# RESEARCH ARTICLES

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## HEAVY METALS (Zn, Cu, Cd, Pb) IN SOME BENTHIC INVERTEBRATE SPECIES AND IN SEDIMENT FROM THREE COASTAL AREAS IN THAILAND AND MALAYSIA

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**Abstract**

*The concentrations of zinc, copper, cadmium and lead in sediment and benthic invertebrates, representing three phyla, from some intertidal mud-flats along the Malay Peninsula (Thailand and Malaysia) were investigated. All samples were collected in October and November 1985.*

*Compared to the same type of animals from temperate latitudes, the concentrations of cadmium and lead tend to be considerably higher, whereas both the zinc and copper concentrations are the same or slightly lower.*

*In the edible bivalve mollusc* Anadara granosa *(the bloody cockle) high concentrations of cadmium were found, whereas in* Glauconome virens *very high lead concentrations were measured.*

*No relationship was found between the metal concentration of the sediment and the organisms.*

*Pattani Bay is considerably more polluted, both in organisms and sediments, than the coastal area of Jeram and the relatively unpolluted Ao Ban Don (Bay of Surat Thani).*

### Introduction

Rivers discharge various substances into the sea, both in solution and in colloidal or particulate forms. The effects of these contaminants on the marine environment will be most direct and intensive in the adjacent coastal regions and depend on the nature,

concentration and amounts of the contaminants. Heavy metals introduced into the marine environment by domestic, industrial and mining activities are incorporated into marine sediments. Marine organisms living in these sediments, such as polychaete worms, crustaceans and bivalves, accumulate these heavy metals to varying degrees.

Sediments from river mouths and coastal areas in the upper Gulf of Thailand show quite obvious contamination with lead in the top 10 cm layer of the bottom[1], and an enrichment of cadmium and lead in the surface layer[2].

The contamination of lead and cadmium in the green mussel *Perna viridis* from river mouth areas is quite considerable. Concentrations of zinc and copper are still within acceptable limits[1] and the metal levels in the bloody cockle *Anadara granosa,* the oyster *Crassostrea commercialis* and the green mussel *Perna viridis* from farms in coastal areas of the upper Gulf of Thailand are sufficiently low that the molluscs can be consumed without any danger to human health[3]. Data obtained from analysis of samples taken from widely scattered areas in the Gulf of Thailand and Strait Malacca, show a remarkable similarity in metal levels with regard to fishes, crustaceans and molluscs[4]. There is no consistent evidence of any element being particularly high in any area. The concentration of cadmium, however, covers a wide range, with the highest values associated with the molluscs *Perna viridis* and *Anadara granosa.* The data clearly indicate the impact of human activity on the coastal marine environment. Relatively high concentrations of cadmium in the cockle *Anadara granosa* reflect negative environmental influence on localized areas. However, fish and shellfish from the western coastal waters of Malaysia contain trace quantities of heavy metals, levels far below the limits of acceptability for human consumption[5]. A recent report on eight metals in three bivalve mollusc species in geographically different areas of the Gulf of Thailand did not indicate a major threat to public health[6].

Almost all studies cited relate the concentration of heavy metals with human health. There are however other criteria, more sensitive to the environment, to assess the impact of pollution on natural ecosystems.

Large numbers of migrating birds, especially waders, concentrate on the intertidal mud-flats along the coast of the Malay Peninsula, where they feed on macrobenthic organisms[7,8]. It is therefore of interest to determine the concentration levels of metals in sediment and macrobenthic fauna as possible sources of metal accumulation in migrating birds.

The present study was conducted as part of Interwader's programme in 1985. It deals with the accumulation of zinc, copper, cadmium and lead in the sediment and the uptake in some selected invertebrate species from intertidal mud-flats of the Malay Peninsula.

## Material and Methods

All samples were collected in October and November 1985, from the intertidal

mud-flats of three coastal areas of the Malay Peninsula: Ao Ban Don (Thailand), Pattani Bay (Thailand) and Jeram (Malaysia) (Fig. 1). The macrobenthic species collected, representing three phyla of invertebrates, and the number of specimens in each sample are given in Table 1. Shrimps were sampled using a small hand-net, whereas worms and bivalve molluscs were collected by sieving from sediment obtained with a sediment corer. Specimens of the green mussel and crabs were collected by hand.

The samples of invertebrates were dried at 60-70 °C and prepared for sending to the Netherlands Institute for Sea Research. The samples were freeze-dried to constant dry weight and homogenized. About 400 mg of sample, measured to the nearest 0.1 mg, were decomposed by an acid destruction bomb technique[9]. For the decomposition of the organic material, 3 ml of 65% nitric acid was added and the bombs were kept for 2 hours at 120 °C. The decomposed samples were transferred quantitatively to polypropylene tubes and diluted with double-distilled water to 10 ml. All samples of organisms obtained were decomposed in duplicate.

Surface sediment was sampled with teflon-coated tubes, in which the sediment was stored up to processing. The grain-size distribution in the sediment was determined

**TABLE 1.** SPECIES SAMPLED FOR METAL ANALYSES; N = NUMBER OF SPECIMENS IN EACH SAMPLE.

| Phylum | Class | Suborder | Species | Sampling sites | N |
|---|---|---|---|---|---|
| Annelida | Polychaeta | | *Dendronereis* spp. | All areas | 20 |
| Arthropoda | Crustacea | Natantia | *Exopalaemon styliferus* (H.Milne Edwards) | Jeram | 30 |
| | | | *Metapenaeus lysianassa* (De Man) | Laem Nok, Jeram | 30 |
| | | | *Palaemon semmelinkii* (De Man) | Ban Changoe | 30 |
| | | Brachyura | *Uca dussumiera spinata* (Crane) | Campus, Jeram | 10 |
| | | | *Uca rosea* (Tweedie) | Jeram | 10 |
| | | | *Dotilla wichmanni* (De Man) | Ban Da To | 30 |
| | | | *Metaplax longipes* (Stimpson) | Ban Changoe | 10 |
| Mollusca | Bivalvia | | *Anadara granosa* (L.) | Jeram | 20 |
| | | | *Glauconome virens* (L.) | Laem Nok, Campus | 20 |
| | | | *Potamocorbula fasciata* (Reeve) | Ban Da To | 100 |
| | | | *Perna viridis* (L.) | Campus, Gulf Thailand | 10 |

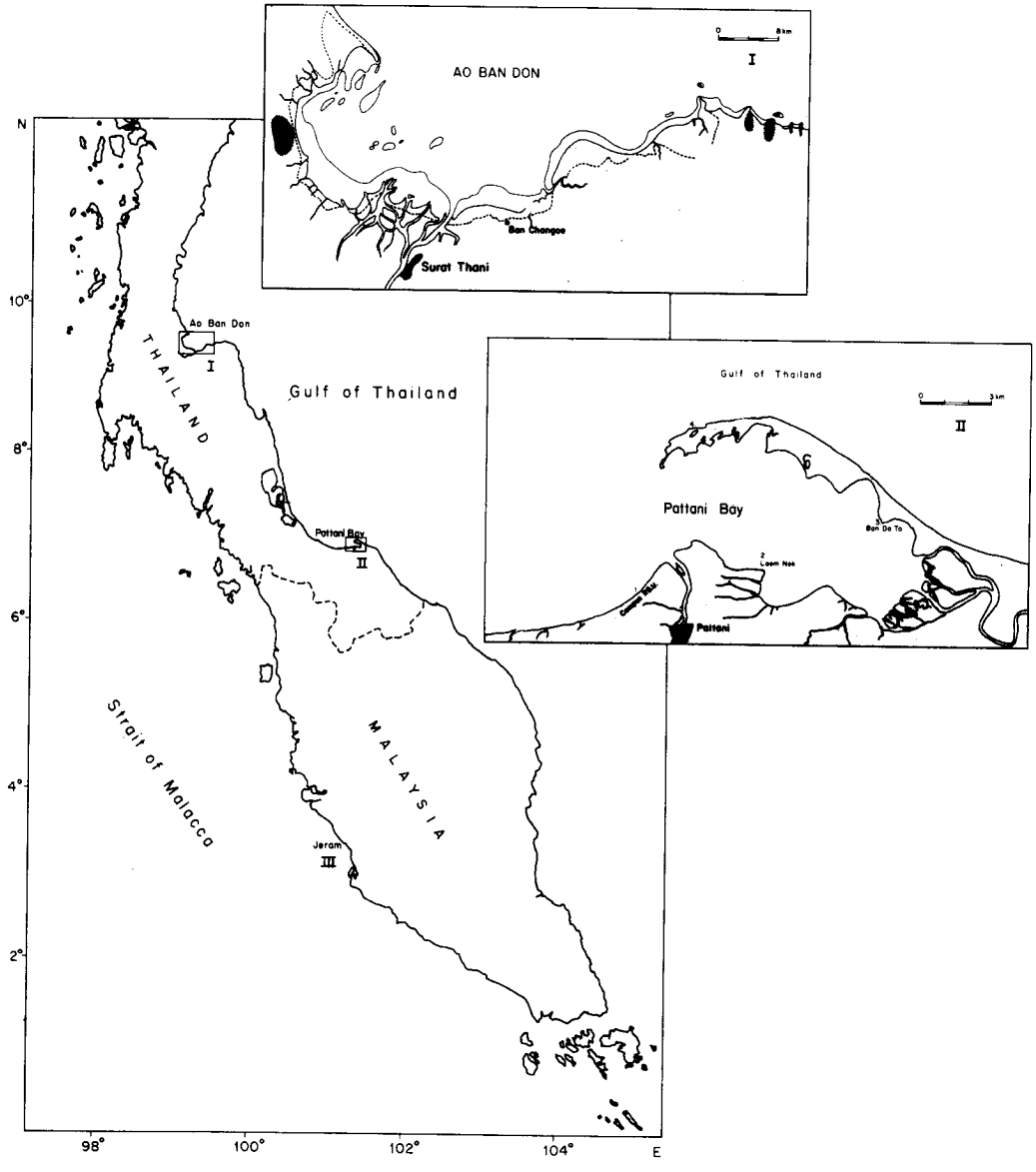


**Fig. 1.** Sampling locations in Thailand and Malaysia; I. Surat Thani Bay - Ao Ban Don, sampling site on mud-flats near Ban Changoe, II. Pattani Bay, sampling sites in the intertidal zones near Pattani river mouth at Campus P.S.U. (1), at Laem Nok (2), Ban Da To (3) and in the Gulf of Thailand (4), III. Coastal intertidal mud-flats near Jeram.

after weighing 10 to 20 gram dry sediment and subsequent sieving with demineralized water (to disintegrate aggregates of fine material) over a sieve with a pore size of 63 µm, dilution to 1 l and filtering over a preweighed 0.45 µm filter. The rest of the sediment suspension (grain-size > 63 µm) was dried at 70 °C and sieved (pore sizes 500, 315, 200 and 100 µm) for 10 minutes at 50 Hz. After weighing the different fractions, grain-size distribution was established (Table 2).

**TABLE 2.** THE TEXTURE OF THE SEDIMENTS FROM FOUR COASTAL AREAS OF THE MALAY PENINSULA.

| Grain-size fraction (µm) | Ao Ban Don (Ban Changoe) % | Pattani Bay Campus % | Pattani Bay Ban Da To % | Jeram % |
|---|---|---|---|---|
| < 63 | 42.5 | 82.0 | 16.2 | 80.8 |
| 63-100 | 19.9 | 7.9 | 3.4 | 6.8 |
| 100-200 | 19.3 | 8.0 | 34.1 | 5.6 |
| 200-315 | 7.2 | 0.4 | 31.9 | 5.4 |
| 315-500 | 7.1 | 0.4 | 11.8 | 1.4 |
| > 500 | 4.0 | 1.3 | 2.6 | 0 |

The metal content of the total sediment and the silt fraction was determined after decomposition of about 500 mg of sediment (measured to the nearest 0.1 mg) with a mixture of 1 ml aqua regina (1 part $HNO_3$ + 3 parts HCl) and 5 ml HF in a teflon destruction bomb[10], for 2 hours at 120 °C, and subsequent dilution with 30 ml saturated $H_3BO_3$-solution and double-distilled water to 50 ml.

Zinc and copper were measured with a flame atomic absorption spectrometer (Perkin-Elmer, model 403). Cadmium and lead were measured with a heated graphite-furnance atomizer (HGA 500) coupled to an atomic absorption spectrometer (Perkin-Elmer, model 5000). To calculate the metal concentrations, the standard addition method was applied and calibration curves were made with standard solutions of copper, zinc, cadmium and lead, respectively. The AAS-measurements were performed in duplicate on each sample.

To avoid contamination of the samples, all chemicals used were suprapur grade (Merck). All materials used were rinsed for 12 hours with 6 N HCl and subsequently rinsed three times with double-distilled water.

## Results and Discussion

### *Zinc*

The zinc concentration measured in the whole body of polychaete annelids, crustacea and molluscs from different areas are given in Figure 2. Of all species analysed, highest concentrations were found in specimens of the polychaete *Dendronereis* spp., with values ranging from 68 to 147 $\mu g.g^{-1}$ dry weight. Shrimps and crabs showed considerably lower values, varying between 67 - 85 $\mu g.g^{-1}$ dwgt. and 54 - 90 $\mu g.g^{-1}$ dwgt., respectively. A relatively high zinc concentration was found in bivalves from the coastal area at Jeram. Lowest concentrations of zinc (about 40 $\mu g.g^{-1}$ dwgt.) were found in the bivalve mollusc *Perna viridis* from the Gulf of Thailand. The mean concentration of zinc was lowest in organisms from Ao Ban Don.

In comparison with the concentration level in organisms, the concentration of zinc in the silt fraction (grain size $< 63$ $\mu m$) of the sediment was remarkably high, with mean values varying between 100 and 250 $\mu g.g^{-1}$ dwgt. Comparison of the zinc concentrations in the silt fraction of sediment from the three areas showed the samples from Pattani Bay to have the highest concentrations.

### *Copper*

The concentration of copper differed considerably among the species, with highest concentrations in crustaceans, varying between 60 and 140 $\mu g.g^{-1}$ dwgt., both in shrimps and crabs. (Fig. 2). Much lower concentrations were found in polychaetes and molluscs, varying between 14.1 and 22.3 $\mu g.g^{-1}$ dwgt. and 4.8 and 17.0 $\mu g.g^{-1}$ dwgt., respectively.

Organisms from Pattani Bay invariably showed the highest copper concentrations. In all cases, the concentration was lowest in specimens sampled in Ao Ban Don. In the silt fraction of the sediment, highest copper concentrations were also recorded in Pattani Bay, with mean values between 40 and 70 $\mu g.g^{-1}$ dwgt.

### *Cadmium*

The concentrations of cadmium found in worms (0.7 - 2.4 $\mu g.g^{-1}$ dwgt.), shrimps (about 0.7 $\mu g.g^{-1}$ dwgt.) and crabs (0.7 - 1.8 $\mu g.g^{-1}$ dwgt.) from Pattani Bay and Jeram were considerably higher than the concentration in corresponding species from Ao Ban Don and in shrimps from Jeram (Fig. 3). In these areas, the concentrations were about 0.17 $\mu g.g^{-1}$ dwgt. in worms, about 0.26 $\mu g.g^{-1}$ dwgt. in shrimps and about 0.12 $\mu g.g^{-1}$ dwgt. in crabs. Pronouncedly high concentrations were measured in bivalve molluscs from Pattani Bay and Jeram, with values ranging from 1.4 to 4.5 $\mu g.g^{-1}$ dwgt.

In the silt fraction of the sediment, highest concentrations were found in Pattani

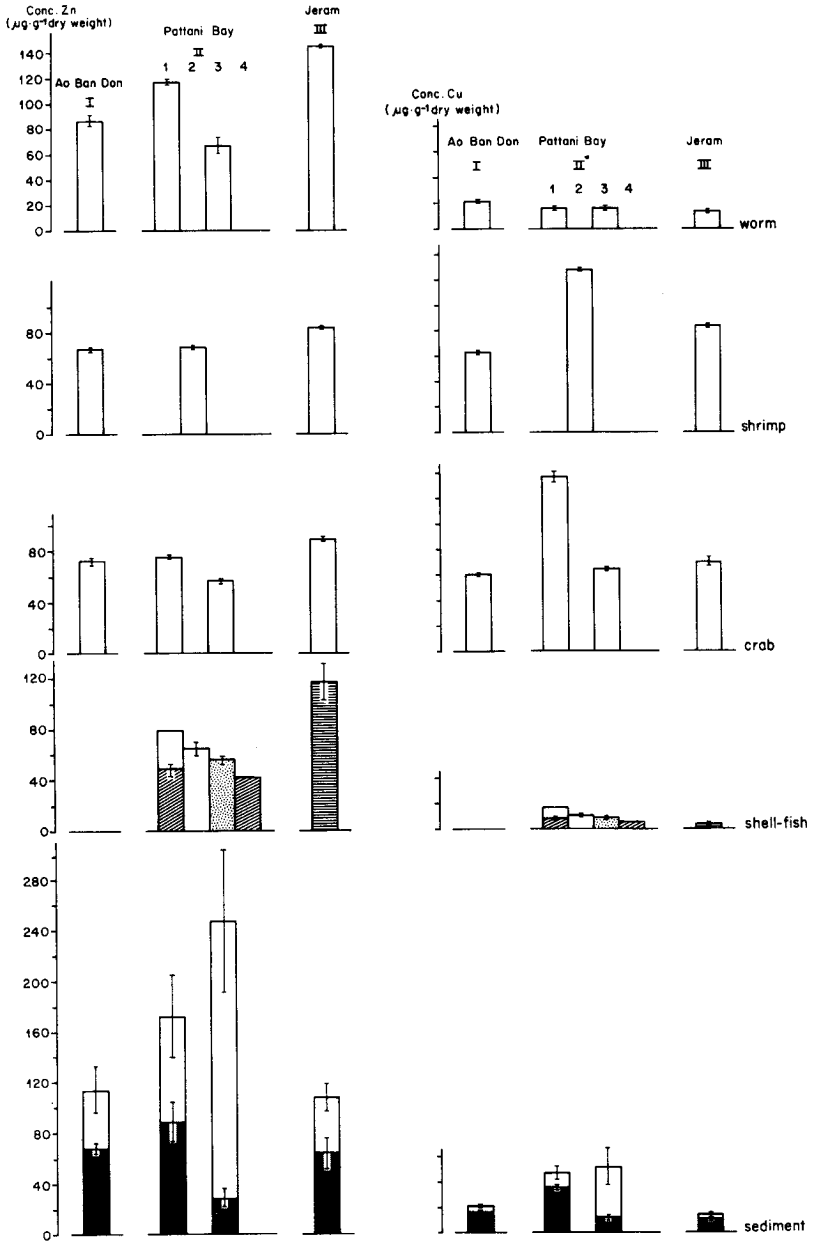


**Fig. 2.** The concentration of zinc and copper ( $\mu g.g^{-1}$ dry weight) in benthic invertebrates and sediment from three coastal areas of the Malay Peninsula. In four species of bivalve molluscs metals were analysed: *Perna viridis* ( ▨ ), *Glauconome virens* ( □ ), *Potamocorbula fasciata* ( ▩ ), and *Anadara granosa* ( ▤ ). Sediment analyses: total column shows the concentration in the silt fraction (grain size < 63 μm); shaded part shows the concentration in total sediment.

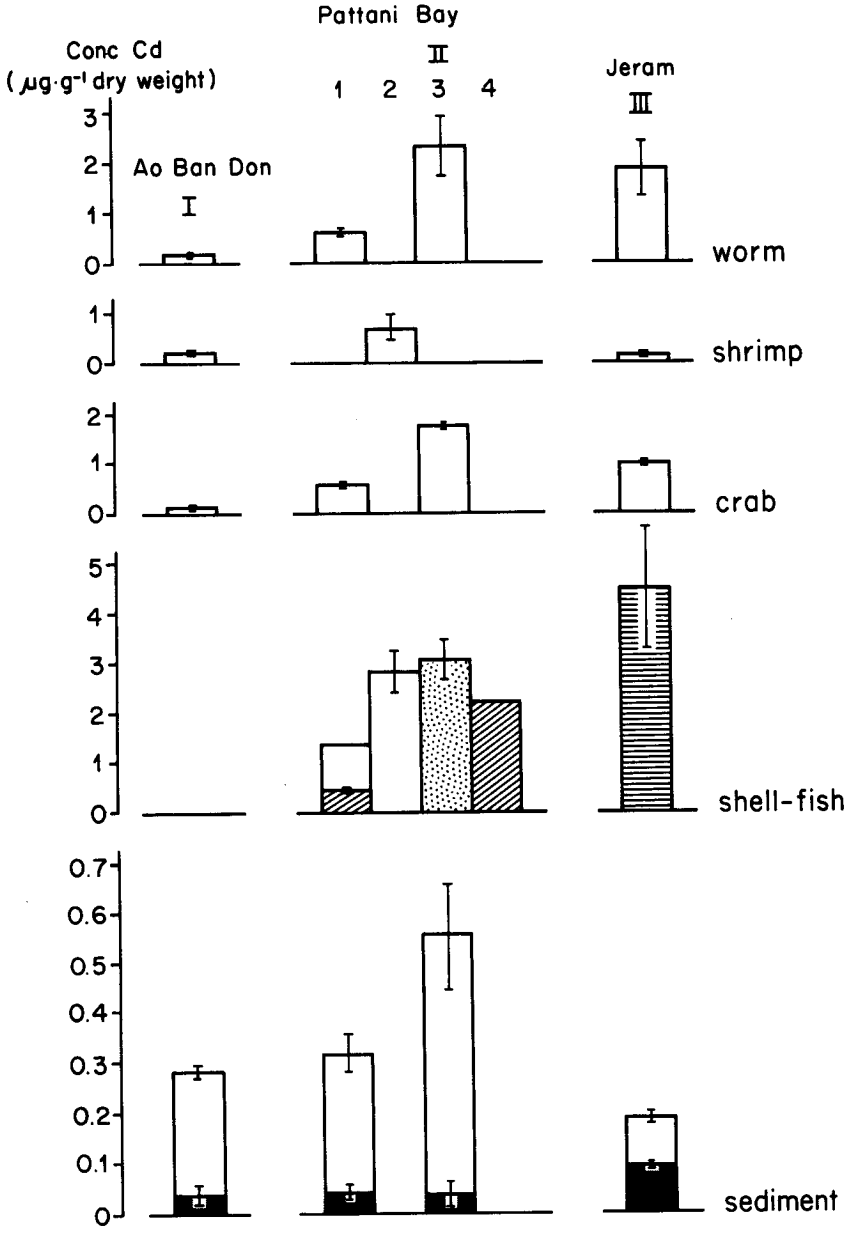


**Fig. 3.** The concentration of cadmium ( $\mu g.g^{-1}$ dry weight) in benthic invertebrates and sediment from three coastal areas of the Malay Peninsula. In four species of bivalve molluscs metals were analysed: *Perna viridis* ( ▨ ), *Glauconome virens* ( □ ), *Potamocorbula fasciata* ( ▩ ), and *Anadara granosa* ( ▤ ). Sediment analyses: total column shows the concentration in the silt fraction (grain size < 63 μm); shaded part shows the concentration in total sediment.

Bay, with mean values varying from 0.32 to 0.56 $\mu g.g^{-1}$ dwgt. These concentrations are almost a factor 10 lower than those in bivalve molluscs from the same area.

*Lead*

The polychaete *Dendronereis* spp. and the bivalve *Glauconome virens* from Pattani Bay showed relatively high lead concentrations, with values ranging from 16.7 - 20.8 $\mu g.g^{-1}$ dwgt. and 12.0 - 18.4 $\mu g.g^{-1}$ dwgt., respectively. These values exceed WHO standards for food.

The concentration of lead found in other species from the three areas varied between 0.7 and 5.6 $\mu g.g^{-1}$ dwgt.

Highest lead concentrations were found in sediments from Pattani Bay. In the grain size fraction $< 63$ $\mu m$, concentrations amounted to 242 $\mu g.g^{-1}$ dwgt. near the mouth of Pattani River and to 95 $\mu g.g^{-1}$ dwgt. at the mud-flats of Ban Da To, which is still twice as high as the concentrations at Jeram (48 $\mu g.g^{-1}$ dwgt.) and Ao Ban Don (40 $\mu g.g^{-1}$ dwgt.).

The total amount and the concentration of heavy metals in seawater, both in solution and adsorbed to suspended matter, may vary in relation with rainfall seasonality, due to an increased river run-off of fresh water. However, the concentration of heavy metals in sediment and organisms reflects the contamination-level of the environment over a longer period. Highest concentrations of the metals analysed were found in organisms and sediments from Pattani Bay. This estuarine area receives a significant amount of fresh water, polluted by industrial and mining activities, from the outflow of the Pattani River. The metal concentrations measured in the lower reach from the diversion dam of Bang Lang to the river mouth ranged from 40 - 120 $\mu g.l^{-1}$ for zinc, was about 30 $\mu g.l^{-1}$ for copper and varied between 3 - 10 $\mu g.l^{-1}$ for cadmium and between 20 and 130 $\mu g.l^{-1}$ for lead[11].

On the contrary, lowest metal concentrations occurred in the benthic invertebrates and sediments both from Ao Ban Don and the coastal area of Jeram. Apparently, the rivers debouching into Ao Ban Don (M. Luang and M. Kirirath), originating in relatively unpolluted agricultural areas without industry and mining, have minor or no impact on the estuarine environment of Ao Ban Don. The coastal intertidal mud-flats in the area of Jeram are not directly exposed to riverwater inflow.

Comparison of data on nereid polychaetes from the literature and the present study shows that the concentrations of zinc and copper in *Dendronereis* are in the same range or slightly lower than in nereid worms from temperate latitudes (Table 3). However, the concentration of cadmium and lead are generally higher and agree with the concentrations found in *Nereis* from estuaries of rivers which drain the old mëtalliferous mining areas of south-west England[12-15].

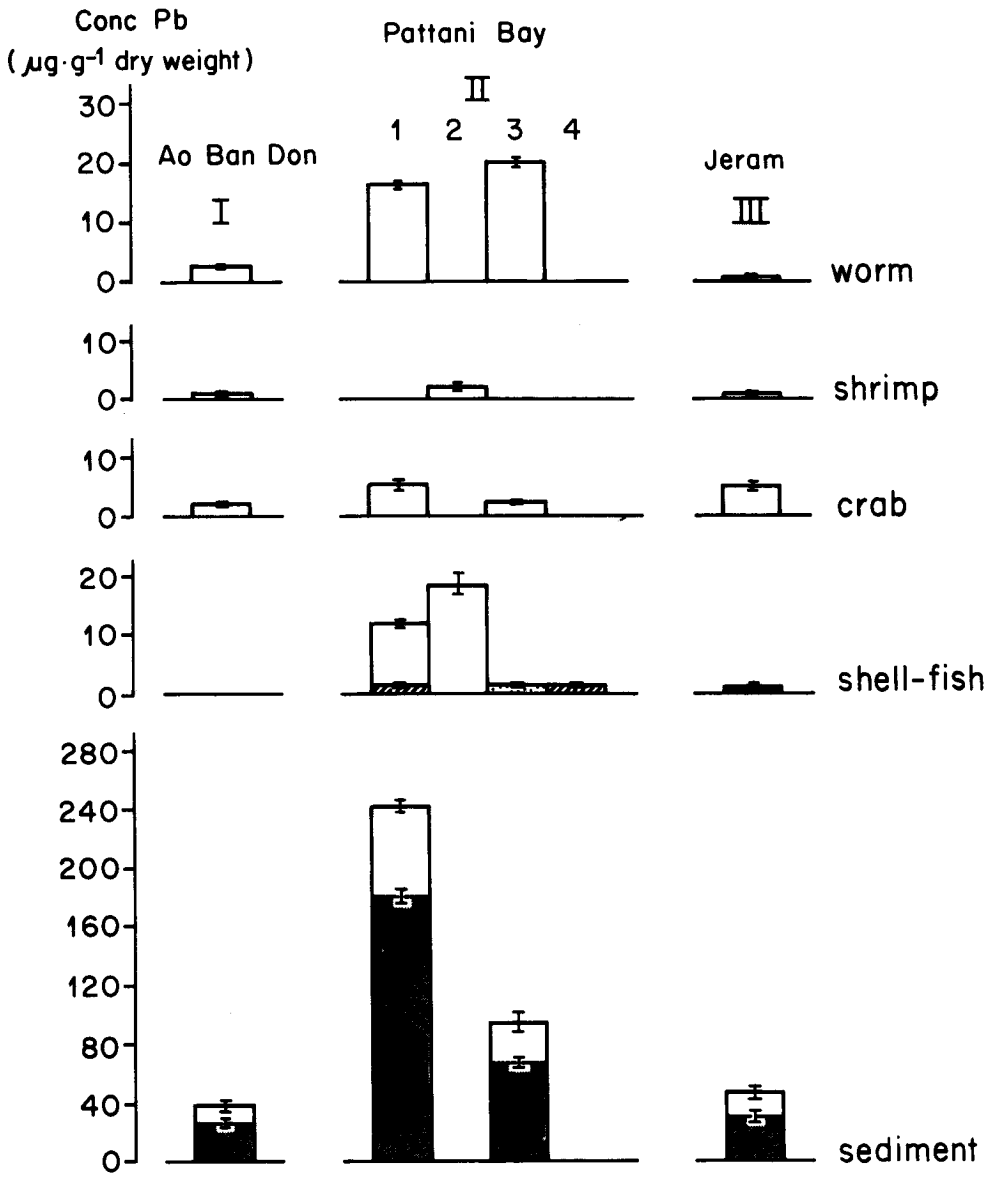


**Fig. 4.** The concentration of lead (μg.g$^{-1}$ dry weight) in benthic invertebrates and sediment from three coastal areas of the Malay Peninsula. In four species of bivalve molluscs metals were analysed: *Perna viridis* ( ▨ ), *Glauconome virens* ( □ ), *Potamocorbula fasciata* ( ▩ ), and *Anadara granosa* ( ▤ ). Sediment analyses: total column shows the concentration in the silt fraction (grain size < 63 μ m); shaded part shows the concentration in total sediment.

**TABLE 3.** REPORTED CONCENTRATIONS OF METALS ( $\mu G.G^{-1}$ DRY WEIGHT) IN SOME NEREID POLYCHAETES.

| Reference | species | Zn | Cu | Cd | Pb |
|---|---|---|---|---|---|
| 12. | *N. diversicolor* | 155-199 | 28-1142 | - | 0.7- 5.9 |
| 13. | | 130-350 | - | 0.08-3.6 | - |
| 14. | | 170-258 | 22- 78 | 0.1 -3.4 | 2.1-261 |
| 15. | | 147-334 | 12- 780 | - | - |
| 16. | | 92-423 | 11- 45 | 0.30-0.70 | - |
| 17. | | 96-196 | 12- 42 | 0.10-0.70 | - |
| | *N. virens* | 65-171 | 7- 20 | 0.01-0.50 | - |
| | *N. diversicolor* | 61-127 | 8- 24 | 0.09-0.38 | - |
| 18. | *Dendronereis* | 68-147 | 14- 22 | 0.17-2.35 | 0.9- 21 |

The concentration of zinc in the crustacea, being the same in shrimps and crabs (mean value 74 $\mu g.g^{-1}$ dwgt.), is lower than in polychaetes and molluscs. These concentrations are in the same range as crustacea from the Dutch Wadden Sea[16,17]. Compared to species of other phyla, crustacea have a very high content of copper, being incorporated in the blood pigment, haemocyanin. However, it can be concluded that the concentrations of copper in crustacea from the Malay Peninsula are higher (92 $\mu g.g^{-1}$ dwgt. in shrimps and 83 $\mu g.g^{-1}$ dwgt. in crabs) than in shrimps from the Dutch Wadden Sea[16,17] (52 $\mu g.g^{-1}$ dwgt.).

The bivalve molluscs *Perna viridis* and *Anadara granosa* are widely grown commercially in shallow waters of the upper Gulf of Thailand. A number of studies have been carried out to measure the concentration levels of metals in the whole body, and in general it was concluded that there was no threat to human health[1-6]. Comparison of these and the present data with some concentrations measured in bivalves from intertidal mud-flats of the Dutch Wadden Sea (Table 4) leads to the following conclusions. The concentration of zinc in bivalve molluscs from the Malay Peninsula is relatively low, except for the bloody cockle *Anadara granosa* (almost 120 $\mu g.g^{-1}$ dwgt.). The concentration of copper is low in *Anadara* (about 5 $\mu g.g^{-1}$ dwgt., but *Glauconome virens* shows relatively high concentrations (11 - 17 $\mu g.g^{-1}$ dwgt.). The cadmium concentration in *Anadara* (4.5 $\mu g.g^{-1}$ dwgt.) is pronouncedly high. The other species show slightly increased concentrations compared to the concentrations found in organisms from the Dutch Wadden Sea. The bivalve *Glauconome virens* accumulates lead to considerably high concentrations, up to 18 $\mu g.g^{-1}$ dwgt. The same conclusion can be drawn when data are compared of the corresponding species from other regions, both temperate and subtropical[14,19-21].

**TABLE 4.** THE CONCENTRATION OF SOME METALS (μ $G.G^{-1}$ DRY WEIGHT) IN BIVALVE MOLLUSCS FROM COASTAL AREAS OF THE MALAY PENINSULA (REF.: 3, 6 AND 18) AND THE DUTCH WADDEN SEA (REF.: 16 AND 17). MEAN CONCENTRATION VALUES WITHIN PARENTHESIS.

| Ref.: | species | Zn | Cu | Cd | Pb |
|---|---|---|---|---|---|
| 3. | *Perna viridis* | 95-253 (140) | 5.7-17.9 (9.6) | 0.09-0.46 (0.18) | 0.19- 1.31 (0.59) |
| | *Anadara granosa* | 79- 92 (88) | 1.4- 2.2 (1.9) | 1.88-2.77 (2.30) | 1.64- 2.16 (1.92) |
| 6. | *Perna viridis* | 61- 75 (69) | 9.4-15.6 (11.3) | 0.88-6.86 (3.16) | 0.31- 1.53 (0.89) |
| | *Andara granosa* | 65-125 (96) | 4.9- 8.8 (6.6) | 1.33-6.47 (2.68) | 0.37- 1.41 (0.82) |
| 16. | *Mytilus edulis* | 61-205 (109) | 6.4-18.0 (10.3) | 1.1 -4.4 (2.07) | -- |
| | *Cerastoderma edule* | 66-101 (80) | 3.2- 6.2 (4.2) | 0.10-1.10 (0.72) | -- |
| | *Mya arenaria* | 71-131 (95) | 5.4-23.8 (12.7) | 0.4 -1.0 (0.65) | -- |
| 17. | *Mytilus edulis* | 55- 78 (62) | 5.5- 8.9 (7.1) | 0.22-1.60 (0.83) | -- |
| | *Cerastoderma edule* | 56- 71 (65) | 2.9- 4.3 (3.5) | 0.13-0.25 (0.18) | -- |
| | *Mya arenaria* | 64-118 (88) | 9.5-14.9 (11.5) | 0.09-0.36 (0.17) | -- |
| 18. | *Perna viridis* | 42- 49 (46) | 6.0- 8.2 (7.1) | 0.51-2.22 (1.35) | 1.11- 1.50 (1.29) |
| | *Anadara granosa* | 103-132 (118) | 4.7- 4.8 (4.8) | 3.29-5.72 (4.51) | 0.43- 1.77 (1.10) |
| | *Glauconome virens* | 65- 80 | 11.0-17.2 | 1.4 -2.8 | 12.0 -18.4 |
| | *Potamocorbula fasciata* | 56 | 9.3 | 3.1 | 1.4 |

The concentration of the metals in the sediment is largely determined by its concentration in the silt fraction, especially at percentages of the silt fraction below 50% (Fig. 5); at higher percentages of the silt fraction, the concentration of zinc, copper and lead reach a constant level, whereas the cadmium concentration still tends to increase, but at a much lower concentration level.

There is no evidence of a relation between the metal concentration in the whole body of any of the species and the concentration of metals either in the total sediment or its silt fraction (grain size $<63$ μm).

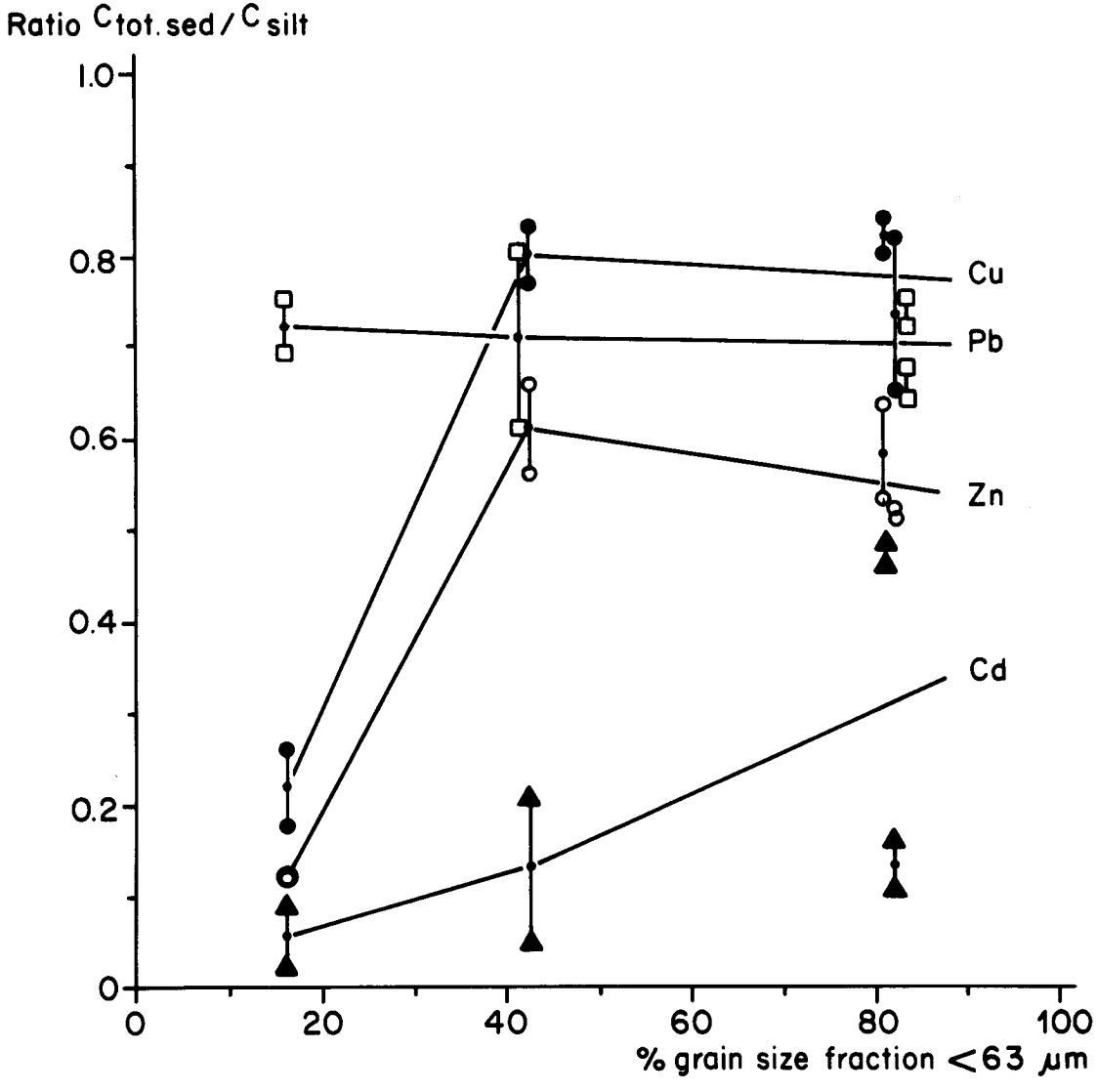


**Fig. 5.** The relation between the percentage of the silt fraction (grain size < 63 µm) of the total sediment and the ratio *Ctot.sed. / Csilt,* being the metal concentration of the total sediment divided by the concentration of the same metal in the silt fraction: zinc (O), copper (●), cadmium (△) and lead (□).

Different species of benthic invertebrates show different uptake patterns for different metals. An increased risk of bioaccumulation and a possibly harmful effect of elevated metal concentrations in benthic invertebrates on migrating birds depend on their feeding-habits, food-preference and length of stay. With respect to human health, it can be concluded that the concentration of cadmium, both in crustaceans and molluscs, does not yet reach harmful levels. However, in some areas (e.g. Ban Da To and Jeram) the cadimum concentration approximates to the maximum admissible levels (1.5 and 5.0 $\mu g.g^{-1}$ dry weight in crustaceans and molluscs, respectively, according to the Dutch standards for acceptable intake, which are based on WHO standards, and take into account the general consumption pattern). In contrast, lead concentrations do exceed the maximum admissible levels in crabs and molluscs (respectively 2.5 and 10 $\mu g.g^{-1}$ dry weight) collected from sampling sites in Pattani Bay.

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