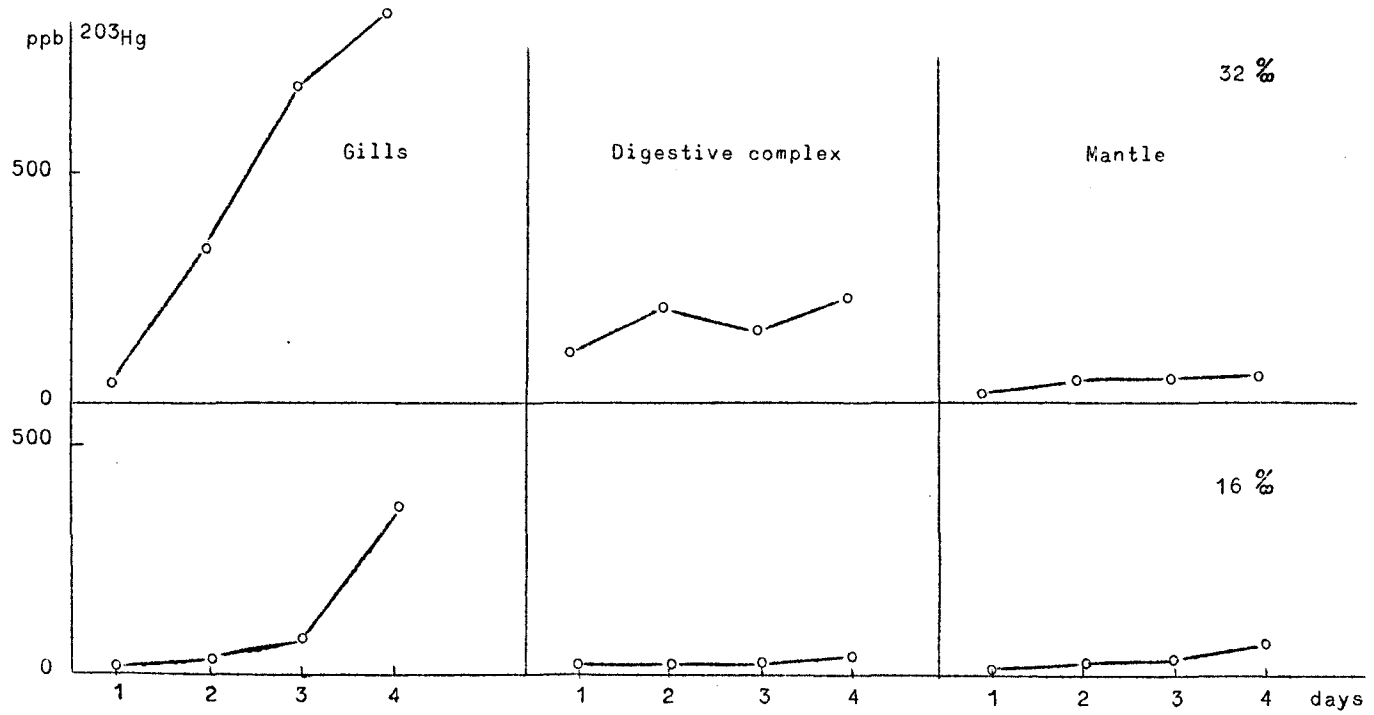


fig. 13.
Effect of salinity on the uptake of Hg by Mytilus edulis exposed to sea water containing 5 ppb Hg.

c) Kinetics of the release of the ^{203}Hg burden

Mussels intoxicated during 24 h in sea water containing 7 ppb ^{203}Hg are placed in Hg-free sea water, changed every day. No Hg is found in the water, the Hg seems to be eliminated in pseudo-feces. Figure 14 shows that a redistribution of the initially incorporated ^{203}Hg probably happens before its release, fastest in the digestive organs.

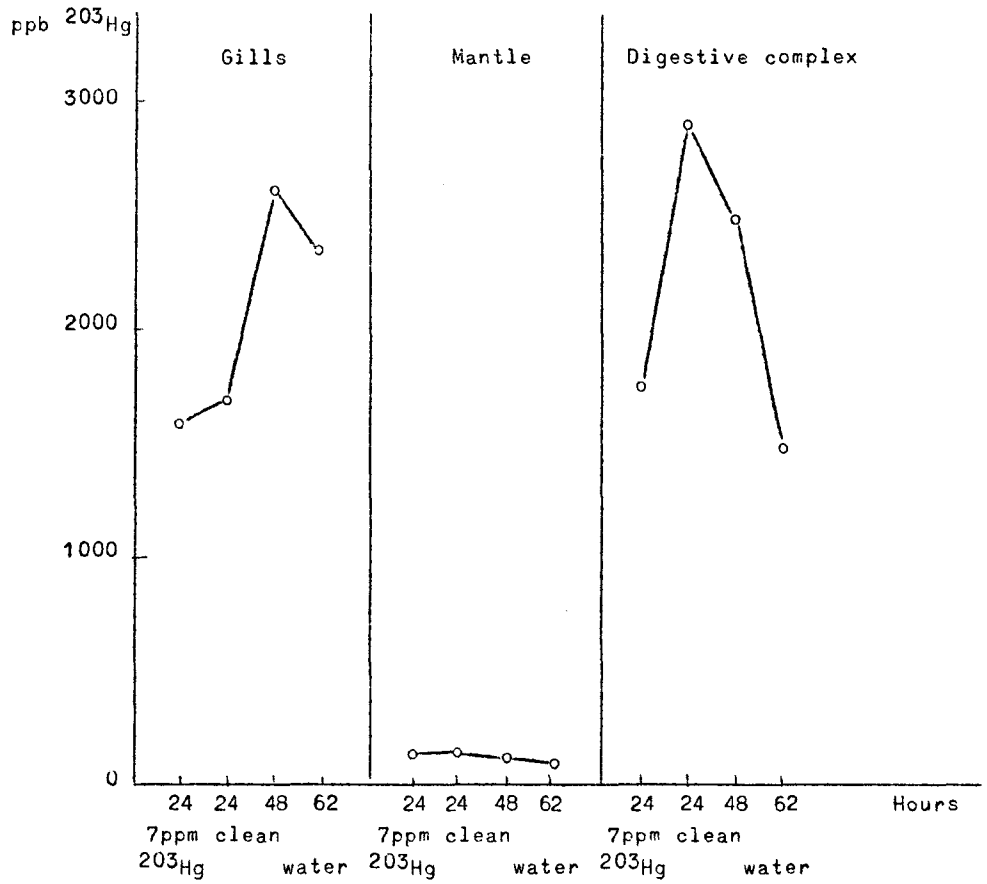


fig. 14.
Release of Hg by Mytilus edulis kept in non contamined sea water after initial intoxication (7 ppb Hg).

The authors have tried to evaluate the amount of ^{203}Hg lost to the atmosphere, adsorbed on suspended particles or on the aquarium walls. In natural sea water their results show that a large proportion of the added ^{203}Hg is quickly adsorbed on particulate matter. They use an

aquarium forming a closed system with traps at the air inlet and outlet (acid $KMnO_4$ solution). Starting at 10 ppb Hg in solution, they end at 8.6 ppb after 65 min ; 9.5 ppb Hg are found on the particles retained on a millipore filter.

With artificial sea water, in presence of mussels, fine particulate matter is formed which adsorbs part of the mercury. Evaporation represents however the most important loss. After 8 days, under these conditions at an initial concentration of 23 ppb the mussels however contain 2.3 ppm ^{203}Hg in the average.

2.2.2.- *Asterias rubens*

Five specimens are exposed in 5 ℓ sea water contaminated with $HgCl_2$ containing ^{203}Hg . The results are expressed as if the total amount of mercury was radioactive.

a) Distribution of ^{203}Hg in the organs (1 ppm Hg in the sea water)

Figure 15 shows that the podia and various parts of the skin accumulate large quantities of Hg . After 15 h the animals are in very bad physiological condition (swelling of disc and arms, loss of pigmentation, cessation of movements). This severe intoxication explains probably the loss of Hg initially accumulated and its release in the water. Digestive organs do not participate at this Hg concentration.

b) Release of ^{203}Hg from *Asterias rubens* initially intoxicated in sea water containing 0.2 ppm Hg

The animals first intoxicated at 0.2 ppm Hg during 24 h are in much better condition than in presence of 1 ppm Hg .

The digestive tract and the podia accumulate Hg ; as in the case of Cu^{++} it seems that at high concentrations the heavy metals block the respiratory system, which limits the entry in other organs. At low concentration the Hg is distributed in organs other than the podia.

Figure 16 shows the release of the Hg load which is fast from the podia and the skin, but the situation is quite different for the stomach and the pyloric caeca, showing a redistribution of the toxic material.

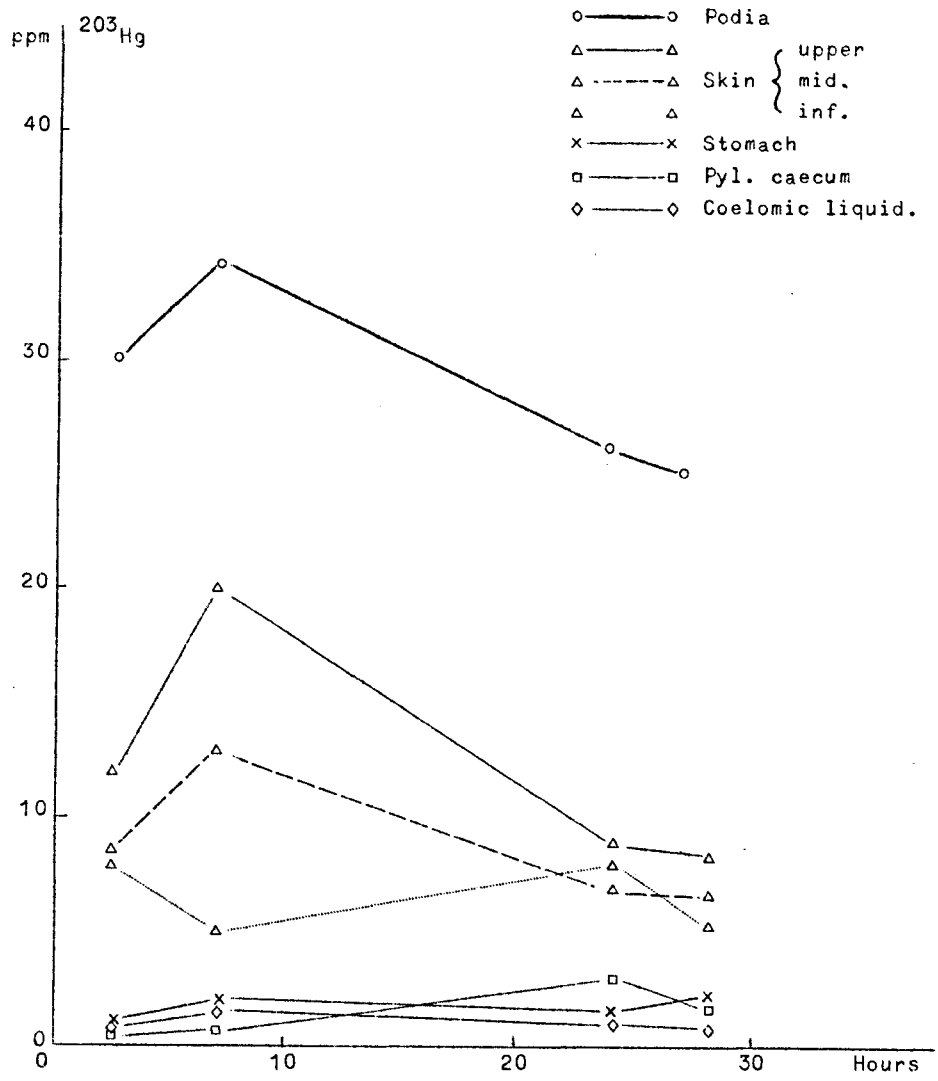


fig. 15.
Uptake of Hg by Asterias rubens exposed to sea water containing 1 ppb Hg.

c) Intoxication of *Asterias rubens* fed on contaminated mussels

Preliminary experiments show that one mussel contaminated in sea water containing 0.8 ppm Hg and fed to one *Asterias rubens* produces an increase of radioactivity in the stomach and the pyloric caeca. The podia and the skin are little affected. The starfish contains about 10 times less Hg than the mussel per gram.

When fed with one mussel exposed to 5 ppb Hg , the starfish accumulates progressively Hg in the pyloric caeca as shown in table 2.

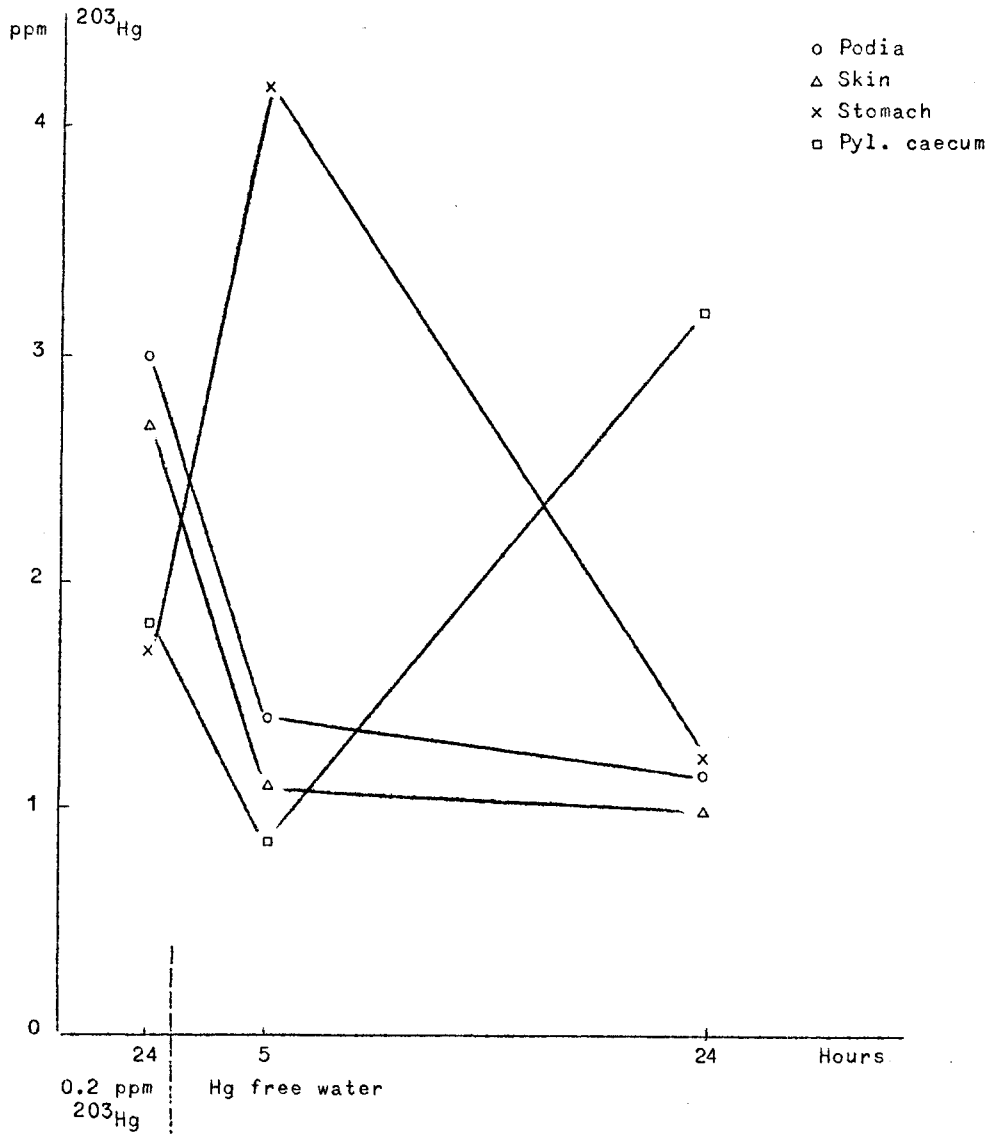


fig. 16.

Release of Hg by Asterias rubens kept in non contamined sea water after initial intoxication (0.2 ppm Hg).

Mucus is formed by the starfish, containing as much as 13 ppb Hg . This process might prove to be an unexpected excretory pathway.

The experiments performed with ^{203}Hg allow to study the effect of very low concentrations of Hg . They show that direct intoxication is more effective than intoxication by ingestion of contaminated food in the case of *Asterias rubens*. They also show how fast Hg is taken from water in the gills of mussels. Conditions similar to those created

Table 2

^{203}Hg content (ppb) in the organs of Asterias rubens fed with contaminated mussels

Organs	Controls		48 hours digestion		106 hours digestion		127 hours digestion	
Podia	0.27	0.51	0.98	0.48	1.47	2.34	0.85	1.22
Skin	1.11	0.54	0.98	1.24	0.63	1.55	1.48	0.87
Stomach	0.17	0.41	0.91	1.80	1.29	13.37	1.50	0.60
Pyl. caecum	0.09	0.17	6.20	4.65	10.10	3.89	55.17	14.13
Rect. caecum	0.32	1.50	2.29	1.04	1.65	6.79	13.60	6.50
Gonad	0.14	0.33	1.39	5.00	0.80	-	6.40	0.39

in the laboratory are seldom found in natural environment, since Hg is probably adsorbed on particulate matter in the water column or in sediments where anaerobic conditions might lead to very stable forms but where bacteria might also produce dangerous methylated forms. However, direct intoxication is a very fast process, which exists even at very low concentrations of free ions and it cannot be overlooked as one of the important entry routes of heavy metals in marine animals, many of which filter continuously enormous amounts of water through their respiratory system. Some do also retain suspended matter eventually loaded with heavy metals. Release of mercury in the sea results probably in most cases in local effects, because of fast adsorption on particulate matter : mussels collected at 3 km from an outlet have been found to contain 0.93 ppm Hg [Fimreite *et al.* (1971)], the specimens found at 11 km distance contained only 0.11 ppm Hg .

d) Cr, Cd, Cu, Pb, Mn, Zn and Fe content of *Mytilus edulis* collected in the Scheldt [Vanden Bossche (1975)]

The mussels have been collected, 100 at a time and grouped 4 by 4 in function of their length (varying between 2 and 6 cm) downstream, at Perkpolder, Terneuzen and Hoofdplaat. The specimens have been taken in January and May to show eventual seasonal effects in connection with sexual maturity (May).

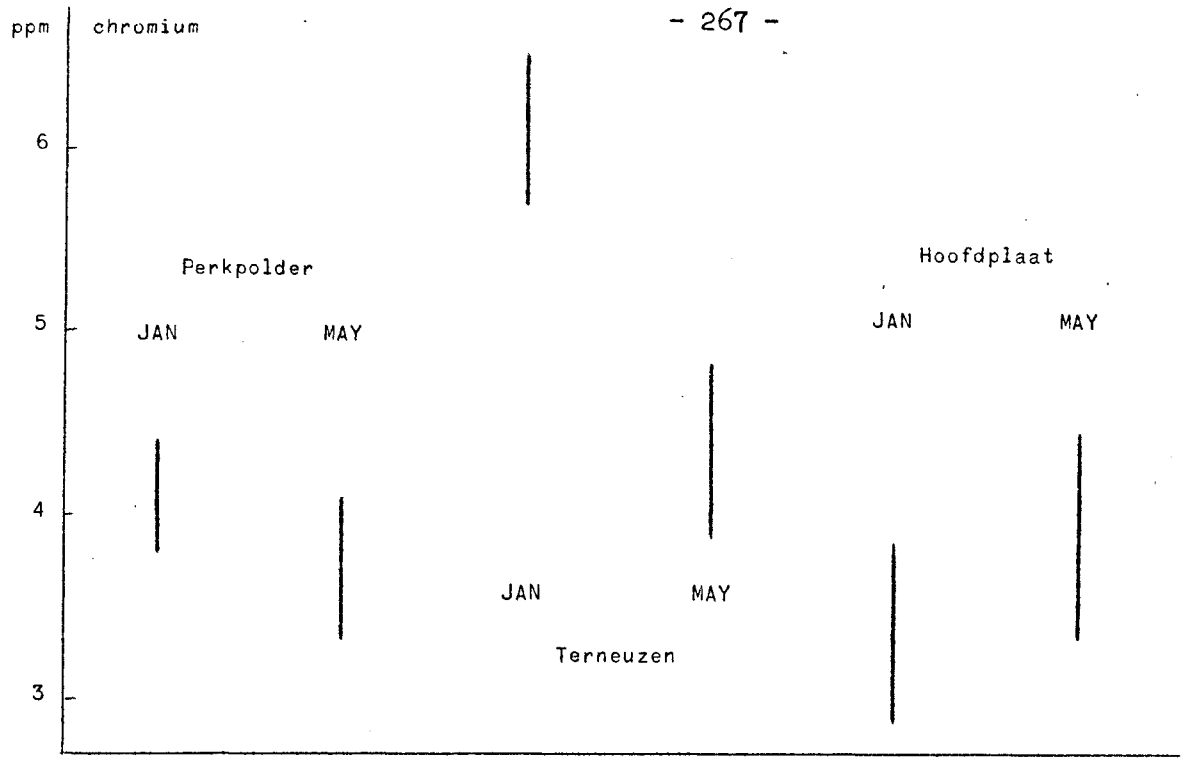

ppm
chromium
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fig. 17.

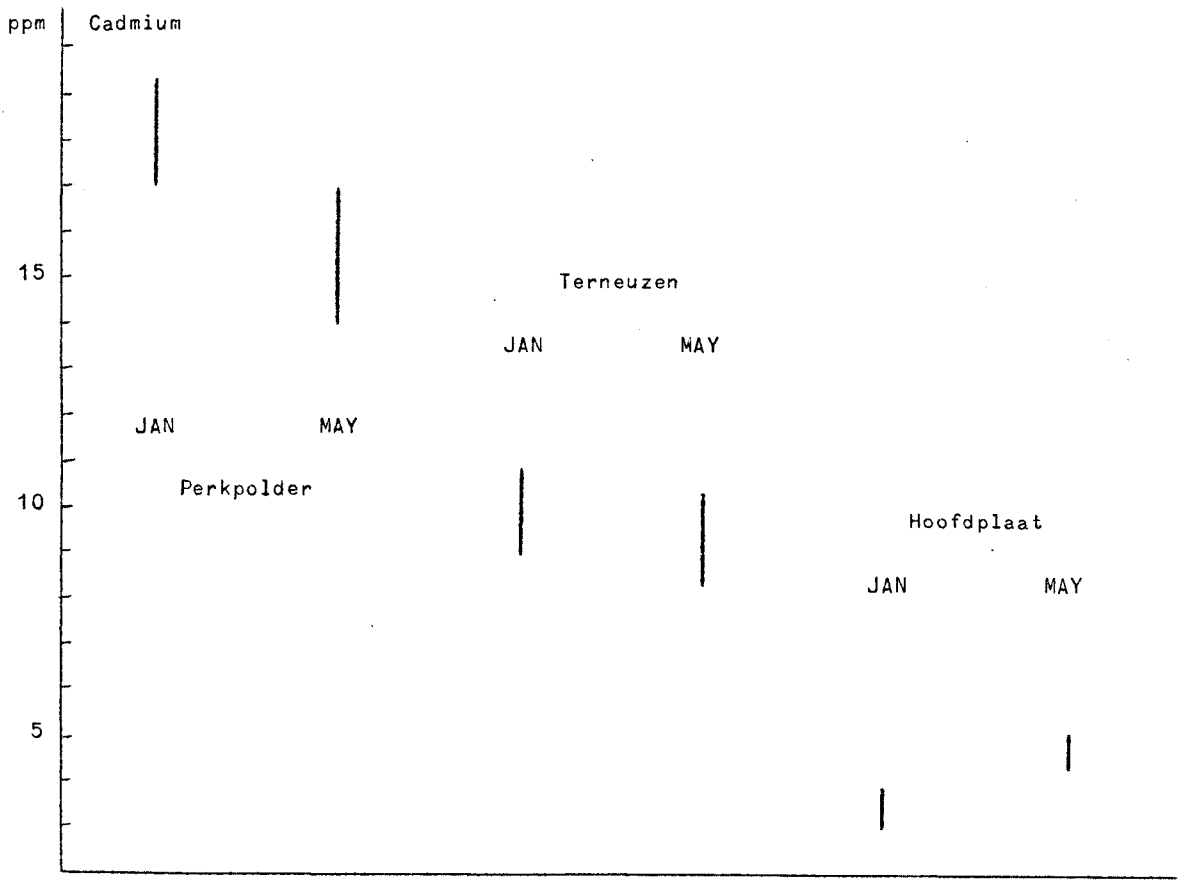
ppm
Cadmium
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fig. 18.

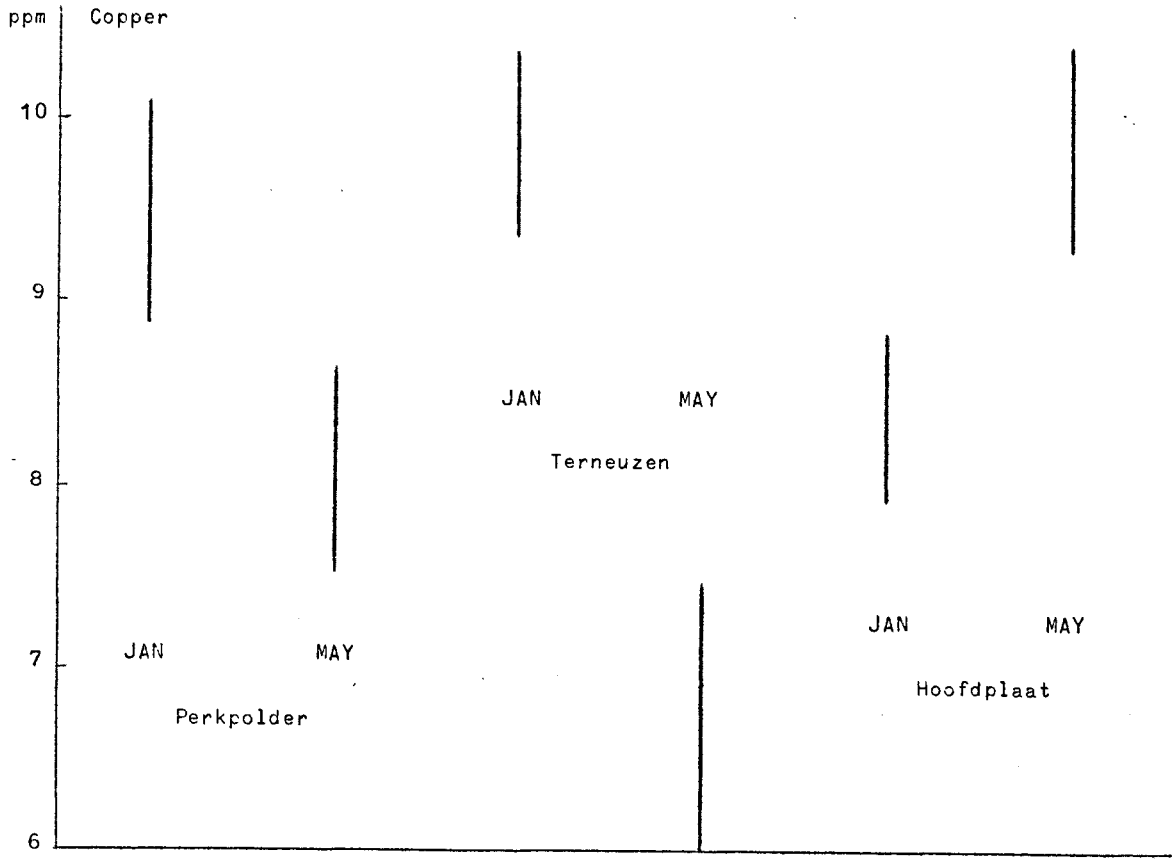
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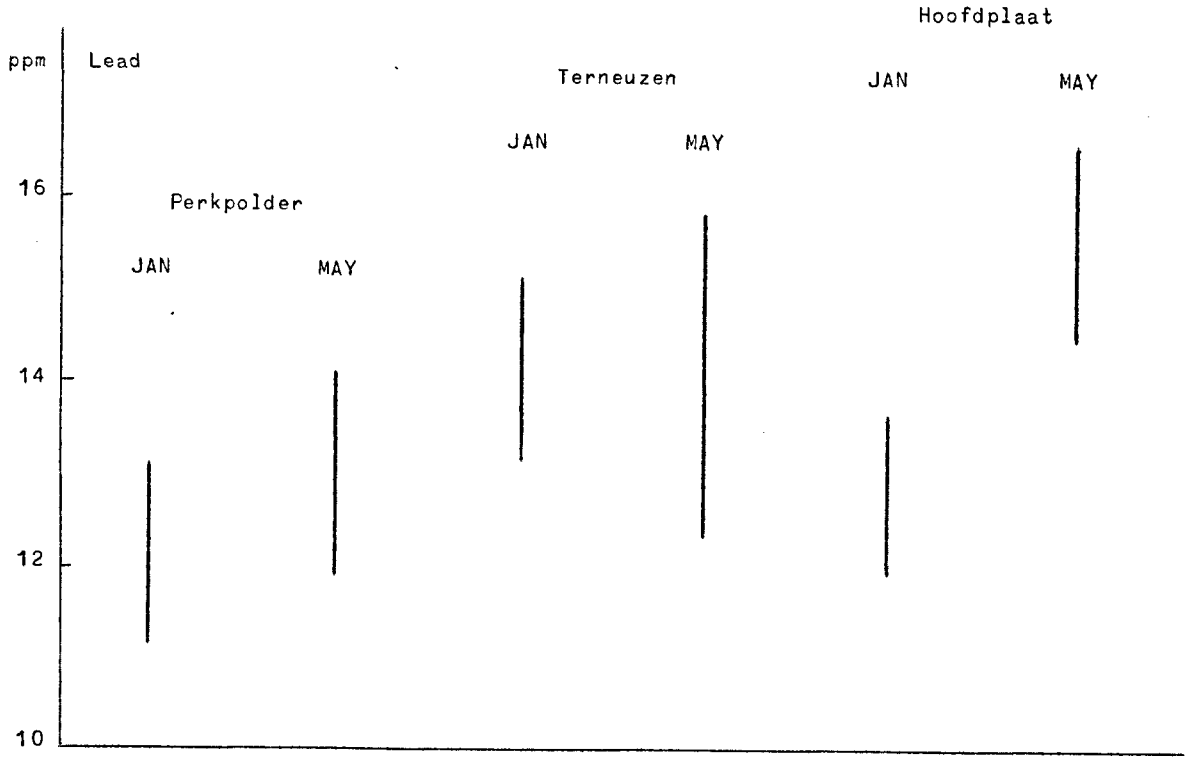
fig. 19.


ppm
Lead
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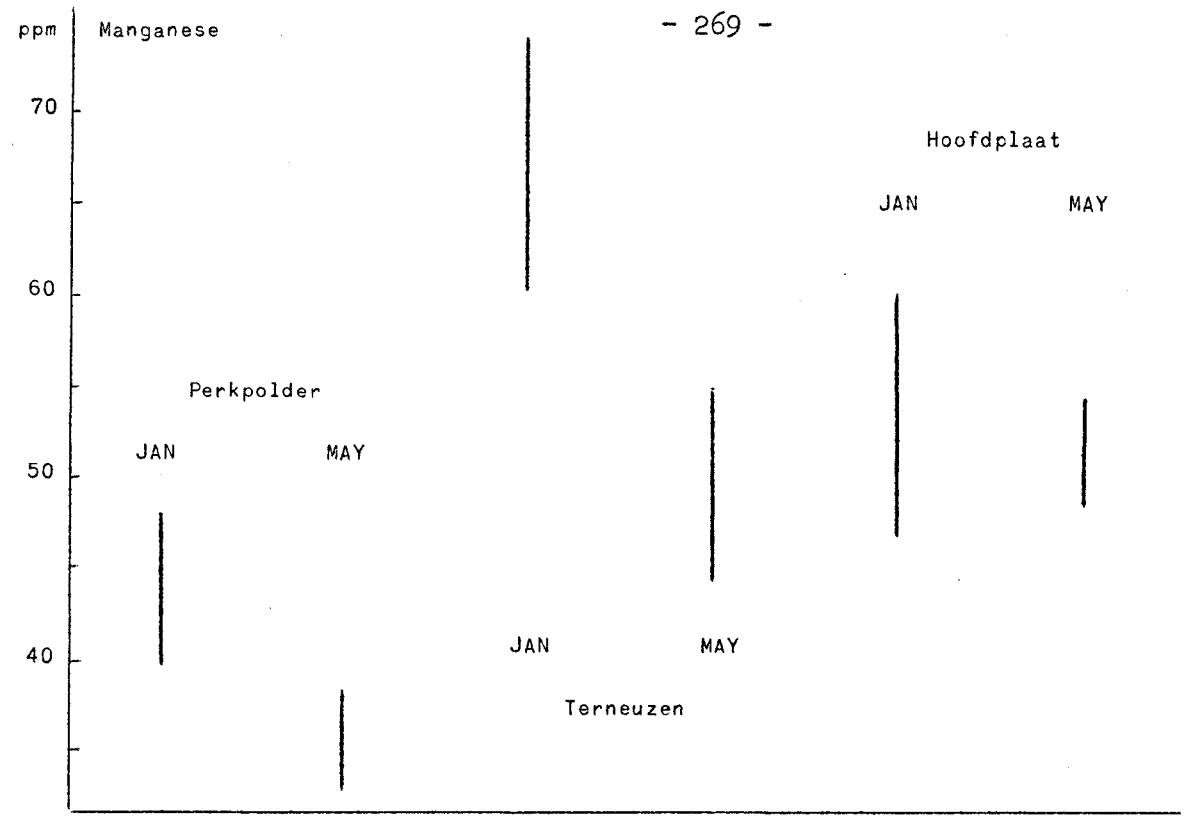
Hoofdplaat
JAN
MAY

fig. 20.


ppm
Manganese
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Perkpolder
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MAY
JAN
MAY
Terneuzen
Hoofdplaat
JAN
MAY

fig. 21.

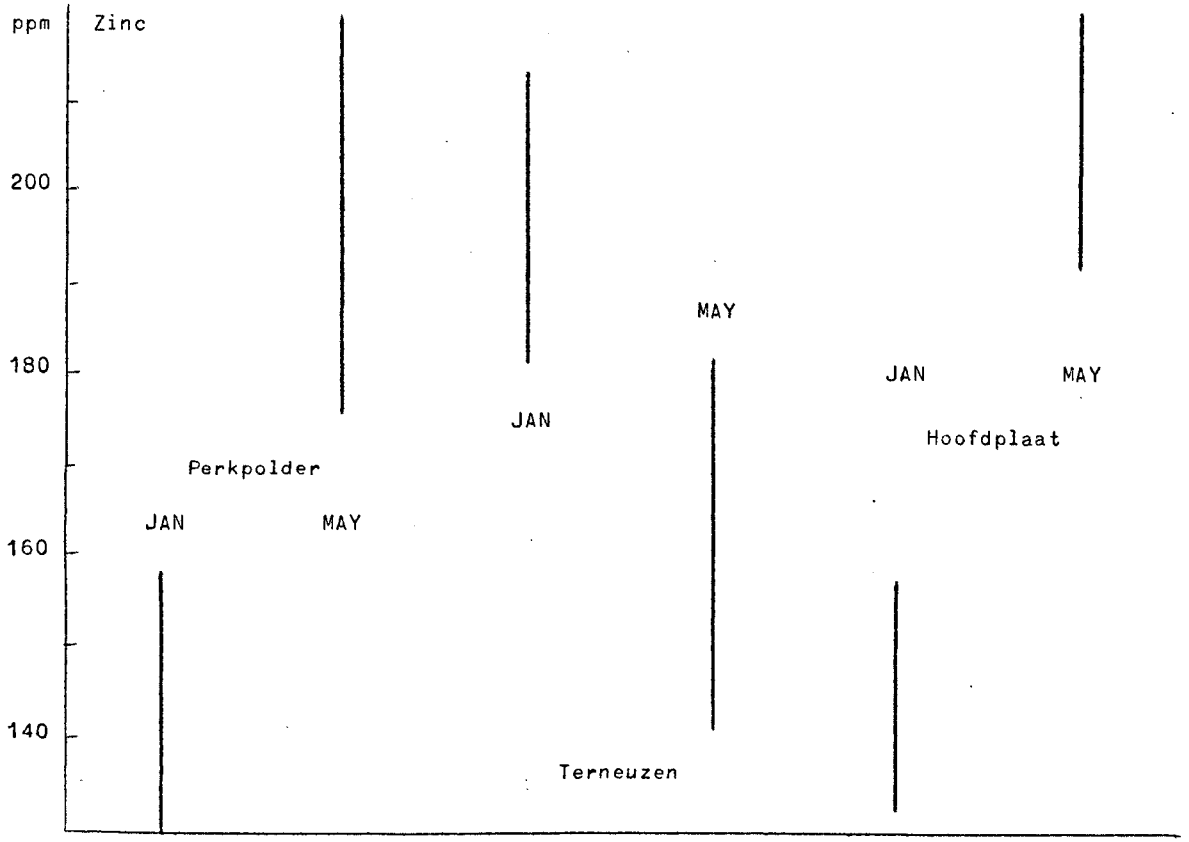

ppm
Zinc
200
180
160
140
Perkpolder
JAN
MAY
JAN
MAY
Terneuzen
JAN
MAY
Hoofdplaat

fig. 22.

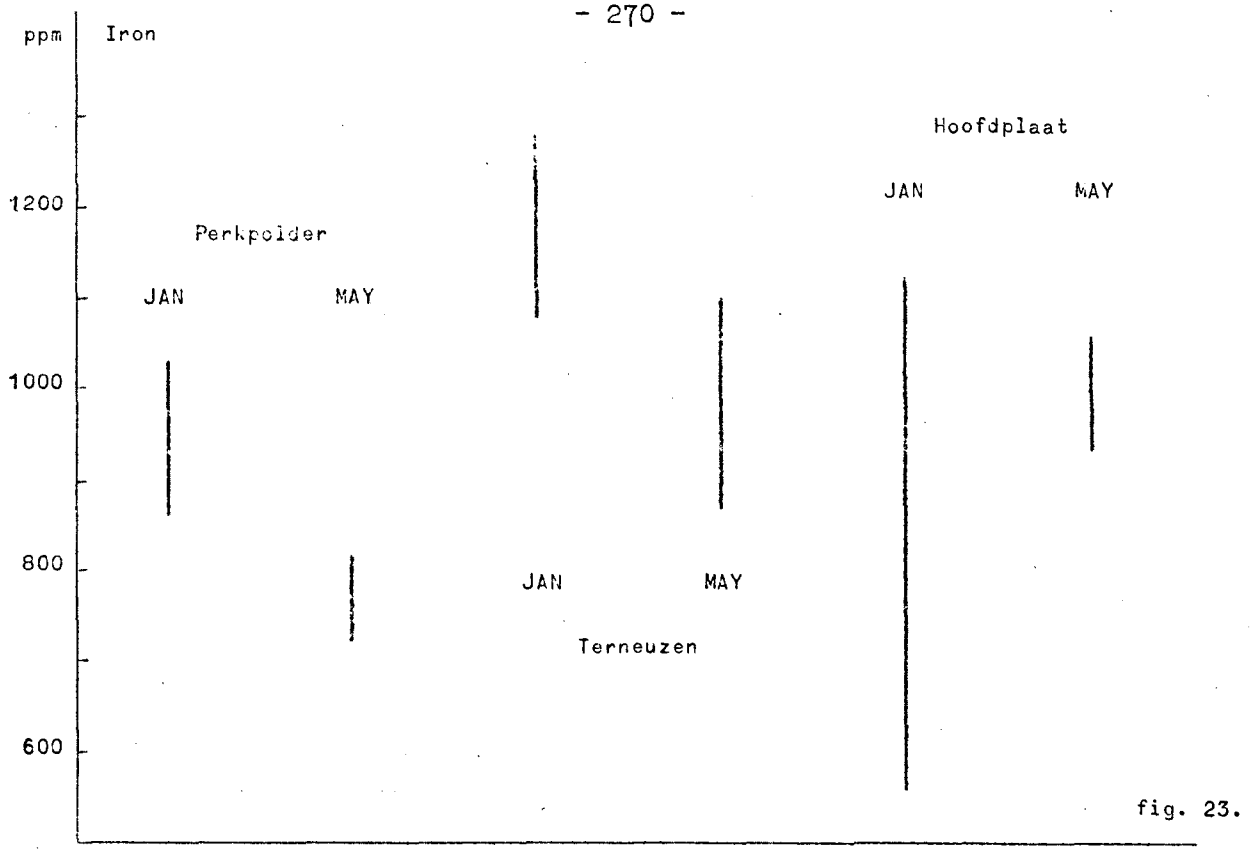


fig. 17-23.
Cr, Cd, Cu, Pb, Mn, Zn, Fe content of Mytilus edulis collected in the Scheldt.

The mussels kept in deep freezer are dried at 115 °C during 24 h, ashed at 450 °C. The ashes are suspended in 3 cm^3 HCl and 1 cm^3 HNO_3. After heating and slight dilution, the solution is filtered on Sartorius 25 mm ∅ SM 12804 filters and brought to 25 cm^3. It is analysed by atomic absorption (Perkin Elmer 303).

The results are indicated in figures 17 to 23 expressed in ppm dry weight (µg/g).

There seems to be no correlation between the metal concentrations and the size of the animals. There is no sign of increased accumulation in older specimens. None of the results show a simultaneous increase or decrease of all metals at one locality. There is no way to decide which place is more contaminated.

Comparison of the values found in January and May however shows some regularity : the concentration of Cd, Cu, Fe, Mn and Cu is less in May at Perkpolder and Terneuzen. This is however not true for Zn and Pb at Perkpolder.

It is to be noted that the results at Hoofdplaat in January might be too low because of the use of a different filtering technique; the Cd concentrations at Perkpolder in January are probably too low because of a defect of the Cd lamp : the mean value should be around 16 ppm .

The results confirm the finding of Perpeet and Vloebergh (1973) regarding the absence of correlation with size; the date for Cu, Zn, Fe are lower than theirs, the data for Pb, Cd and Cr are in the same range.

The fact that lower concentrations are found in May might indicate the existence of some excretory mechanism, but this is only speculation since no data are available about the metal concentrations in the water, in the sediments, in suspended matter, at the same localities, at the same time in the year.

3.- Vertebrates (fish)

3.1.- Accumulation and excretion of Hg by *Myoxocephalus scorpius* (scorpion fish) [Bouquegneau (1975)]

Myoxocephalus scorpius, also called *Cottus scorpius* is of little importance as food resource, but plays an important ecological role. It is extremely voracious, feeding on crustaceans, fish eggs and larvae. It is found along the North Atlantic and the North Sea coasts.

The fishes are kept in natural sea water and exposed to sublethal doses of 0.1 ppm and 1 ppb Hg ($HgCl_2$ or CH_3HgCl). The water is changed every day. The methodology is the same as described in the previous work on the eel *Anguilla anguilla* [Bouquegneau (1973a)].

3.1.1.- Total Hg burden and body distribution in non-intoxicated fish

Table 3 shows the distribution of Hg in specimens from the region of Ostend and Den Helder (Netherlands). No significant difference is found between the fish originating from these two localities, but the total body burden is very high (1.1 ppm Hg), most of which located in the muscles.

Figure 24 indicates that there exists a correlation between the muscle content and the body weight, but no correlation is found between

Table 3

Distribution of Hg in non-intoxicated Myoxocephalus scorpius

Organs	Weight (g)	Concentration of Hg (ppm)					Hg burden (µg)
		(1)	(1)	(2)	(2)	$\bar{m} \pm ES$	
Muscles	71.5	0.9	1.3	1.7	1.4[1]	1.3 ± 0.2	93.0
Skin	10.5	0.4	0.6	0.5	0.2	0.4 ± 0.1	4.2
Stomach	5.1	0.4	0.7	0.7	0.6	0.6 ± 0.1	3.1
Gills	4.6	0.7	0.8	1.1	0.9	0.9 ± 0.1	4.1
Bones	3.2	-	0.4	0.8	0.9	0.7 ± 0.2	2.2
Liver	2.4	0.5	1.5	2.2	1.0[2]	1.3 ± 0.4	2.6
Gonads	0.8	-	0.4	0.4	0.7	0.5 ± 0.1	0.4
Intestine	0.7	0.4	0.5	0.9	0.8	0.7 ± 0.1	0.5
Kidney	0.4	0.8	1.0	1.0	0.7	0.9 ± 0.1	0.4
Bile	0.3	-	0.3	-	0.4	0.4 ± 0.1	0.1
Spleen	0.2	0.5	0.7	1.0	0.8	0.8 ± 0.1	0.2
Heart	0.2	0.4	0.8	0.9	0.3	0.6 ± 0.1	0.1
Brain	0.1	-	1.4	1.3	0.8	1.2 ± 0.2	0.1
Body weight	100	Total amount of Hg in 100 g fish					111.0 µg 1.1 ppm

(1) : 2 living specimens from the Fisheries Institute in Ostend.
(2) : 2 living specimens from the Marine Station of Den Helder.
1 Including the 25 results from fig. 1 [Bouquegneau (1975)].
2 Including the 25 results from fig. 2 [Bouquegneau (1975)].

the liver Hg concentration and the body weight. Correlation however does exist between muscle and liver burden : the more Hg in the muscles, the more in the liver.

3.1.2.- Total Hg burden and body distribution in fish intoxicated during one month in sea water containing 1 ppb Hg ($HgCl_2$ or CH_3HgCl)

Table 4 gives the distribution of Hg in fish kept during one month in sea water containing 1 ppb Hg ($HgCl_2$ or CH_3HgCl) . The results clearly demonstrate the accumulation of Hg in the various organs, the gills being especially affected in the case of HgCl ,

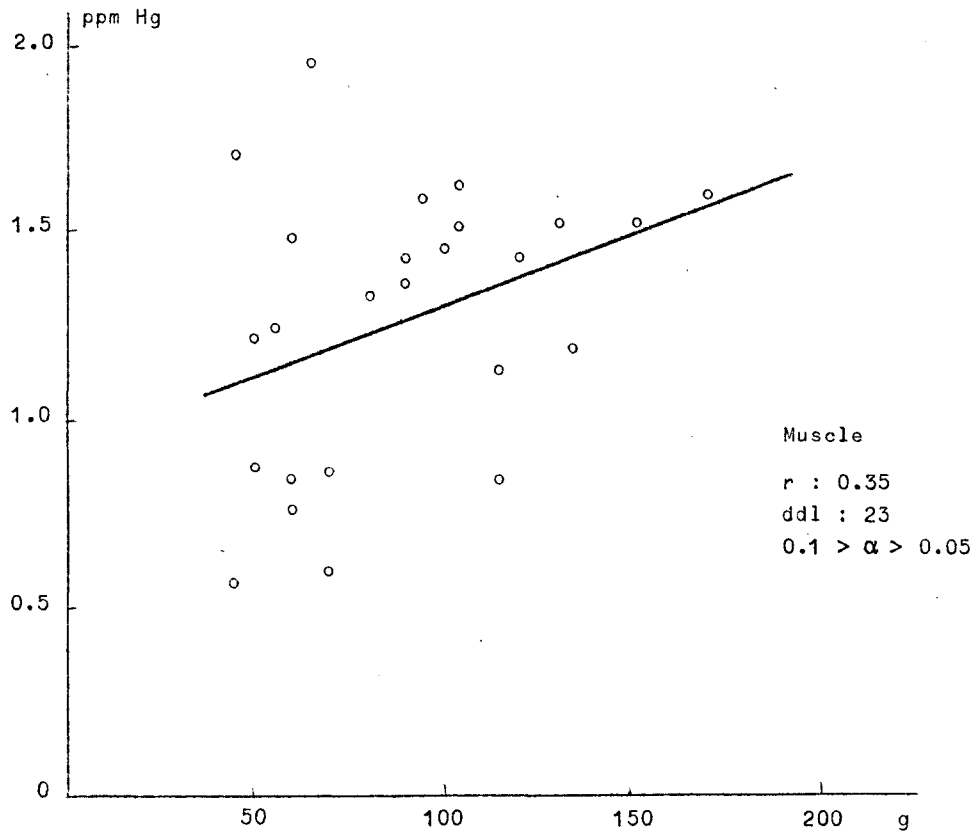


fig. 24.
Relationship between the concentration in muscles from Scorpion fish and body weight.

as observed in sea water adapted eels. The overall effect is more important with CH_3HgCl , but the distribution differs from that observed in the case of $HgCl_2$.

3.1.3.- Kinetics of Hg accumulation and excretion

Fish are first kept during 24 days in sea water containing 0.1 ppm Hg ($HgCl_2$) ; some specimens are placed in non-contaminated water after 8 days and the Hg content of the main organs is followed in both batches.

Figure 25 shows the kinetics of accumulation and release in muscle, liver and gills.

Table 4

Hg burden in Myoxocephalus scorpius kept during 30 days in sea water containing 1 ppb Hg ($HgCl_2$ or CH_3HgCl)

Organs	Weight (g)	Hg burden (µg)		
		Controls	1 ppb Hg in sea water during 1 month	
			$HgCl_2$	CH_3HgCl
Muscles	71.5	93.0	100.1	135.9
Skin	10.5	4.2	4.2	13.7
Stomach	5.1	3.1	3.1	5.1
Gills	4.6	4.1	12.0	11.5
Bones	3.2	2.2	1.9	2.6
Liver	2.4	2.6	4.6	7.4
Gonads	0.8	0.4	0.4	1.0
Intestine	0.7	0.5	0.5	0.8
Kidney	0.4	0.4	0.6	0.8
Bile	0.3	0.1	0.3	0.4
Spleen	0.2	0.2	0.2	0.5
Heart	0.2	0.1	0.2	0.5
Brain	0.1	0.1	0.2	0.2
Body weight	100			
Hg body load		111.0 µg	128.3 µg	180.4 µg
Hg body concentration		1.1 ppm	1.3 ppm	1.8 ppm
Concentration factor		-	(1.3 - 1.1).100 = 200	(1.8 - 1.1).100 = 700

The Hg distribution has been followed as shown in table 4 for the other body parts. The total concentration factor reaches 51 , 75 and 98 respectively after 8 , 16 and 24 days in presence of 0.1 ppm Hg ($HgCl_2$) , it falls to 46 and 35 when the fish is kept, after 8 days intoxication during 8 and 16 days in non polluted water.

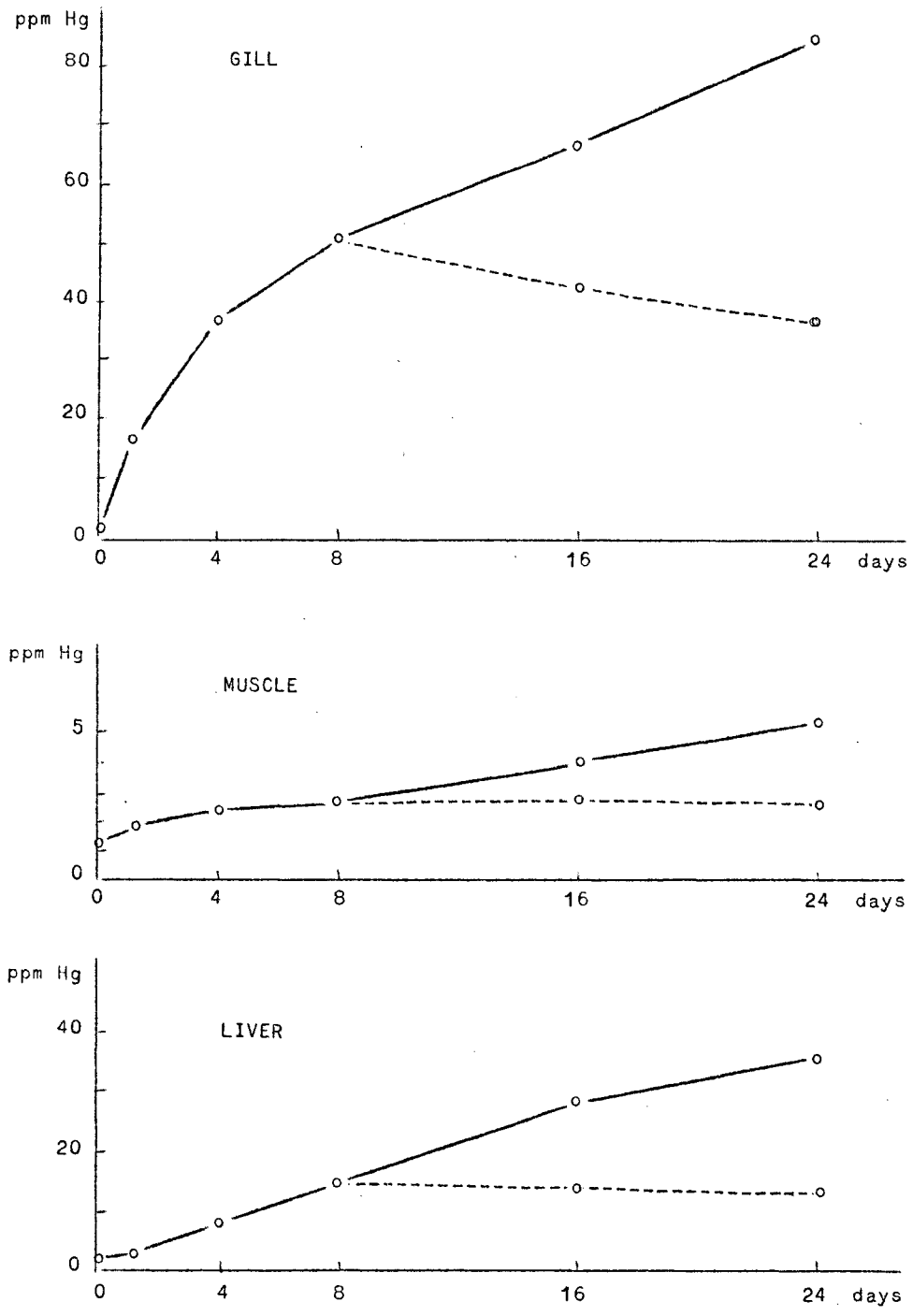


fig. 25.

Accumulation of Hg in the gills, muscles and liver of Scorpion fish kept in sea water containing 0.1 ppm Hg (heavy line).

Increase of Hg concentration when the fish are kept in non contamined water after 8 days intoxication (dotted line).

The curves show that for a given intoxication time the rates of accumulation in the different organs can be classified as follows starting with the slowest one : muscle and stomach, bones, brain, intestine and skin, bile, heart, liver, spleen, kidney, gills. Elimination of Hg is more complicated :

a) the gills, skin, intestine, spleen tissues lose Hg rather quickly; the kidney, liver and bone tissues show a somewhat greater half life.

b) the muscles, heart and stomach tissues practically do not release their Hg load within the time of the experiment, and the half life of Hg is very long in these organs. The same conclusion was reached for eels [Bouquegneau (1973a)].

c) the Hg content of the bile continues to increase when the intoxicated fish is decontaminated, indicating that Hg is eliminated from the liver.

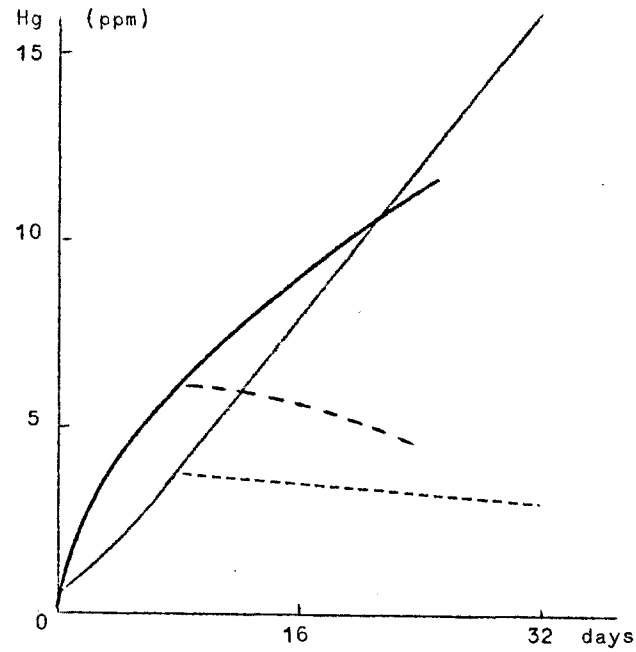


fig. 26.
Accumulation and release of mercury by Scorpion fishes (heavy lines) and sea water adapted eels (thin lines). Both are intoxicated in sea water containing 0.1 ppm Hg ($HgCl_2$).

It is possible from the different accumulation and release curves to construct a diagram showing the kinetics of intake and removal of Hg in

the total body as shown in figure 26, together with the results obtained for sea water adapted eels [Bouquegneau (1973a)]. It is obvious that the scorpion fish accumulates Hg much faster than eels at the start of the experiment; elimination is also faster.

The above results reveal that *Cottus scorpius* has an abnormally high burden of Hg in natural conditions. The intoxication experiments indicate that Hg accumulates in muscles and is stored there with an extremely long half life; liver is more active, accumulates and releases Hg much faster.

The fact that the amounts found in fish living in normal conditions are of the same order of magnitude in both liver and muscle indicate that the polluting levels at Ostend and Den Helder are rather the same regarding Hg.

Direct accumulation, through the gills, is very important and although intoxication through the food chain cannot be discarded and is difficult to ascertain, both effects cumulate.

The fact that the scorpion fish accumulates and eliminates Hg faster than eels is related to the difference in the relative weight of muscles, gills and liver in both fishes : respectively 71.5 % and 84.8 % , 4.6 % and 1.9 % , 2.4 % and 0.9 % .

It also seems evident that the excretory mechanisms are more efficient in *Cottus scorpius* than in *Anguilla anguilla* : the Hg concentration in the kidney still increases in eels submitted to desintoxication [Bouquegneau (1973a)] and remains stationary in scorpion fish.

3.2.- The mechanism of resistance of fish to Hg-poisoning [Bouquegneau *et al.* (1975)]

It has been shown by Bouquegneau (1973a) that eels intoxicated by Hg at sublethal doses of $HgCl_2$, accumulate large quantities of Hg and become resistant to otherwise lethal concentrations.

This type of adaptation mechanism can be identified as being related to the formation in different organs of the eel of metallothionein-like proteins.

Eels adapted to sea water are exposed to 0.4 ppm Hg ($HgCl_2$) during two weeks. The organs of control animals and intoxicated

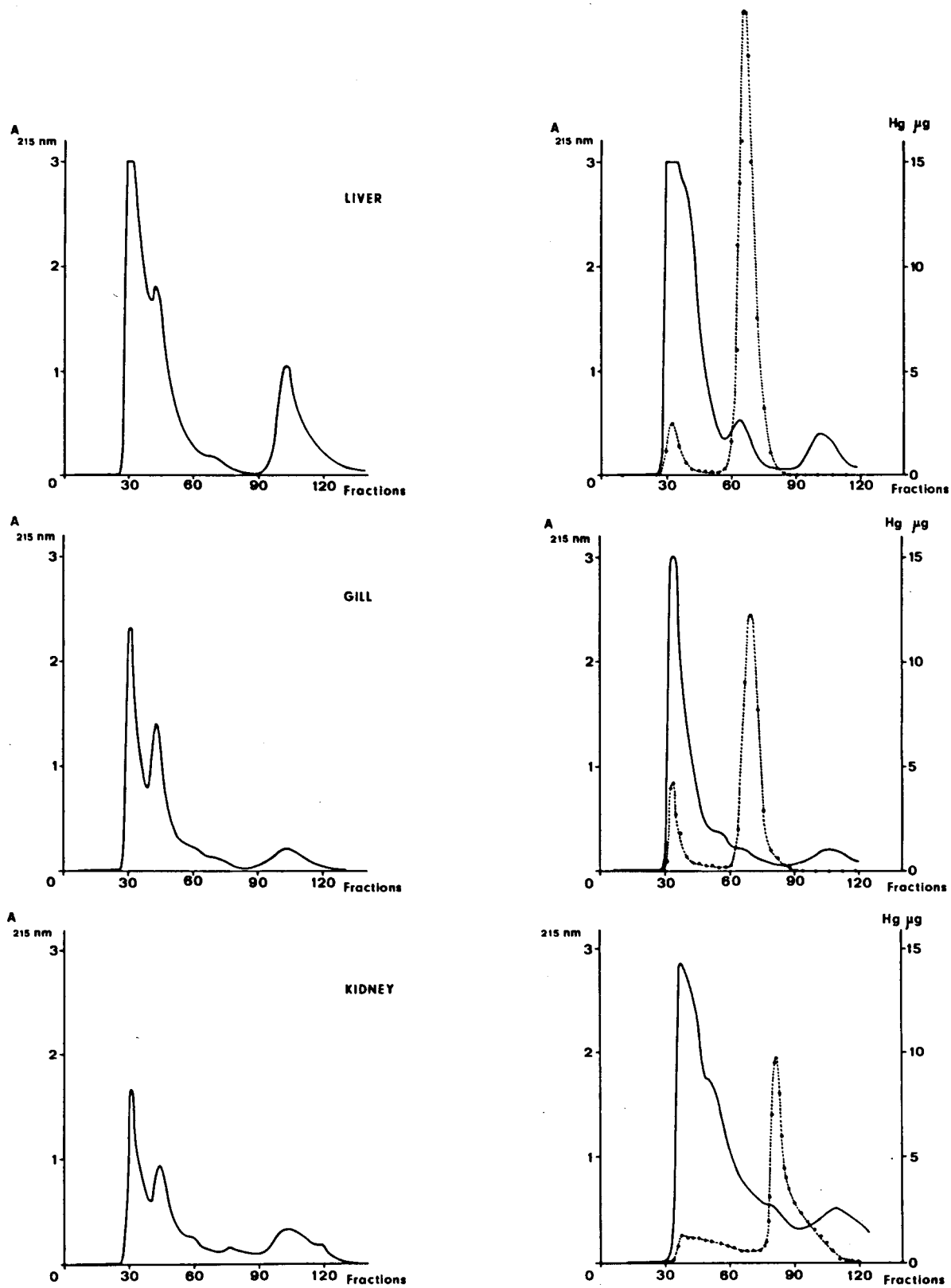


fig. 27.

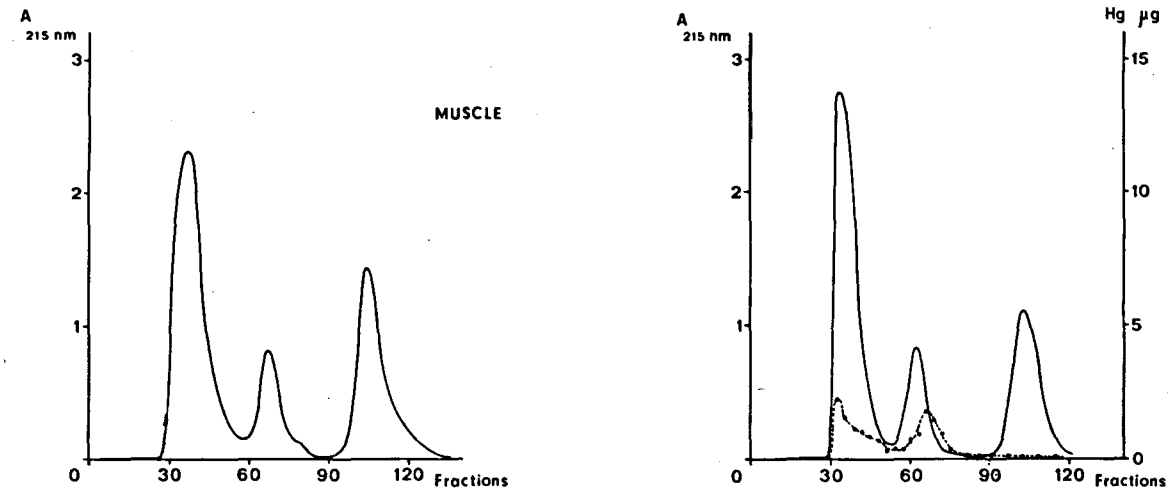


fig. 27.
Elution profiles on Sephadex G 75 columns (5 x 50 cm) of the extracts of different eel tissues. Left side, control fish; right side, intoxicated fish. Hg concentration is expressed in µg/9 mℓ fractions (dotted line).

specimens are homogenized in 3 volumes of 0.5 M sucrose, the extracts are centrifuged and chromatographied on Sephadex G75 columns equilibrated in NH_4HCO_3 0.05 M . Elution is monitored at 254 nm ; the amount of Hg is determined in the fractions as described earlier [Bouquegneau (1973a)]. Amino acid analyses are made using the procedure of Benson and Patterson (1965) and a Beckman amino acid analyser Model 120 B.

Figure 27 shows the typical distribution of Hg in the various fractions obtained from liver, gill, kidney and muscle of chronically intoxicated eels.

Except in the muscle sample most of the Hg is found bound to a protein fraction having a molecular weight close to 10000 daltons.

The mercury carrying fraction of liver extracts shows an unusual protein spectrum, similar to those produced by metallothioneins (fig. 28). Removal of Hg (70 %) by dialysis against chelating agents produces a spectral change also typical of these proteins.

Table 5 shows the amino acid composition of the Hg binding protein from eel liver. The low level in aromatic amino acid residues and the high content in cysteine residues is also characteristic of metallothioneins although the amount of cysteine is smaller than in the proteins extracted from kidney and liver of mammals and birds exposed to Cd and Hg intoxication.

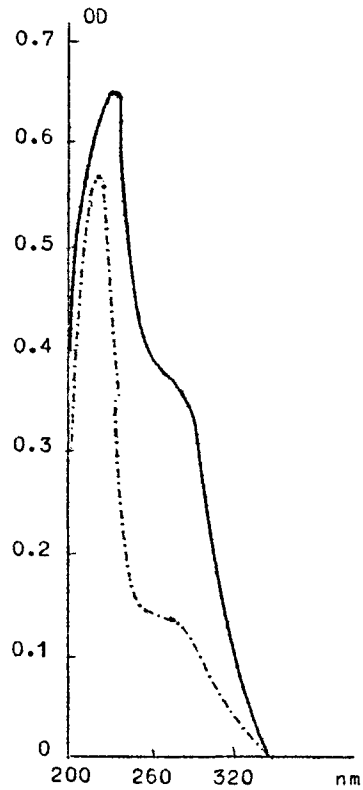
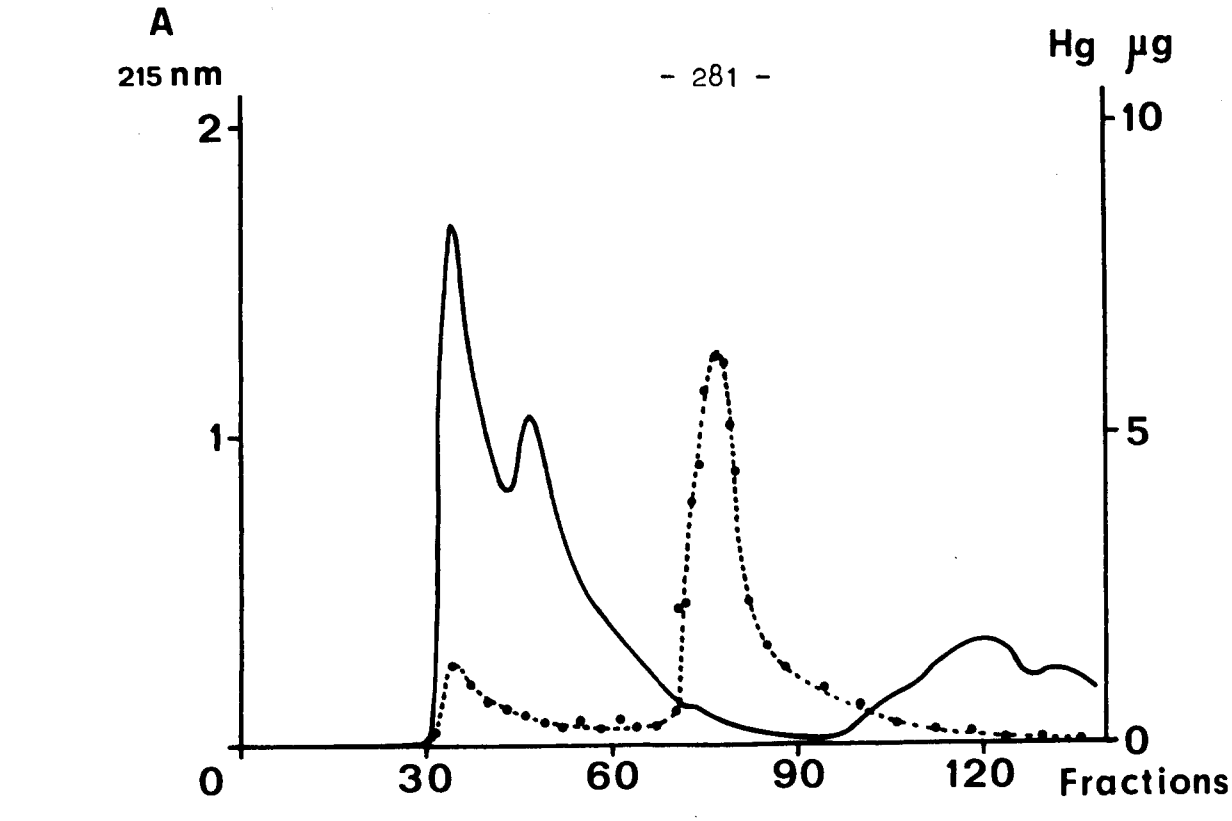


fig. 28.

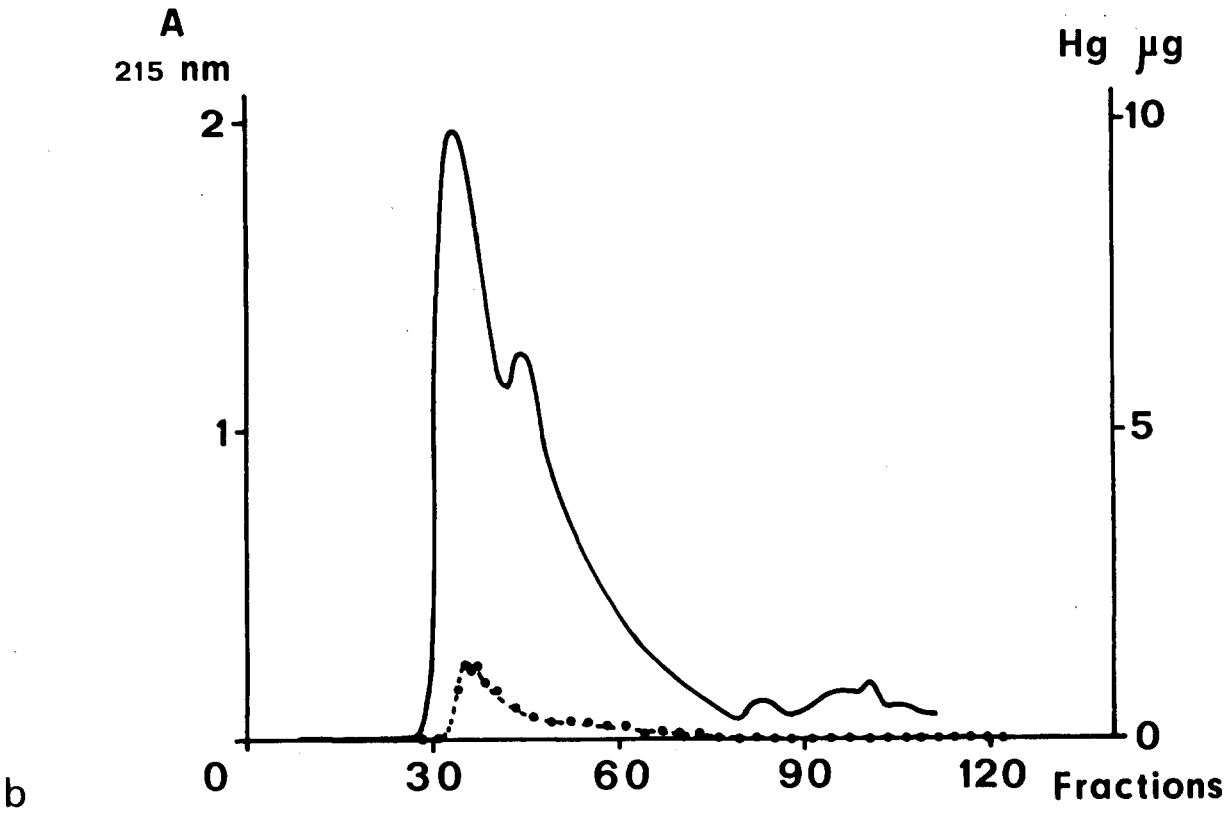
Ultra-violet absorption spectra in NH_4HCO_3 0.05 M of the Hg binding protein of eel liver in presence (———) and absence (·-·-·-·-) of mercury (see text).

To test the role of these proteins in the adaptation mechanism developed by chronically intoxicated eels, the Hg distribution in the different gill protein fractions obtained from eels exposed to 0.4 ppm Hg during 8 days has been compared to the Hg distribution in extracts from acutely intoxicated eels (10 ppm — 5 h) .

Figure 29 shows the elution profiles indicating that in acutely poisoned eels, the Hg is absent in the low molecular weight proteins, revealing that the amount of metallothionein is extremely low. In this case one gram tissue contains 61 µg Hg ; 46 µg are found in the insoluble fraction, the rest in the supernatant is bound to high molecular weight proteins. At sublethal doses one gram tissue contains after 8 days 120 µg Hg ; 23 µg in the insoluble fraction, 97 µg in the supernatant, 78 µg bound to the metallothionein fraction.



a



b

fig. 29.
Elution profiles on Sephadex G 75 columns (5 x 50 cm) of the gill extracts prepared from 1 g gill tissue of chronically (a) and acutely (b) intoxicated eels. Hg concentration is expressed in µg/9 mℓ fractions.

Table 5

Amino acid composition of Hg binding protein in eel liver

Amino acid	N_o of residues/molecule	
	calculated	assumed
Lys	9.60	10
His	1.10	1
Arg	1.70	2
Asp	8.08	8
Thr	6.50	7
Ser	7.60	8
Glu	10.20	10
Pro	10.20	10
Gly	11.10	11
Ala	11.20	11
* Cys (half)	8.60	9
Val	4.80	5
** Met	0.83	1
Ile	3.10	3
Leu	4.60	5
Tyr	0.00	0
Phe	1.06	1
Trp	-	-
Total	100.30	102.0

* determined as cysteic acid
** determined as methionine sulfone

It is concluded that Hg most probably induces the synthesis of a low molecular weight protein containing a large amount of cysteine capable of binding Hg and acting as a protective agent against damage caused to other proteins where SH groups play an important role with respect to their enzymatic activity, linked for instance to the active transport of Na. It is known [Bouquegneau (1973b)] that acutely intoxicated eels die from disruption of the osmotic balance, the Na content of the blood increasing drastically.

3.3.- The effect of Cd on eels (*Anguilla anguilla*) adapted to sea water [Lambot (1975)]

Sea water adapted eels are exposed to natural sea water containing varying concentrations of Cd ($CdCl_2$) . The tissues are mineralized in HNO_3 65 % (2.5 cm^3 per g fresh tissue). The diluted extract is analysed using atomic adsorption techniques (Perkin-Elmer 103 and 303); samples with low Cd content are analysed by polarography after dry mineralization under activated oxygen (Tracer-lab 600). Mineralization with $HNO_3 + H_2O_2$ and extraction (APDC-MIBK) have also been used.

3.3.1.- Mortality curves

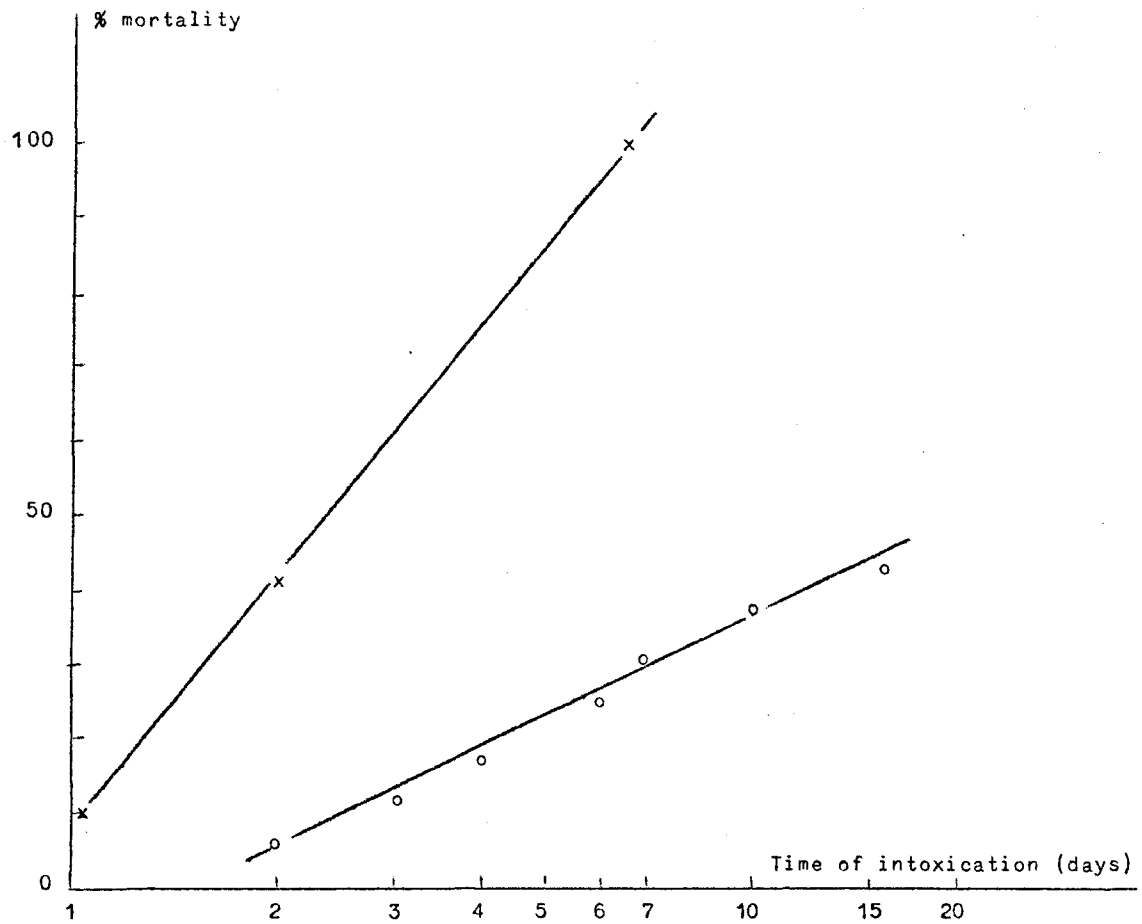


fig. 30.
Mortality curves for eels intoxicated in sea water containing 30 ppm Cd (o) or 80 ppm Cd (x).

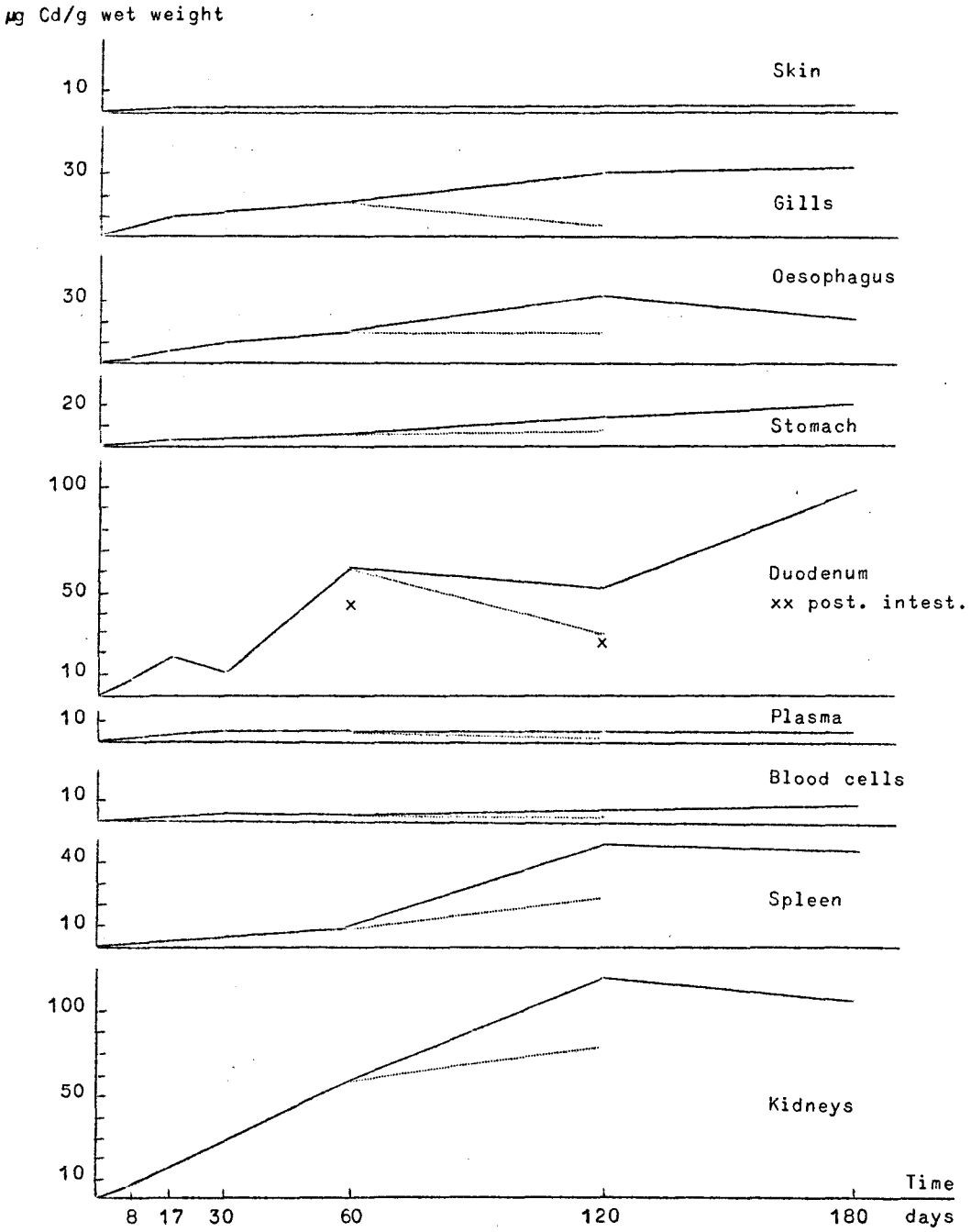


fig. 31a.

Accumulation and release of Cd in and from the organs of eels kept in sea water containing 13 ppm Cd (dotted line : Cd levels in the animals kept in non polluted sea water after 60 days intoxication).

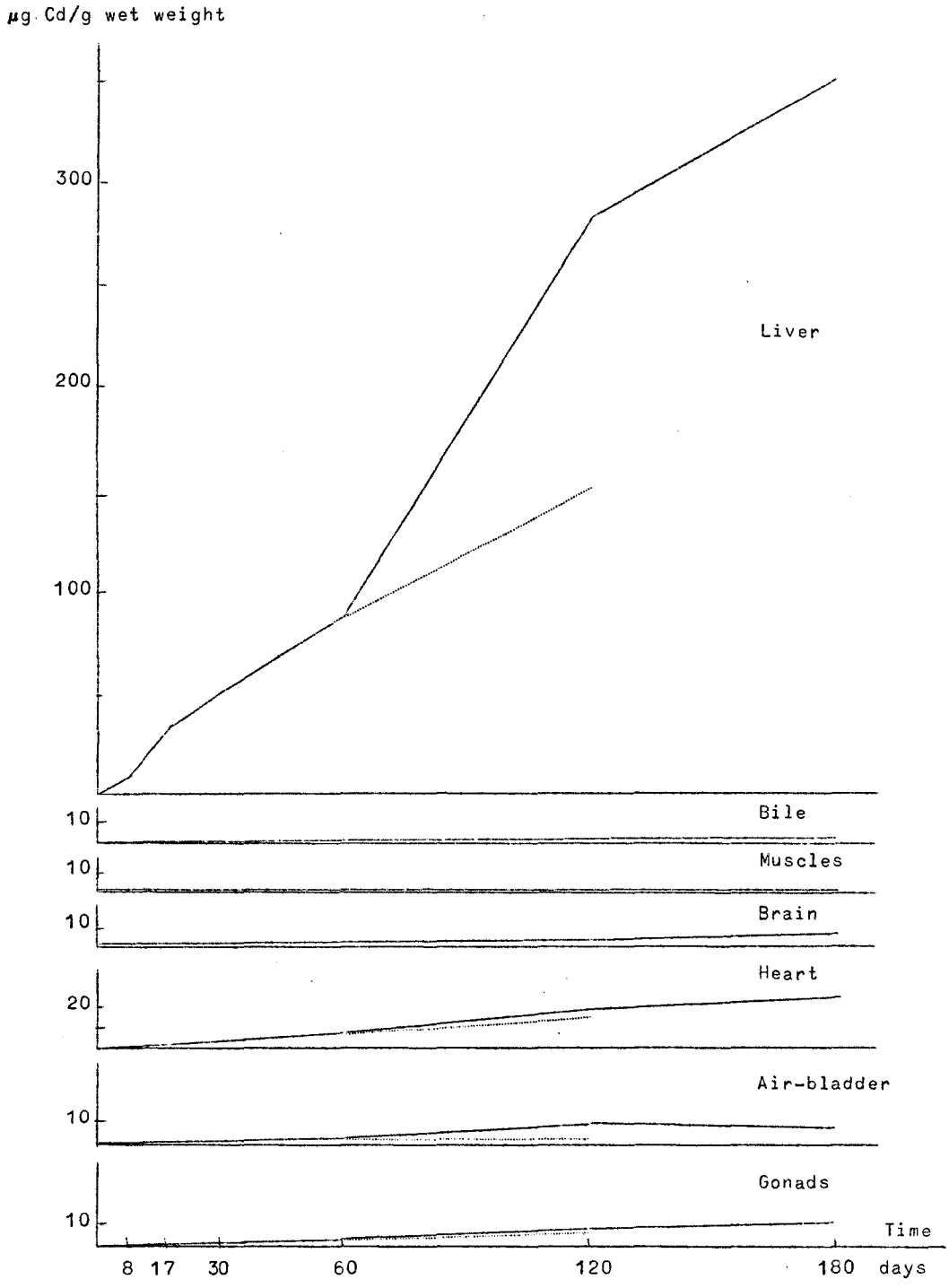


fig. 31b.
Accumulation and release of Cd in and from the organs of eels kept in sea water containing 13 ppm Cd (dotted line : Cd levels in the animals kept in non polluted sea water after 60 days intoxication).

Figure 30 shows mortality curves at lethal doses of 30 and 80 ppm Cd. In the sublethal range eels can be kept four months in water containing 13 ppm Cd . They are thus extremely resistant; a dose of 5 ppm Cd is lethal for *Cottus scorpius*.

3.3.2.- Kinetics of accumulation and release of Cd

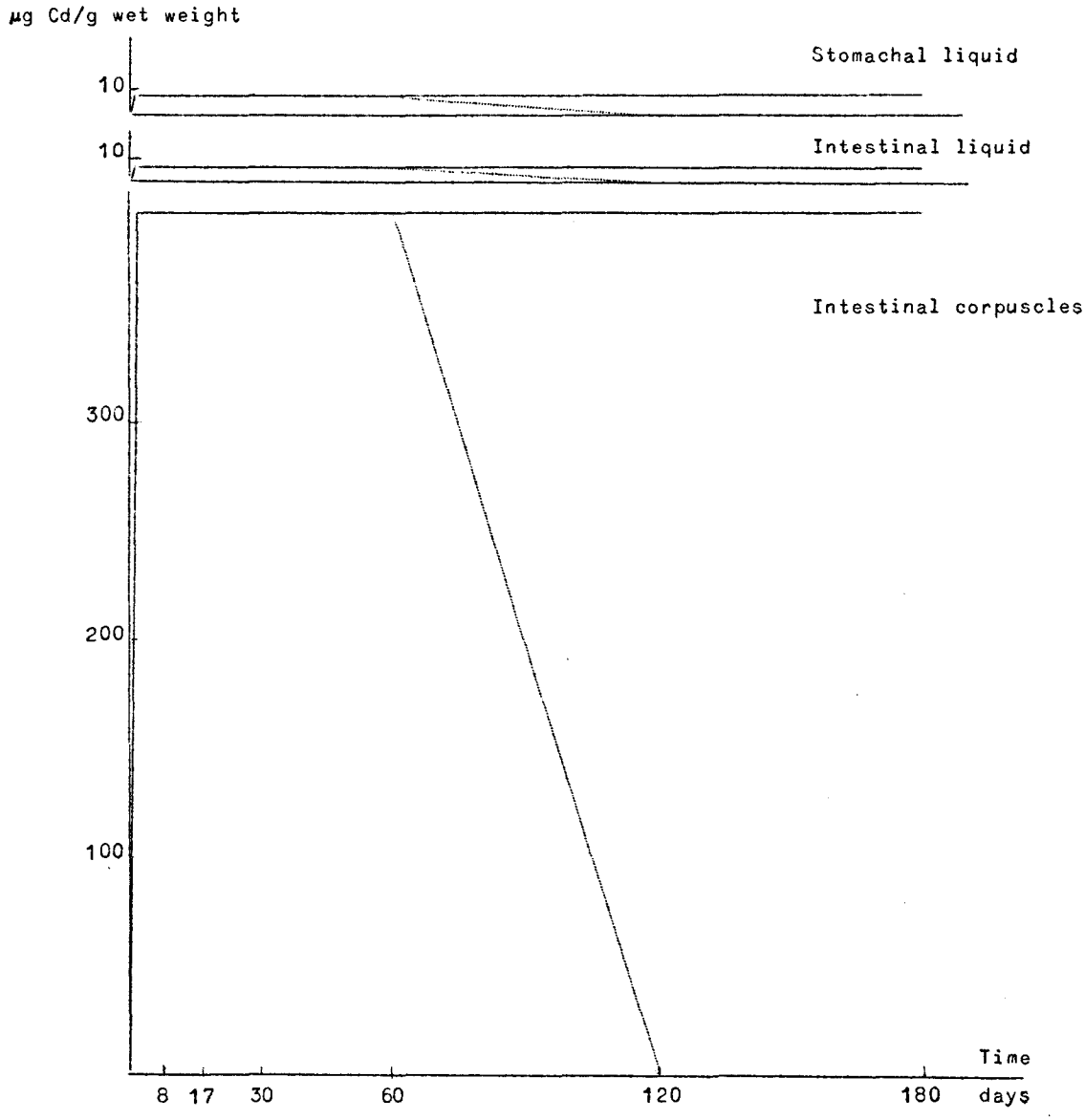


fig. 31c.
Accumulation and release of Cd in and from the organs of eels kept in sea water containing 13 ppm Cd (dotted line : Cd levels in the animals kept in non polluted sea water after 60 days intoxication).

Figure 31 (a,b,c) shows the accumulation of Cd in different organs and body fluids of eels exposed to 13 ppm Cd , some of the animals being kept in non contaminated water after 60 days intoxication.

Accumulation is highest in the liver, kidneys and duodenum. Release is negligible when the loaded animals are kept in normal sea water.

The case of intestinal corpuscles will be dealt with later in this report.

Table 6 gives the distribution of Cd in animals kept during 60 days in sea water containing 0.013 , 0.13 and 13 ppm Cd . Concentration

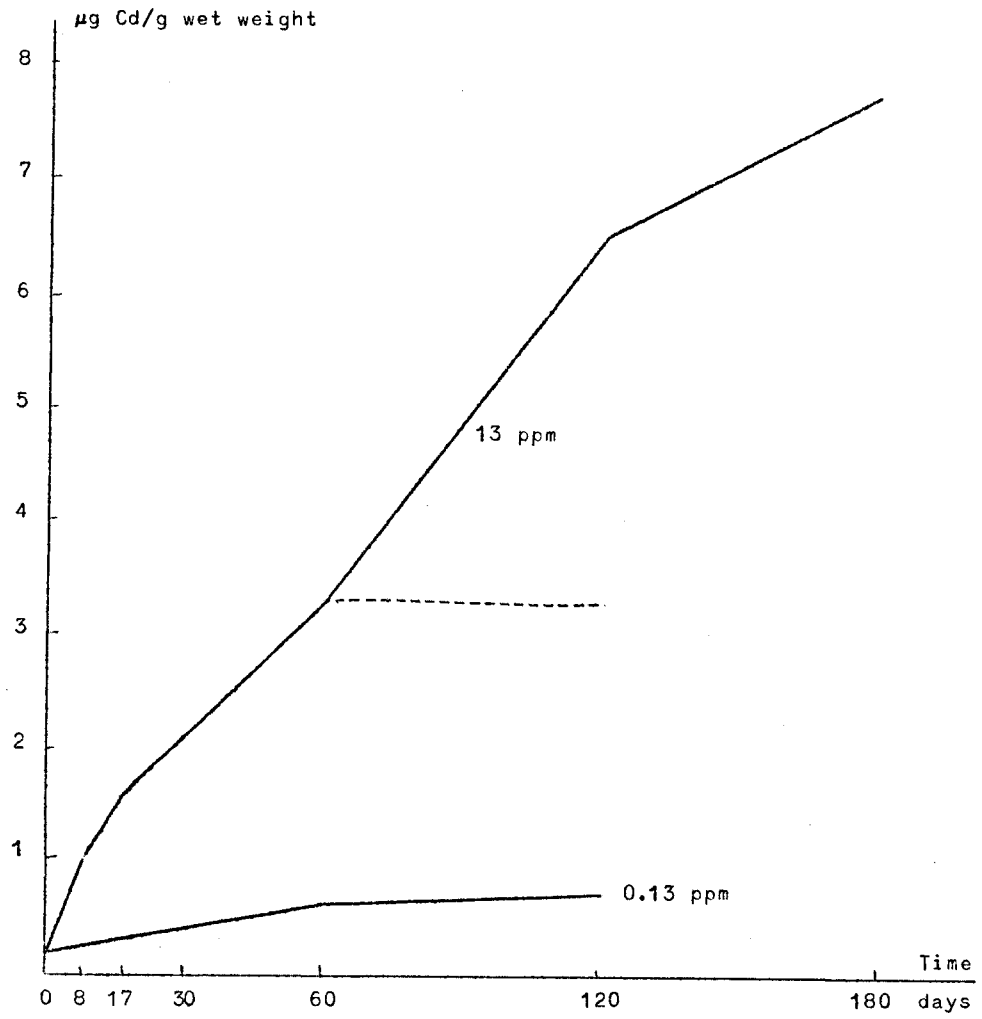


fig. 32.

Accumulation and release of the total Cd load in eels exposed to sea water containing 13 ppm and 0.13 ppm Cd (dashed curve : Cd load in animals kept in non polluted water after initial intoxication).

Table 6

Distribution of Cd in eels intoxicated during 60 days in sea water containing 0.013 , 0.13 or 13 ppm Cd

Cd concentration in sea water	0.013 ppm	0.13 ppm	13 ppm		0.013 ppm	0.13 ppm	13 ppm
Organs	Cd concentration ppm (wet weight)*			Weight of organs(g)	Total amount of Cd (µg)		
Muscles	< 0.5	< 0.5	0.6	75.5	7.5 ?	15.1 ?	43.0
Skin	0.2	0.4	1.2	10.6	2.1	4.2	12.7
Bones	-	-	-	6.6	0.7 ?	1.3 ?	3.8 ?
D.T. Oesophagus	< 0.3	1.5	14.5				
Stomach	< 0.3	-	5.2	Σ = 2.1	1.9	17.4	65.3
Duodenum	2.2	19.2	60.8				
Post. intest.	0.9	4.3	43.8				
Liver	0.9	6.0	88.3	1.2	1.1	7.2	106.0
Kidneys	1.6	21.8	56.2	0.7	1.1	15.3	39.3
Gonads	< 0.2	0.5	3.2	0.7	< 0.1	0.3	2.2
Plasma	0.1	0.3	3.1	0.6	< 0.1	< 0.1	1.8
Gills	0.7	2.6	16.5	0.5	0.3	1.3	8.2
Intest. liquid	< 0.1	< 0.5	5.6	0.4	< 0.1	< 0.1	2.2
Blood cells	0.5	0.3	2.6	0.3	0.1	0.1	0.8
Spleen	0.5	1.7	9.7	0.2	0.1	0.3	1.9
Air bladder	0.4	-	2.5	0.2	< 0.1	-	0.5
Bile	0.4	0.4	1.2	0.1	< 0.1	0.1	0.1
Heart	< 0.2	-	7.8	0.1	< 0.1	-	0.8
Stomachal liquid	-	-	9.7	0.1	< 0.1	< 0.1	1.0
Intest. corpuscles	2.0 ?	22.0	396.0	0.1	0.2	2.2	39.6
Brain	< 0.3	0.5	1.8	< 0.1	-	-	-
Total body	0.15	0.63	3.29	100	15.1	63.5	329.2
Concentration factor	11.6	4.9	0.25				

* 1 ppm = 1 µg/g .

factors $\frac{\text{ppm Cd in tissue or animal}}{\text{ppm Cd in water}}$ are higher the lower the Cd concentrations of the sea water.

Figure 32 shows total body burden versus time at 13 ppm and 0.13 ppm .

It can further be shown that at 13 ppm , after 120 days exposure, the muscles (0.6 ppm Cd) only contain 7 % of the total Cd body load, although they represent 75 % of the body weight. The liver with a weight corresponding to 1 % of the body contains 50 % of the total Cd.

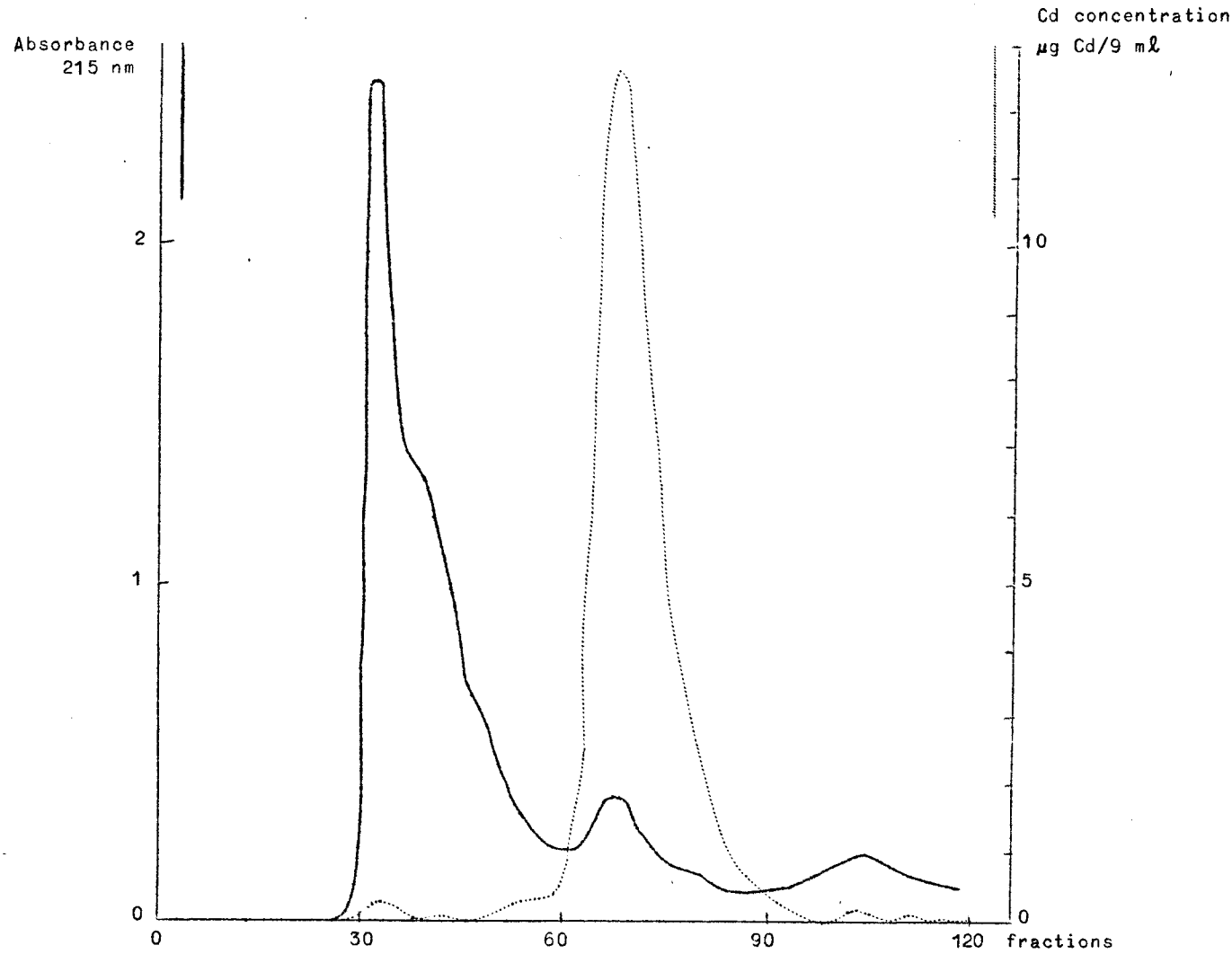


fig. 33.
Eel intoxicated during 120 days in sea water containing 13 ppm Cd . Absorbance and Cd distribution in fractions collected from liver extracts passed on Sephadex G 75 [see Bouquegneau et al. (1975)].

Figure 33 shows that Cd is bound, as Hg, to a metallothionein, the existence of which has also been recently demonstrated in plaice (*Pleuronectes platessa*) by Coombs (1974).

At lower doses (0.13 ppm) the kidneys, the liver and the digestive tract contain 50 % of the total Cd body burden but the duodenum and the kidneys contain more Cd than the liver. In non-intoxicated fish where the total amount of Cd is 0.1 - 0.2 ppm the concentration is also maximum in the kidneys. Storage in the liver seems to happen only at high Cd concentration ranges.

Comparison with Hg intoxicated eels is shown in table 7 and shows how different the two metals are distributed between the organs.

Table 7

Comparison between the distribution of Hg and Cd in the organs of eels exposed to contaminated sea water

Organs	0.13 ppm Cd 60 days	0.1 ppm Hg 32 days
	Total Cd in µg	Total Hg in µg
Muscles	15.1	1136.3
Skin	4.2	163.6
Digestive tract	17.4	36.3
Gills	1.3	126.7
Liver	7.2	43.7
Kidneys	15.3	92.6
Total body	63.5	1614.4
Concentration factor	4.9	162

At acute lethal Cd doses (90 ppm), the Cd level in plasma reaches about 15 ppm in 10 h, the levels in the other organs remain below or are equal to those observed during prolonged sublethal actions.

3.3.3.- Intestinal corpuscles

Both in sea water adapted eels and scorpion fishes, white mucus corpuscles (~ 1 μ diam) are observed in the intestine. They are also present in fresh water eels.

In eels intoxicated with Cd these corpuscles are found to contain enormous amounts of Cd (see table 8).

Table 8

Cd content of intestinal corpuscles from eels intoxicated at different Cd concentrations

Cd concentration in sea water (ppm)	Cd concentration in the intestinal corpuscles (ppm) (wet weight)			Maximum concentration factor
	Min	Max	m	
0.005 (= SW from the aquarium)			< 4	
0.013			< 4	< 307
0.13	5	27	22	207
0.9	7	71	55	79
13.0	141	859	396	66
90	33	3397	942	38

Although their weight fraction is very small (0.1 g) they carry after 8 days intoxication at 13 ppm half the total Cd found in the animal. After 120 days the corpuscles carry as much Cd as all the muscles.

Corpuscles from non intoxicated eels retain Cd *in vitro* : put into distilled water containing 90 ppm Cd , they contain 2.188 ppm Cd after 6 h treatment. The same is observed in sea water.

The corpuscles taken after 6 h from eels exposed to 90 ppm Cd contain about the same amount (3000 ppm Cd) as found in corpuscles directly exposed to a solution containing identical Cd concentration. The same is true at other Cd concentrations and it thus seems that Cd is simply adsorbed on the corpuscles directly from the sea water ingested by the animal. The effect is to considerably lower the Cd concentration in

the intestine : in one experiment at 90 ppm Cd in the sea water, the Cd concentration was found to be 45 ppm in the stomacal liquid and only 1.3 ppm in intestinal liquid; the corpuscles carried 3000 ppm Cd . The presence of corpuscles would therefore protect the intestinal wall and greatly limit the entry of Cd by this route.

Table 9

Composition of intestinal corpuscles of sea water adapted eels

Element	ppm (dry weight)
Mg	126,000
Ca	75,000
Na	20,500
S	5,400
K	1,300
Sr	700
P	600
Si	150
As	74
Zn	46
Ti	31
Fe	27
F	20
Al	15
Cr	11
Br	10
Ba	5
Sb	5

The average composition of the corpuscles is given in table 9. Their content in Ca and Mg is very high, and these metals are probably present under the form of carbonates, precipitated in an organic matrix formed from mucous secretions and cellular debris.

The Cd concentration observed in muscle animals in non contaminated sea water (1 - 5 ppb Cd) is of the order of 0.03 - 0.10 ppm, rising sometimes to 0.4 ppm . After 120 days in sea water containing 13 ppm Cd, the concentration in the muscles only reaches 0.6 ppm . Direct intoxication by Cd has thus probably little effect in natural conditions on the average level found in fish muscles. However it is obvious that the

viscera although their weight fraction is small can carry an important part of the total burden and their Cd content should be controlled. Whether ingestion of contaminated food has a bearing on the Cd level in fish remains an open question and awaits further experimentation.

Fish seem to be protected against the toxic effects of Cd by the formation of metallothionein at least in the liver and also by the presence of intestinal corpuscles capable of adsorbing large amounts of Cd, subsequently eliminated.

4.- General conclusions

1) Whether in invertebrates or vertebrates, and in the case of diatoms direct intoxication quickly rises the heavy metal burden of exposed animals or plants; the release when back in non-contaminated water, at least in animals, seems to be a comparatively slow process involving the redistribution of the heavy metals within the body, the half life being largest in muscle. With diatoms Zn seems to quickly adsorb on the cell walls; its subsequent fate depends strongly on the growth aptitude retained under intoxication.

2) Marine species seem in general extremely resistant in presence of heavy metals and are capable of accumulating large amounts of these, becoming a potential danger to man as food. The resistance can be explained in the case of fish by the production of low molecular weight proteins containing large amounts of SH groups having high affinities for heavy metals like Hg, Cd, etc. These metallothioneins normally control the cell content in essential metals like Zn and Cu. Their synthesis is enhanced when large amounts of heavy metals are present in water. It should be interesting to look for these proteins in marine invertebrates. If the aquatic animal life is capable of synthetizing more rapidly large amounts of such proteins then terrestrial animals or plants do, then the danger of consuming contaminated marine food at a rate greater than the rate at which mammals for instance are capable of producing metallothionein might be the final due to determine tolerable contamination levels in aquatic

animals. The whole problem of tolerable doses would boil down to an estimate of the defense capacity of terrestrial animals first, in terms of the kinetics of their protective proteins production. By no doubt many marine species can accumulate enormous doses of heavy metals, lethal to man, without difficulties. Little damage is therefore expected to be caused to marine life by dumping heavy metals in the sea. The danger of eutrophication, with all its ecological changes, appears far more important.

Both types of pollution have an impact on man, the former because it lowers the quality of marine food, the other because it lowers the amount of consumable food produced. On the other hand, in heavily eutrophicated regions heavy metals easily bind to organic suspended matter and sediments, anoxic conditions lead to stable compounds and the potential danger of heavy metals is lowered. Regression of eutrophication might lead to the release of heavy metals and subsequent direct contamination of marine life at all levels. The kinetics of intake are generally fast compared to the release mechanisms : to trap heavy metals in eutrophicated areas and subsequently to release them by suddenly controlling PO_4 , NO_3 , etc. sources, would simply substitute to continuous dumping a sort of square wave action which obviously should be avoided by determining the optimal rate of reversal to normal conditions.

3) Water quality tests either using phytoplankton, larvae, fish, etc. will all give different results depending on how the test is carried out and the type of organism used. The author believes that field surveys are far more efficient to determine the intensity of pollution and its type.

4) Direct intoxication is obviously important for marine species ingesting or filtering great amounts of water; the effect of pollutants moving through the food chain is still difficult to assess, although many results seem to indicate that direct contamination is more effective. Further studies are needed to clarify this point.

5) Adsorption and desorption of heavy metals on particulate matter, organic or inorganic, on living cells, on particles inside animals (like

those formed to bind large amounts of Cd in fish) are to be studied more carefully; in the case of phytoplankton, at least for diatoms, this process might prove to be an important entry route not only for heavy metals but for other pollutants.

6) More studies are needed on the benthic fauna physiology with respect to accumulation of toxic substances and possible rate of release in correlation with local substrate and water quality.

7) Efforts should be continued to obtain kinetic data as required for modelling marine systems [Nihoul (1975)] at all levels of marine life and to try and group animals and plants with respect to their main physiological behaviour (oxygen and CO_2 consumers or producers, filter feeders, scavengers, detritus feeders, predators, etc.) to try and evaluate the rate of energy flow through the globalized system together with the fate of the toxic substances resulting from man's impact on the marine ecosystem.

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