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Cadmium Bioavailability to Three Estuarine Animals in Relation to Geochemical Fractions to Sediments

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Abstract. The effects of sediment characteristics and geochemical fractions on the biological availability of cadmium to estuarine animals were studied. Grass shrimp (*Palaemonetes pugio*), blue mussels (*Mytilus edulis*) and a polychaete (*Nereis virens*) were exposed to Cd-treated sediment for 14 days. The test populations were evaluated for mortalities, oxygen consumption (except *N. virens*), and bioaccumulation of Cd. Sediment Cd was extracted sequentially to determine the exchangeable (EP), easily reducible (ERP), organic-sulfide (OSP), moderately reducible (MRP), and acid extractable (AEP) geochemical phases.

The shrimp displayed significant respiration effects; the shrimp and mussels bioaccumulated Cd, particularly in the Cd-sand treatments. The polychaete did not bioaccumulate Cd. The OSP contained the greatest concentrations of Cd in all treatments. The Cd-sands were characterized by relatively higher levels of EP and ERP than for silts or clays. A statistical model was developed that describes the bioaccumulation of Cd as a function of EP and ERP geochemical fractions of sediment Cd for *M. edulis*.

When contaminants such as heavy metals are introduced into estuarine ecosystems, they often accumulate in the sediments (Lyons and Fitzgerald 1980; Santschi *et al.* 1980; Ray 1984). As a result, ecosystems such as seaports or other industrialized coastal areas that have chronic inputs of metals have highly contaminated sediments. This characteristic has led to concerns over the ecological effects that may be associated with sediment quality. Of particular concern are toxic effects and the potential for bioaccumulation of metals in biota exposed to the sediments. Since Cd is a toxic element found in many industrial effluents, considerable interest and research has been generated concerning its flux in the water-sediment-biota system (Ray 1984). Although organisms can accumulate Cd from sediments, the mechanisms controlling bioavailability are poorly understood (Ray 1984; Ray and McLeese 1987). Few studies have demonstrated strong correlations between sediment metal concentrations and toxicity or bioaccumulation potential (Neff *et al.* 1978; Rule 1985). In a study of 136 combinations (sediments-invertebrates-metals-salinities), only 36% of these showed a significant relationship between metal concentrations extracted from the sediments and in the tissues of the exposed organisms (Neff *et al.* 1978). These and other workers have generally used the total (or bulk) metal sediment content or a hot, concentrated acid extraction for sediment metals (Kersten and Forstner 1987; Rubinstein *et al.* 1983), so the lack of correlation does not seem surprising. Studies of dredged material have suggested that metals may be more biologically available in coarse sediments that display relatively low acid extractable concentrations than in finer textured sediments with greater extractable concentrations (Alden *et al.* 1988; Butt *et al.* 1985; Rule 1985).

Cadmium and Hg uptake by *Mytilus edulis* and *Mytilus demissus* was related to the amounts in a dilute HCl extract and total organic matter from sediments consisting mostly of sand with detritus or estuarine peat (Breteler and Saksa 1985). The major chemically active sediment form in these studies was the organic matter and its relationship with uptake was found as expected.

The uptake of heavy metals (including Cd) is related to the geochemical sediment phase in model, single phase systems. Cooke *et al.* (1979) and Davies-Colley *et al.* (1984), measured bioaccumulation from aerobic systems containing only iron oxide, manganese oxide, inorganic $CaCO_3$, biogenic $CaCO_3$, or a silicate clay mineral. These authors have given insight into binding/desorption and bioavailability from specific mineral phases but have not addressed multiphase systems in which these different mineral forms will compete for metals (Luoma & Bryan 1981) or anaerobic sediments where metal sulfides will constitute a major phase (Davies-Colley *et al.* 1984).

In order to further explore the relationship between sediment Cd and its uptake by estuarine organisms, a laboratory study was conducted to determine the biological availability of Cd to grass shrimp (*Palaemonetes pugio*), blue mussel (*Mytilus edulis*) and the polychaete worm (*Nereis virens*). The objectives of the study were to (1) determine the effects of sediment type, sediment Cd concentration, and their interaction on mortalities, respiration rates, and Cd bioaccumulation in the test species, (2) determine if added Cd affected the body burdens of Cu, Fe, Mn, Ni, Pb, or Zn, and (3) determine the relationship between the geochemical fractionation of Cd in the sediments and mortality, respiration and bioaccumulation.

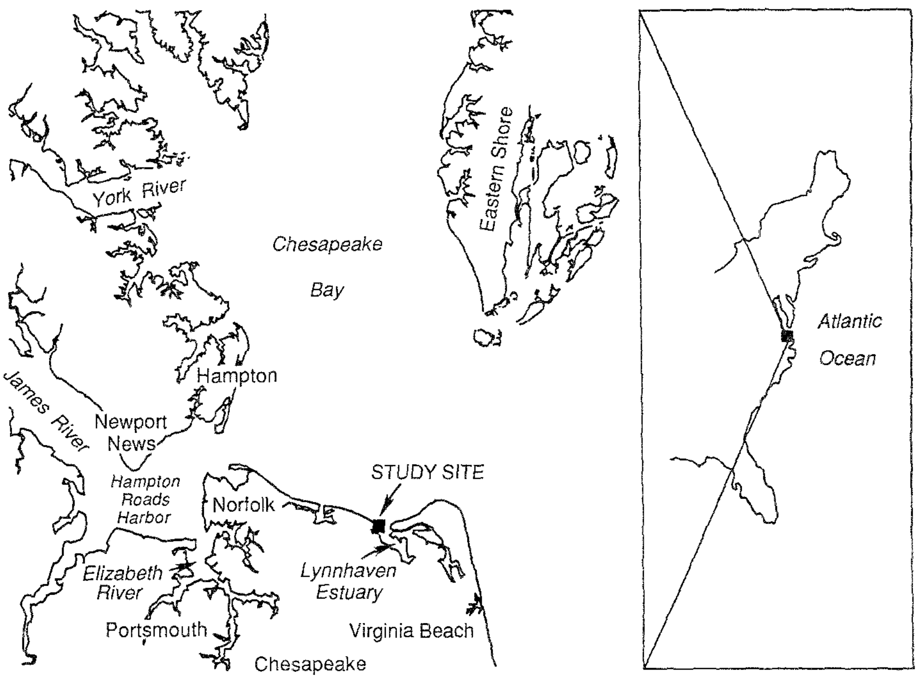

Fig. 1. Study area

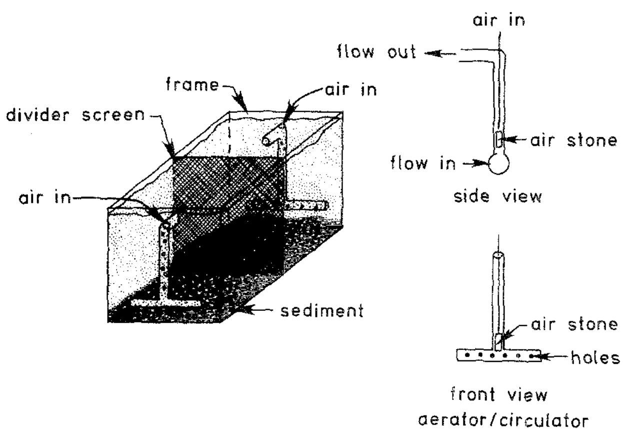

Fig. 2. Aquarium with divider screen and aerator/circulator

Materials and Methods

Sand, silt, and clay textured sediments were taken from the Lynnhaven Estuary, Virginia Beach, VA (Figure 1). This system is relatively free of direct industrial inputs, is surrounded by residential areas, and drains directly into the Chesapeake Bay. Each sediment was wet sieved through a 4 mm plastic mesh, thoroughly mixed, and the percent solids determined. Within 24 hr after collection, 2.5 L portions of sediments were placed in 4-L plastic jars and an appropriate amount of 1,000 mg/ml Cd standard solution was added to give 0 mg/kg (background), 5 mg/kg, and 10 mg/kg concentrations (dry weight basis) for each sediment type. After shaking for 24 hr the samples were placed in 30-L aquaria with 20 ppt salinity artificial sea water. After a 24-hr settling period, a plastic screen divider was placed in the center of each aquarium and an aerator/circulator was added to each end of the tank (Figure 2). The test animals were introduced to the aquaria 48 hr after the circulating systems were started. Each 30-L aquarium contained two test populations which were exposed to separate plots of sediments in independent circulation cells of water flow. Preliminary statistical evaluations were conducted on the biological and geochemical data in order to determine whether the communication of water through the screen (primarily via diffusion) affected the "within aquarium" replicates relative to the "between aquarium" replicates. Neither means or variances were significantly different between the "within" and the "between" effects for any of the treatments, so a total of six replicates were considered for each treatment.

The grass shrimp were collected in the same area as the test sediments; the blue mussels at the Chesapeake Light tower, east of the Chesapeake Bay mouth, and the polychaetes were purchased from a commercial aquaculture firm. All organisms were acclimated for seven days at the test conditions of 20°C and 20 ppt salinity before the experiments. Ten each of the grass shrimp and blue mussels and five each of the polychaetes were added to each half of the aquaria. Organisms were taken at this stage for background metal concentrations and prepared for analysis as described below.

The 14-day experiments followed the solid phase bioassay methods described by the US EPA and COE (1978). Seventy-five percent of the water was changed every other day and the organisms were not fed during the experiments.

At the end of the 14-day exposure, the respiration rates of the shrimp and mussels were measured, using aquaria water and a flow-through respiration system that has been successfully used in previous tests of the sublethal effects of dredged materials (Alden *et al.* 1988). Live/dead counts were made and all live organisms were dupurated for 24 hr in freshly prepared 20 ppt salinity water to purge sediments from their guts. *Palaemonetes pugio* and *N. virens* were rinsed quickly with deionized water, blotted, frozen for 48 hr, and dried at 60°C. *M. edulis* was washed with deionized water, frozen for 48 hr, shucked, and the tissue and fluids dried at 60°C. The dry tissue was stored less than one month before digestion. Organisms were digested in 22.4 *M* redistilled HNO_3 + 30% H_2O_2.

Sediment samples were collected at the beginning and at the end of the experiment. Samples were collected from three random locations in each half of each aquarium and composited to form two replicate samples per tank. The samples were centrifuged to remove excess water, remixed and subsampled for moisture determination. A sequential selective extraction series (modified from Brannon *et al.* 1976) was conducted as shown in Figure 3. Wet sediments (5 g dry wt) were subjected to the sequential extraction series in Figure

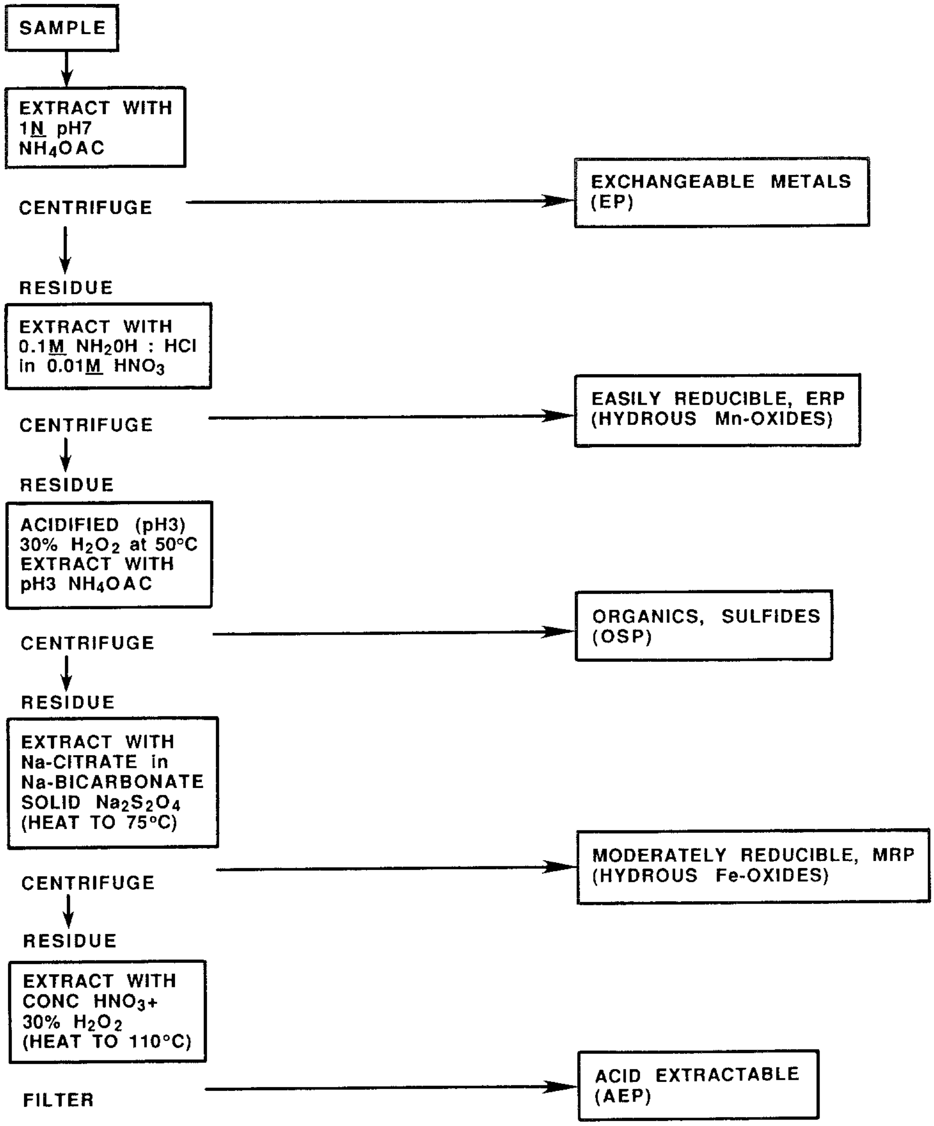

Fig. 3. Selective extraction scheme

3. Volumes of extractant were 50 or 60 ml for the different reagents. Details and discussion of the procedure can be obtained from the authors.

Sand, silt, and clay distribution in the sediments was determined by sieve and pipette methods after removal of soluble salts and dispersion with sodium hexametaphosphate (Gee and Bauder 1986). Sediment organic carbon was measured by a wet dichromate oxidation technique (Nelson and Sommers 1982).

All metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) were determined by atomic absorption spectrophotometry (AAS), using a Perkin-Elmer Model 603 and deuterium background correction. Standards were prepared in the appropriate matrix for the various extracts. Quality control materials were SRM 1566 (Oyster Tissue) from the National Bureau of Standards and WP-386 concentrate from the US EPA for spiking the selective extraction matrices. All QC results were within the acceptable limits for all of the materials and matrices.

The biological data were analyzed by a 2-way ANOVA and Duncan's Range Test and, if indicated, by a regression-based ANOVA of main effects and interactions. A stepwise regression was utilized to determine relationships between the Cd body burdens and the geochemical fractions, and sediment characteristics of the treatments. All statistical analyses were conducted with the SAS statistical package (SAS 1985).

Results and Discussion

Although there was a relatively high sand content of all sediment types (Table 1), the very fine size of the sand in the "silt" and "clay" types yielded field characteristics of these textures. Grain size in the "sand" texture was mostly in the coarsest (1–2 mm) range. Organic carbon content increased with decreasing particle size.

In the majority of the native sediments, Cd was in the acid extractable phase (AEP) and organic sulfide phase (OSP) (Figure 4A). The Cd in the AEP is interpreted to be bound by the most resistant oxides and structurally in other resistant minerals. Cadmium in the OSP was most likely in the form of sulfides (Davies-Colley *et al.* 1984; Gardiner 1974; Lee and Kittrick 1984). No correlation of Cd with organic carbon was found for any of the sediment-treatment-extraction combinations. The large majority of applied Cd resided in the OSP, with the second greatest amount in the easily reducible phase (ERP) (Figure 4B, C). There were similar amounts of Cd in the ERP of the respective sediments for both the 5 and 10 mg/kg treatments, indicating that the

Table 1. Mean values of sedimentological characteristics of test sediments. Standard errors of the means are in parenthesis (n = 18)

Sediment type	% Sand	% Silt	% Clay	% Organic carbon
Sand	95.9 (0.4)	1.2 (0.3)	2.4 (0.2)	0.2 (0.03)
Silt	74.5 (0.9)	19.3 (0.6)	6.9 (0.3)	1.2 (0.08)
Clay	59.6 (1.4)	19.4 (1.0)	11.0 (0.4)	1.7 (0.09)

binding capacity of this phase was met or exceeded with 5 mg/kg of added Cd. The form of Cd in this phase is uncertain; this is commonly defined as the Mn-oxide phase but the concentration of extractable Mn was very low (<1 mg/kg). Significant amounts of Fe (>100 mg/kg) were extracted in the ERP and it is possible that the Cd was associated with the easily reducible forms of Fe. Cadmium in the exchangeable phase (EP) was detected only in the sand (avg. of 0.04 ± 0.005 mg/kg for treated sands). Equivalent, low levels of Cd were found in the AEP of 5 and 10 mg/kg treatments for the respective sediment types, indicating little reactivity (Cd binding) of this phase.

Mortalities of the test species were low (<15%) and not significantly related to sediment type (TRT1), Cd concentration (TRT2), or their interaction. Body burdens of Cu, Fe, Mn, Ni, Pb, or Zn showed no significant difference for any test organisms from any of the Cd treatments (Table 2). The initial postulate that added Cd might affect the uptake of these metals was not valid. Cadmium content of *N. virens* did not vary with the sediment type or Cd treatment, unlike the results of Ray *et al.* (1980). An average body burden of 1.04 mg/kg (dry wt) was obtained in this test species.

The ANOVA and Duncan's tests indicated that the Cd concentrations in the shrimp from the 5 and 10 mg/kg treatments were significantly higher ($p < 0.05$) than the controls (Figure 5) but the sediment effects were not significant ($p = 0.2$). The body burdens of blue mussels were directly related to the added Cd, with pronounced effects in the sand treatments (Figure 6).

Respiration rates of the blue mussels did not display significant relationships to the Cd treatments or sediment types. Gilfillan *et al.* (1985) reported that respiration rates of *M. edulis* were not significantly related to body burdens of Cd, even though some of the mussels in their study were taken from contaminated areas and the "scope for growth" indices of the populations were indirectly correlated with tissue cadmium levels. Therefore, respiration does not appear to be an effective condition index for mussels exposed to sublethal levels of Cd.

In a review of the literature, Eisler (1985) reported that cadmium causes respiratory disruption in certain marine species. The disruption manifested itself as a reduction in respiration in crustaceans (Vernberg *et al.* 1974) and an increase in respiration in fish gill and tissue (Calabrese *et al.* 1975). In the present study, significant disruption was observed to occur in both directions: significantly elevated respiration rates for shrimp populations exposed to the 5 mg/kg treatments and significantly depressed rates for those in the 10 mg/kg treatments, particularly those involving the sandier sediments (Figure 7). This pattern follows the "general adaptation syndrome" (Selye 1976; Sinderman 1985): an increase in metabolism in response to the stress of moderate levels of a contaminant; and a decrease in metabolism when the exposure period and/or the concentration is sufficient to cause a collapse in the respiratory compensation response. Similar responses have been observed for grass shrimp exposed to various types of contaminants (Cantelmo *et al.* 1978; Rao *et al.* 1979; Dillon 1981; Alden *et al.* 1988).

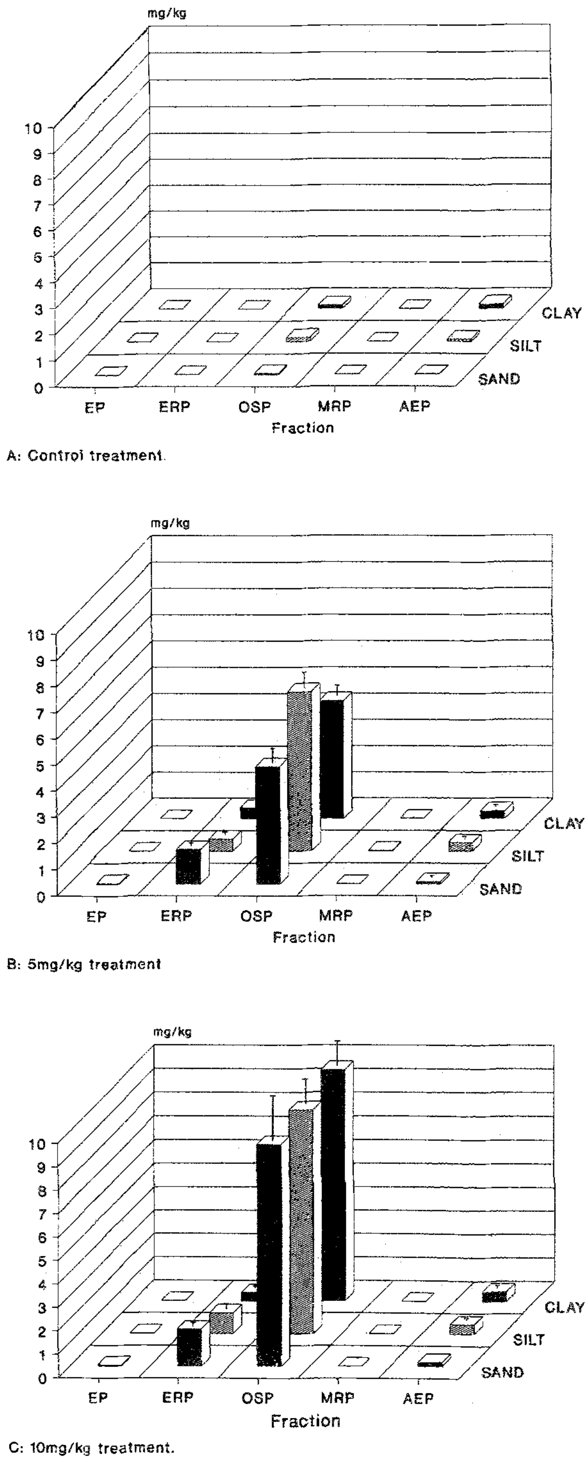

Fig. 4. Cadmium concentrations (mg/kg) in the test sediments. Errors bars represent one standard error of the mean

Since the 10 mg/kg treatment in the sands produced the

Table 2. Grand means (mg/kg dry wt) and standard error of the means for elemental analysis of exposed organisms. Numbers in parenthesis are the number of observations

Element	*M. edulis*	*P. pugio*	*N. virens*
	(59)	(59)	(44)
Cd	6.18 ± 0.62	1.19 ± 0.04	1.04 ± 3.9
Cu	11.30 ± 0.68	121.31 ± 0.97	8.98 ± 0.18
Fe	903 ± 260	44.0 ± 2.5	304 ± 7
Mn	10.60 ± 1.6	7.70 ± 0.44	4.20 ± 73
Ni	23.80 ± 1.1	5.55 ± 0.21	1.49 ± 0.17
Pb	8.35 ± 0.46	9.79 ± 0.22	4.06 ± 2.49
Zn	204 ± 20	96.5 ± 1.6	121 ± 14

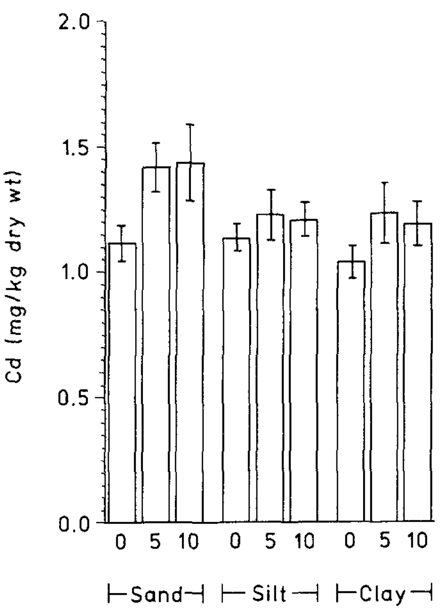

Fig. 5. Cadmium concentrations in *Palaemonetes pugio* after exposure to sediment/Cd treatments. Error bars show one standard error of the mean (n = 6)

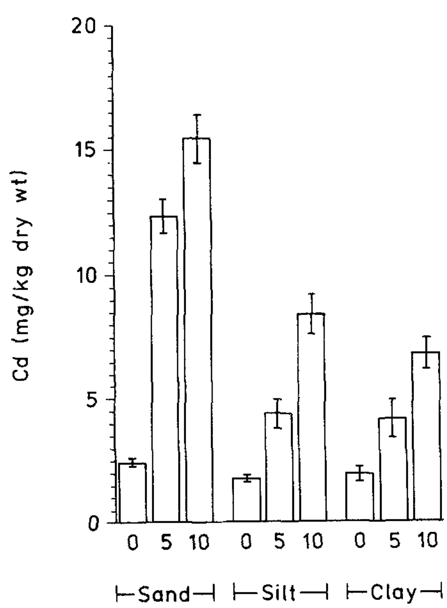

Fig. 6. Cadmium concentrations in *Mytilus edulis* after exposure to sediment/Cd treatments. Error bars show one standard error of the mean (n = 6)

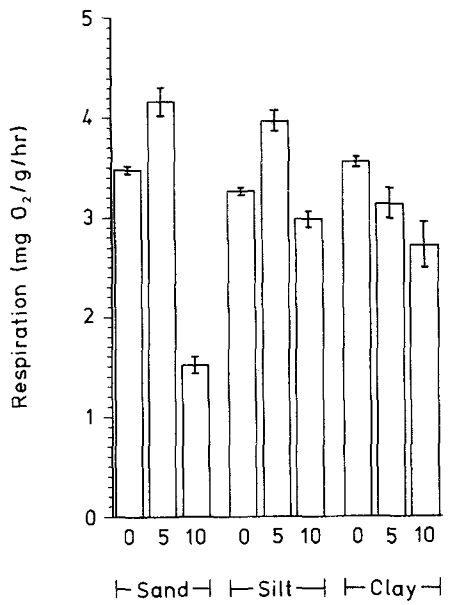

Fig. 7. Respiration rates of *Palaemonetes pugio* exposed to sediment/Cd treatments. Error bars represent one standard error of the mean (n = 6)

greatest respiration effects and Cd bioaccumulation, a discriminant analysis was performed to determine how this treatment differed from the others in terms of geochemical fractionation of Cd. The Cd-10 sand was characterized by the highest Cd concentrations in the EP and ERP (Figure 8). A second discriminant analysis was run on residuals from a MANOVA designed to account for the effects of the Cd treatment for all test sediments (*i.e.*, to analyze texture effects). Sands had higher Cd levels in the EP and ERP, regardless of treatment; silts and clays had higher Cd levels in the AEP; and silts had the only detectable Cd-MRP (0.03 ± 0.03 mg/kg for Cd-5) (Figure 9).

From a geochemical interpretation, the EP and ERP are the phases most likely to contain bioavailable elements. Metals in these fractions are the most weakly bound and should be most available to the water column and/or organism uptake. Research suggests that Cd accumulation by some organisms is a function of the amount in the aqueous phase which, in turn, depends upon the amount and leachability of the element in the sediment phase (Ray 1984; Ray *et al.* 1980; Ray and McLeese 1983).

Stepwise regression of the body burden data versus the geochemical fractions and sedimentological characteristics

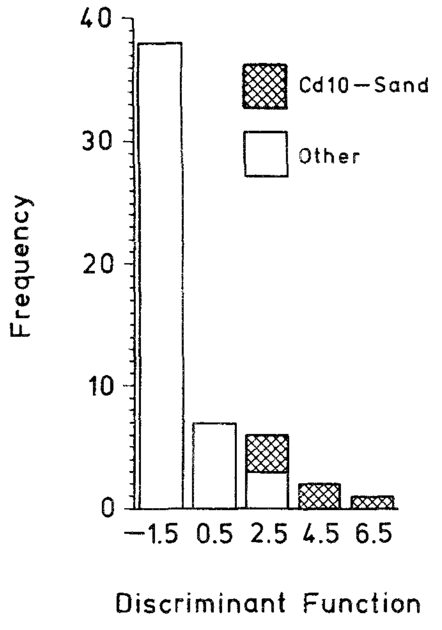

Fig. 8. Discriminant function scores describing differences between the Cd-10 sand and other treatments. The discriminant function label represents those geochemical fractions shown to be significantly ($p < 0.01$) related to the treatment effects in the MANOVA

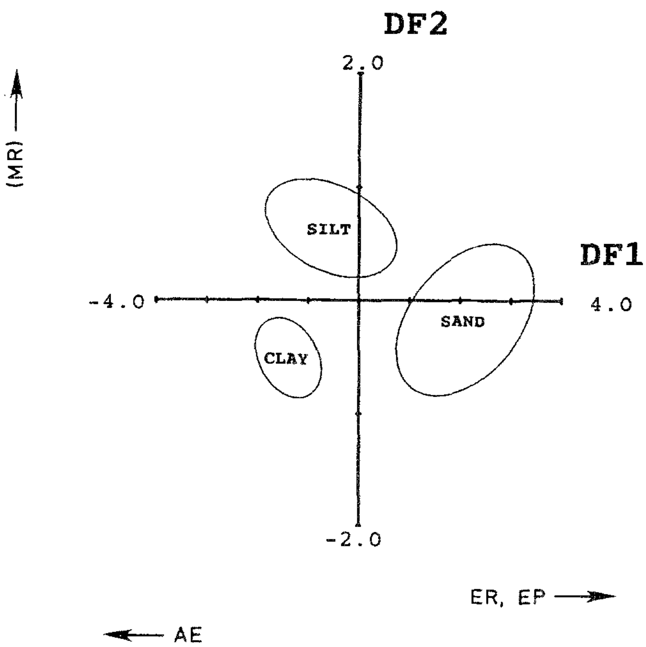

Fig. 9. Confidence ellipses ($\alpha = 0.05$) for discriminant functions describing sediment-related differences in geochemical fractions of cadmium. The discriminant function labels represent geochemical fractions shown to be significantly ($p < 0.01$) related to the sediment types in the MANOVA. Cadmium in the MR fraction was detected only in the silt

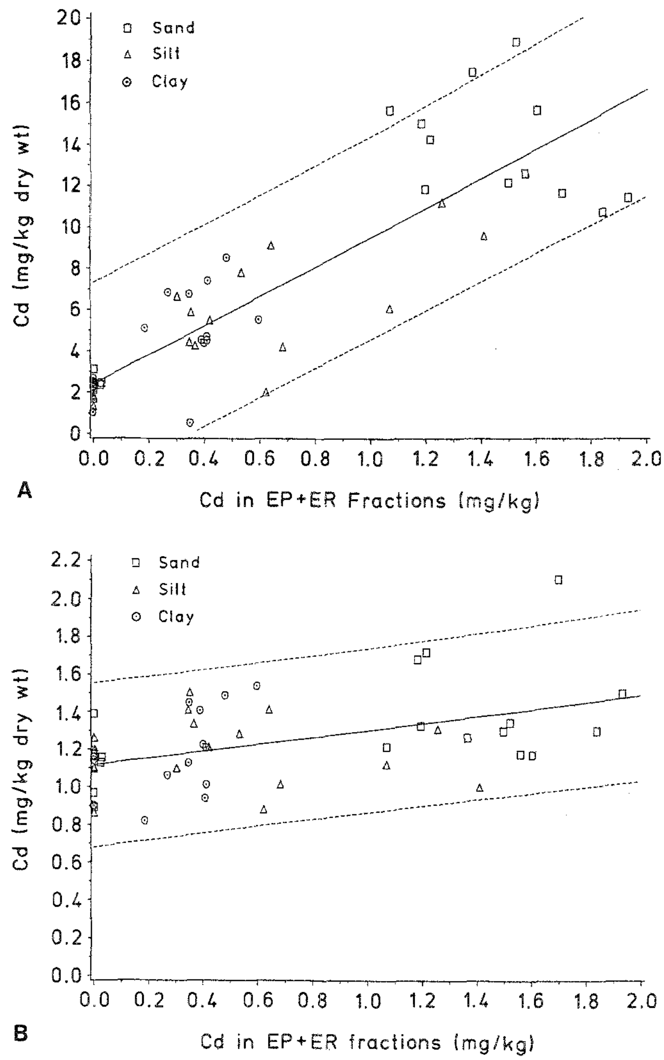

Fig. 10. A. Cadmium concentrations in *Mytilus edulis* versus Cd in EP + ER fractions in sediment ($p < 0.0001$; $r^2 = 0.73$). **B.** Cadmium concentrations in *Palaemonetes pugio* versus Cd in EP + ER fractions in sediments ($p = 0.005$; $r^2 = 0.14$)

indicated that the EP and ERP fractions were the most biologically available. These two fractions combined were significantly related to the Cd content of *M. edulis* ($p > 0.0001$; $R^2 = 0.73$; Figure 10A). The shrimp accumulated far less Cd, but body burden was also significantly related to the EP and ERP ($p = 0.005$; $R^2 = 0.14$; Figure 10B). The 95% prediction limit is shown for each regression line. All other fractions were poorly related, if at all, to the bioaccumulation of Cd. The body burden of mussel Cd is described by the relationship:

$$Cd_{M.\ edulis} = 2.59 + 6.98\ [EP + ERP].$$

This equation represents a successful bioaccumulation model using selective sequential geochemical extraction of estuarine sediments. The regression equation can be slightly refined for each of the sediment types but the above general equation gives very satisfactory results. The major component of the equation is the ERP Cd which accounts for the large majority of the bioavailable Cd.

Summary and Conclusions

The respiration rates of grass shrimp were significantly affected by the cadmium levels with an interaction between the sediment type and Cd treatment (*i.e.*, most elevated rates in the sand-5 mg/kg treatment and, most depressed rates in the sand-10 mg/kg treatment). Cadmium body burdens of the test organisms were significantly higher in tanks with added Cd, particularly in the sand treatments. The sand treatments were geochemically characterized by higher EP and ERP fractions. The EP and ERP fractions of all sediments were directly related to the Cd body burdens of the test species. Despite the fact that the major portion of the added Cd entered the organic/sulfide fraction, the EP and ERP fractions that characterized the sands appeared to be the most biologically available.

There appeared to be a finite amount of Cd which could be incorporated into the ERP, since these concentrations were very similar for both treatment levels and most of the additional Cd in the Cd-10 treatment partitioned into the OSP. Therefore, analytical protocols focusing upon these easily extractable fractions will provide a more ecologically realistic picture of the significance of metals contamination than will bulk sediment analyses or those using strong extraction techniques.

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