

Chemical Extractions of Heavy Metals in Sediments as Related to Metal Uptake by Grass Shrimp (*Palaemonetes pugio*) and Clam (*Mercenaria mercenaria*)

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Abstract. Sediments from four sites in the Hampton Roads Harbor and Elizabeth River system were subjected to solid phase bioassays using *Palaemonetes pugio* and *Mercenaria mercenaria*. Metal levels in both organisms after exposure to the sediments varied little between sites; there was no difference in metal uptake between organisms exposed to the test sediments and to the reference sediment. All of the test sites would be acceptable for ocean disposal with respect to the tested metals. Metals concentrations in *Palaemonetes* were generally greater than *Mercenaria*. The amounts of metals extracted from the sediments were in the order of Conc $\text{HNO}_3 + \text{H}_2\text{O}_2 > 1\text{ N HNO}_3 > \text{DTPA}$. Differences in metal levels in the Conc $\text{HNO}_3 + \text{H}_2\text{O}_2$ and DTPA extracts from different sediments were generally significant and related to sediment type and sampling location. Since there was no significant difference in the concentrations in tissue for either organism, there was no correlation of metal uptake with the sediment extraction method. Out of four sites and metals studied with two test organisms, there were only four instances of bioaccumulation. Data from sediment extractable metals and metal/Fe ratios indicated *Palaemonetes* were enriched with respect to the sediment in Cr, Cu, Ni, Pb, and Zn; *Mercenaria* were enriched with Pb and Zn.

Because of the increasing amounts of toxic metals from anthropogenic sources in the sedimentary environment, various means have been devised for determining their effects on marine organisms. Reaction to the uptake of metals ranges from the

obvious toxic responses to those of chronic and no adverse effects. Levels of metals resulting in chronic responses are difficult to detect or assess. These levels are of importance, since prolonged chronic levels or synergistic effect of various metals and/or other toxicants can result in undue stress on the organisms. The most direct way to assess the degree of biological uptake of toxic metals from sediments is to collect and analyze the sediments and organisms dwelling therein. The collection of sufficient biomass, or of desired species is often difficult; the task is impossible if the sediment in question contains high levels of toxicants, low dissolved oxygen or nutrients, or if other factors result in the presence of few organisms.

Because of these difficulties, laboratory bioassay techniques have been developed for the assessment of responses to chronic metal exposures. The need to standardize procedures, test organisms, and other factors led to the development of the "Implementation Manual" by the US Environmental Protection Agency (EPA) and the US Army Corps of Engineers (COE) (1978). Although there are several advantages to laboratory bioassays, there are also some drawbacks, primary of which is the time and cost of the tests. The Ocean Dumping criteria (EPA and COE 1978) require that for evaluation of sediment toxicity, a three-phase (liquid, suspended-solid, and solid) bioassay be used for each sample. If proper replication of sediment samples and multiple test organisms are used, the task of testing a large number of sediments can become enormous and costly.

Raymond W. Alden, III (personal communication) adopted the suspended solid phase bioassay for use as a screening test for large numbers of sediment

samples. This method was found to work very well as a screening technique; however, the development of a relatively rapid, simple, and inexpensive method for initial screening of sediments for toxic metals availability would be very useful. The possibility that this might be accomplished with a chemical extract of the sediment is very inviting. Although it is realized that a single extraction procedure may not work for all metals or organisms, a series of extractions may be an advantageous alternative to the bioassay method.

Chemical extractants have been successfully used for both major and trace metals in soils (Mortvedt *et al.* 1972). Some researchers and reviewers (Pequegnat *et al.* 1978) argue that research indicates that no simple extractant can be developed to predict biological availability of sediment trace metals. While this is probably true, a more complex chemical extractant scheme may be possible to attain and prove less costly and time-consuming than bioassay techniques. Such a procedure could be very useful for screening purposes.

The variety of potential chemical extractants is great. Those that are most likely to be initially tested are the ones already successfully used for extraction of trace metals in soils. Most of the marine sediment extracting (or leaching) techniques determine the partitioning among various chemical phases, with no direct interest to the bioavailability (Hirst and Nicholls 1958; Chester and Hughes 1969; Gibbs 1973). Diks and Allen (1983) studied the uptake of Cu from suspensions of four river sediments by tubificid worms. They extracted Cu from five different fractions of the sediment and attempted to relate uptake to the Cu concentrations in the five phases. A high correlation was found between Cu uptake and the amount of the element present in the manganese oxide/easily reducible phase. Other researchers have studied dredged material, but were primarily concerned with metal partitioning (Brannon *et al.* 1976; Chen *et al.* 1976).

This method of assessing potential bioavailability is receiving increased interest and attention. Depending on the chemical nature of the sediment, the determination of metals in various chemical fractions may have merit. A major problem in comparing metals extracted from various phases to biological uptake is that the entire fraction of each is dissolved. Biologically-available metals may occur primarily in only one phase or may be the most loosely-bound portions of several phases. A method is needed that will extract those metals easily available to biological organisms, regardless of the phase in which they exist. This paper presents results from the testing of three extraction methods for this application.

The types of chemical extractants may conveniently be divided into three groups: (1) acids, (2) chelates, and (3) salts. The standard sediment extractant utilized in the author's laboratory is hot, concentrated HNO_3 -plus-30% H_2O_2 . While being far from a bulk metal extraction, this is a rather severe treatment. The procedure is expected to completely remove metals associated with hydrous oxides, carbonates, and organic matter and to strongly leach the remaining constituents. Metals thus extracted should be well above the maximum concentrations expected to be bioavailable to any organisms or be released by any chemical changes which might occur in the sediments. A large data base has been established with this extractant on lower Chesapeake Bay area sediments, and it was included in the bioavailability study.

Weak acid (0.01–1.0 *N*) extractants are used by many soil test laboratories for estimating trace metal availabilities. The acidic nature of soils and plant root exudates make such extractants very appropriate. For marine organisms and sediments, these solutions may be less applicable to *in situ* sediment processes. However, acid extractants may simulate conditions encountered in the gut of many organisms that ingest sediments. Gates and Travis (1969) found that pH 4 was the lowest known value for benthic invertebrate gut content. Only small amounts of metal would be released at this pH. Vertebrate digestive tracts are commonly at pH values of 3 or less. There are mechanisms of uptake and organism soil/sediment interactions that are not thoroughly documented. Malo (1977) used 0.3 *N* HCl to extract aquatic sediments but was primarily interested in the metals associated with the "acid extractable oxides." Pequegnat and Presley (1978) used 1 *N* HNO_3 to extract marine sediments as an estimate of bioavailability; the 1 *N* concentration was practical where large amounts of CaCO_3 were present. Because of their work and the fact that a stronger concentration of HNO_3 was used in the first procedure, the 1 *N* concentration was chosen as the second of the test extractants in the current study.

Extractions with chelating agents have been introduced in the field of soil testing (Lopez and Graham 1971; Lindsay and Norvell 1969a, 1969b; Rule and Graham 1976). Chelating agents are of interest as extractants, because of their natural presence in organisms and their particular affinity for trace elements. Two agents widely used for soil extractions are ethylenediaminetetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA); the latter was chosen as an extractant in the present study. Salt solutions (0.10 to 1.0 *N*) have been used as extractants for major metals in soils.

Since marine systems already are at such salt concentrations, these procedures would not be expected to be effective as a primary extractant for trace metals in marine/estuarine sediments.

This report presents data obtained when sediments from dredging sites in the Hampton Roads Harbor and Elizabeth River, Virginia, were extracted by the three procedures discussed above, and compares the results to those obtained in solid phase bioassay toxicity test method utilizing the grass shrimp *Palaemonetes pugio* and the clam *Mercenaria mercenaria*.

Materials and Methods

The Port of Hampton Roads, Virginia, is located within the major metropolitan area encompassed by Norfolk, Virginia Beach, Chesapeake, Portsmouth, Newport News, and Hampton (Figure 1). The waters of this harbor are in one of the most industrialized coastal areas in the eastern part of the United States.

Sediments were collected from four dredging sites in the Hampton Roads Harbor and Elizabeth River (Figure 1). Site D was located at the western end of the Newport News Channel in Hampton Roads Harbor, near major shipyard facilities and ship anchorages. Site E was adjacent to the large naval base in the Harbor. Site H was near the confluence of the Western Branch and main stem of the Elizabeth River, an area downstream of the most heavily industrialized portion of the river. Site P was located in a lightly industrialized area near the upper reach of the Southern Branch of the Elizabeth River. A brief sediment description is presented in Table 1. Ten grab samples were taken at each site, using a 0.76 m³ clamshell grab; 18 L of material from the center of each was composited to obtain test sediment for each site. Sediment was stored overnight at 4°C and bioassays were initiated the day following sediment collection. The ten-day solid-phase bioassays utilized methods described by the EPA and COE (1978). Grass shrimp (*Palaemonetes pugio*) and clam (*Mercenaria mercenaria*) were used as the test organisms. The organisms were collected in non-industrial areas from Virginia's Eastern Shore and acclimated for seven days to the conditions of 20°C and 30 ppt. salinity before the beginning of the bioassay. Organisms were acclimated for 48 hr in 30-L aquaria to reference sediment taken from a potential offshore disposal site located in nearshore shelf waters of the Atlantic Ocean approximately 20 km off the mouth of the Chesapeake Bay. The organisms were exposed to sediments from the four test sites by adding these sediments to the aquaria to simulate ocean disposal. The control group was exposed to reference sediment. All sediment sites were replicated six times. Further details concerning the study area, sediments, and bioassay methods may be obtained from Alden and Young (1982). At the end of the ten-day bioassay, samples of the sediments and both types of organisms were taken from each of the tanks for metals analyses. Sediment samples were dried at <40°C, crushed to pass a 2mm stainless steel sieve, and stored in plastic bags at room temperature until extracted. Both organisms were allowed to depurate for 24 hr in 30 ppt salinity water to remove sediment gut content. *Palaemonetes* were rinsed quickly with deionized water and dried at 60°C. *Mercenaria* were washed with deionized water, shucked and the tissue and fluids dried at 60°C. Tissue samples were analyzed within two weeks of collection.

Organisms (five per sample) were dissolved in 22.4 M redistilled HNO₃ + 30% H₂O₂. Sediment samples were extracted by each of the following methods: (1) Hot (100°C), concentrated (15.4 M) redistilled HNO₃ + 30% H₂O₂ for six hr, filtered through pre-rinsed Whatman No. 42 filter paper; (2) 1 N HNO₃ (after neutralization of carbonates), shaken for two hr at room temperature, centrifuged, if necessary filtered; and (3) 0.005 M DTPA in 0.10 M NaOAc, shaken for four hr, centrifuged, filtered if necessary. Metals analyses for Cr, Cu, Fe, Mn, Pb, Ni, and Zn were performed by atomic absorption spectrophotometry (AAS), using a Perkin-Elmer 603. Standards were prepared in the appropriate matrix for the various extracts. Quality control materials were SRM No. 1654 (River Sediment) and SRM No. 1566 (Oyster Tissue) from the National Bureau of Standards.

Linear regression analyses were performed with the metal concentrations of either *Palaemonetes* or *Mercenaria* as the dependent variable and sediment concentrations from the three extractions as the independent variables. Regression analyses were made in the same manner using metal concentrations normalized to the iron concentration (metal/Fe). One-way ANOVA/Duncan's Range analyses were conducted on the data set to test replicate homogeneity and to compare site means from the sediment extractions and for the tissue concentrations using both the metal concentration and the iron-normalized coefficients. All statistical packages were from the SPSS Manual (Nie 1975).

Results and Discussion

ANOVA/Duncan's Range Test showed that the replicate values for all data sets (sediment extractions and tissue concentrations) constituted statistically similar subsets at the 95% confidence level. Variances for all data sets were homogeneous by the Bartlett's test.

Quality Control Samples

Metal concentrations certified for the river sediment (NBS-SRM No. 1654) are for total metals and the extractant would not be expected to yield the true values. Because of the nature of the material (high organics, fine clay, and quartz sand), concentrations obtained for Cr, Cu, Mn, Ni, Pb, and Zn were within certified ranges. Concentrations obtained for Fe were lower than the certified value, but consistent with past extractions of this material.

Concentrations obtained for the oyster tissue (NBS-SRM No. 1566) were within acceptable ranges for Cu, Fe, Mn, Pb, and Zn. Some difficulties for Cr and Ni were experienced because they were near the detection limit for this analysis.

Organism Uptake-Bioaccumulation

There were 56 instances of possible uptake, but significant uptake occurred in only three cases. The

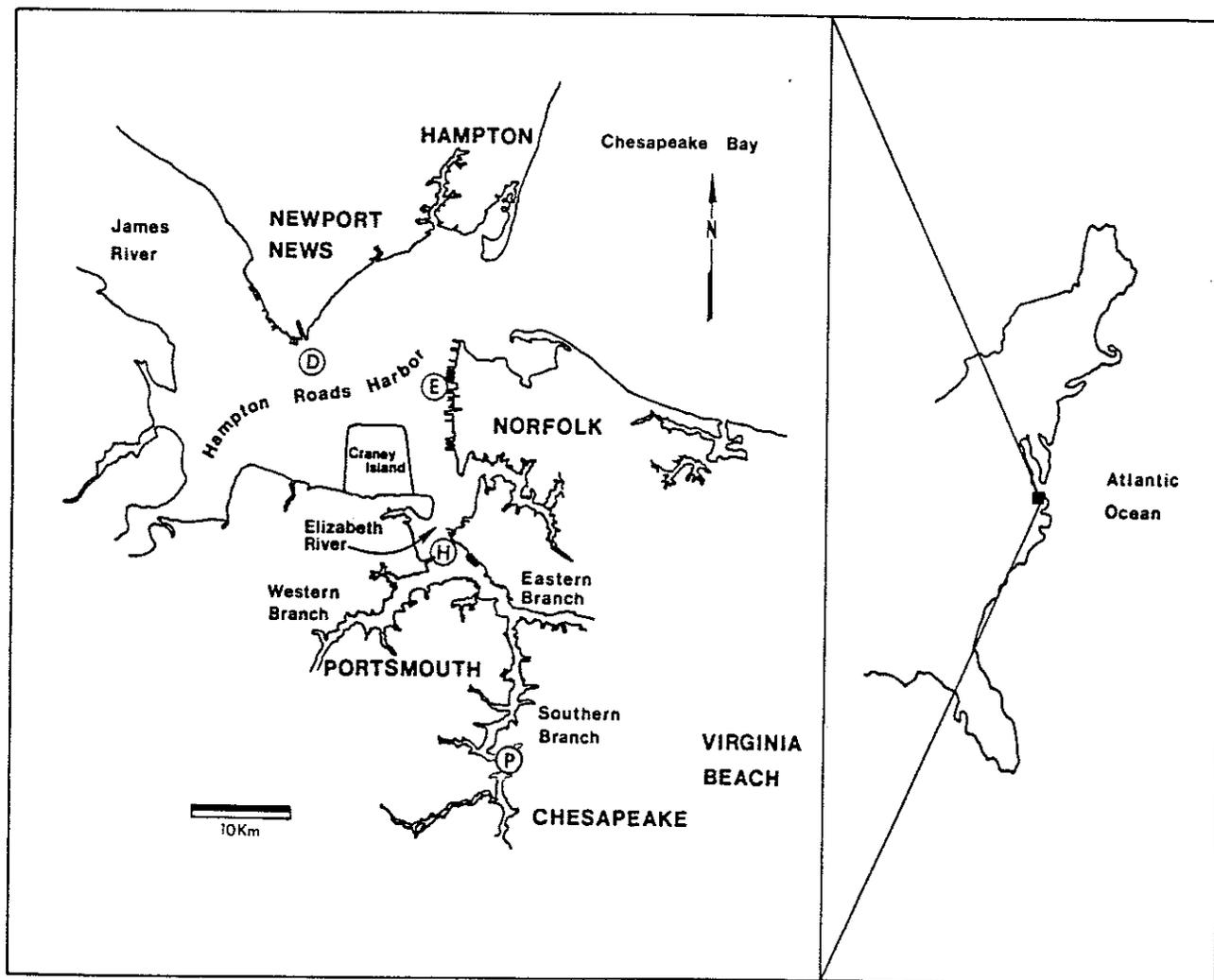


Fig. 1. Study area: Sediment collection sites in the Port of Hampton Roads, Virginia

Table 1. Sediment descriptions

Reference site

Medium to coarse sand; none collected >2mm, numerous small shell fragments, no visible organic matter

Site P

Approximately 17% of sampled material was >2mm and was all shell hash—this was not chemically analyzed

<2mm: about 50% shell debris, the rest is fine sand with very minor amounts of mud—no visible organic matter

Site D

Fine gray mud, no sand, minor trace of shell debris, some organic matter

Site E

Fine gray mud with some very fine sand, minor trace of shell debris, some organic matter

Site H

Fine gray mud, no sand, minor trace of shell fragments, some organic matter

only statistically significant difference between sites in uptake of metals by *Palaemonetes* was for Cu (Table 2). (Note the two significantly different groups, a and b, for Site P and the Reference Site). Organisms from Site P sediments contained higher levels than those from the Reference site and this is the only instance of bioaccumulation by *Palaemonetes*. In many cases, metal concentrations for the shrimp from the test sediments were less than those from the Reference sediment.

There were significant differences in metal uptake between sites by *Mercenaria* in only two cases (Table 2). Clams from Site D had significantly higher Fe concentrations than clams from any other sites, including the Reference sediment. Concentrations for Pb were significantly lower from Site E than from Reference Site organisms; Pb concentrations from all other sites were the same as for the Reference Site. *Mercenaria* concentrations of Zn were greater from Site P exposure than from other sedi-

Table 3. Metal concentrations (mg/kg dry weight) of *Palaemonetes* from acclimation tank (Background) and after exposure to Reference sediment

| | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
|--------------------|-------------|------------|-------------|------------|-------------|------------|------------|
| Background | 1.5 ± 1.2 | 163 ± 10.7 | 40.4 ± 6.6 | 9.7 ± 3.7 | <2.5 | <2.5 | 92.5 ± 3.4 |
| Reference sediment | 12.1 ± 14.2 | 140 ± 7.8 | 47.7 ± 21.4 | 15.5 ± 9.0 | 19.3 ± 21.0 | 25.1 ± 3.0 | 95.1 ± 8.2 |

ments; extractable metals from Site P were very similar to those from the Reference Site. Even so, the biological availability of Cu and Zn was greatest at Site P.

The similarity of metals concentrations in many of the test organisms exposed to the Reference and test sediments suggests either similar bioavailability for all sediments and/or mechanisms of organism regulation of metals content. These observations were also made by Cross *et al.* (1969) in studies with Mn, Fe, and Zn uptake by polychaetous worms.

Tissue concentrations from organisms exposed to the Reference sediment are rather high for several of the metals. In a previous bioassay, *Palaemonetes* were sampled after laboratory acclimation (background) as well as after exposure to the Reference sediments. Reference sediments for both bioassays were taken from the same offshore area. Tissue concentrations for several metals after exposure to the Reference sediment were similar for both data sets. There was an increase in tissue concentrations of some metals (Cr, Mn, Ni, Pb) after exposure to the Reference sediment (Table 3), which may indicate greater amounts of biologically available metals in the Reference sediments than for those at the collection site.

All of the sites tested should be acceptable for ocean disposal with respect to metals. There was bioaccumulation in only one instance for *Palaemonetes* and two instances for *Mercenaria*.

Sediment Extractions

With the exception of Mn at Site P and the Reference Site, metal concentrations extracted by Conc HNO₃ + H₂O₂ were significantly different between the various sites for all metals (Table 2). The amounts extracted were related to both the location and characteristics of the sediments. The (offshore) Reference sediment was a medium- to coarse-grained sand with minor amounts of shell debris. Site P is upriver from the major industrial activity and this is reflected in the relatively low amounts of extracted metals (except for Pb). Material from Site P was approximately 50% shell debris with the balance being fine sand (Table 1). Sites D, E, and

H were fine-grained (silty clays) sediments in the highly industrialized areas of Hampton Roads Harbor and Elizabeth River (Figure 1) and had correspondingly greater levels of extractable metals. Ranking of the sites in order of increasing amounts of Conc HNO₃ + H₂O₂ extractable metals gives Ref Site < Site P < Site E < Site D < Site H. The only exception to this ranking was the greatest level of extracted Mn found at Site D.

Metal concentrations extracted by 1 N HNO₃ showed patterns similar to those obtained with the Conc HNO₃ extraction. For most sites, the amounts extracted by the 1 N HNO₃ were significantly lower than those for Conc HNO₃ for corresponding sediments. The exceptions to this occurred with Mn, Ni, and Pb levels in Site P samples. Only the Pb value appears to be significantly greater for the 1 N than for the Conc HNO₃ extraction and this difference appears to be related to the volume of acid used in the Conc HNO₃ extractant and the sediment carbonate content. Extracted metal levels in the two HNO₃ methods were most similar for the Reference Site and Site P sediments. This similarity is probably due to the high amount of shell and sand material in these samples and the low metal input from anthropogenic sources. The shell material was easily dissolved by either of these acid concentrations.

Using the DTPA extraction, much lower concentrations of metals were obtained than in either of the two HNO₃ methods. One anomalous exception was for Ni extracted from the reference sediment where the DTPA extracted amount was greater than for 1 N HNO₃ and equal to the Conc HNO₃ extracted concentration. For most metals, the DTPA extraction concentrations were significantly different for the various sites. As with the HNO₃ methods, DTPA extracted levels were most similar between Site P and the Reference Site. The DTPA results show that there are different amounts of easily removable (extractable) metals in the sediments; these do not necessarily correlate with the 1 N HNO₃ extraction method (Table 4). The consistently good correlation for extracted Mn and Zn for all methods suggests that the chemical forms of these two differ from the other metals and are similarly affected by the three extractions.

Table 4. Correlation and R² values between extraction methods

| | R values | | | | | | |
|---|-----------------------|-------|-------|-------|-------|--------|-------|
| | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
| Conc HNO ₃ vs DTPA | .1061 | .8727 | .8142 | .9733 | .8147 | .5906 | .8769 |
| Conc HNO ₃ vs 1 N HNO ₃ | .2586 | .7593 | .6725 | .8862 | .0171 | .5285 | .8767 |
| 1 N HNO ₃ vs DTPA | .6214 | .6504 | .3252 | .8881 | .0242 | -.1644 | .9584 |
| | R ² values | | | | | | |
| | Cr | Cu | Fe | Mn | Ni | Pb | Zn |
| Conc HNO ₃ vs DTPA | .0113 | .7642 | .6629 | .9473 | .6637 | .3488 | .7690 |
| Conc HNO ₃ vs 1 N HNO ₃ | .0669 | .5765 | .4523 | .7854 | .0003 | .2793 | .7686 |
| 1 N HNO ₃ vs DTPA | .3861 | .4230 | .1085 | .7887 | .0004 | .0270 | .9185 |

Animal-Sediment Interactions

Since there was essentially no difference in metal uptake from the various sediments, regression analysis of tissue concentrations of both *Palaemonetes* and *Mercenaria* against concentrations extracted from sediments showed no significant correlations. Therefore, the three extractants cannot be used to predict uptake of metals by *Palaemonetes pugio* or *Mercenaria mercenaria* from the sediments used in this bioassay. Sediment extractions showed that the levels of extractable metals varied greatly. DTPA values indicated that there were significantly different amounts of easily extractable metals, yet organism uptake was not a function of these concentrations. Two of the three isolated cases of bioaccumulation occurred on the sediment with the least amount of extractable metals. As indicated in an earlier section, the mechanisms of organism-sediment interaction are not well understood.

Organism Enrichment

When studying organisms collected from various environments, organism enrichment of metals relative to sediment metals is sometimes determined. Organism enrichment of metals is measured by comparing tissue concentrations with sediment extracted concentrations (Cross *et al.* 1969). Certainly the choice of sediment extractant may determine if enrichment occurs, yet this is a convenient method of comparing metal concentrations of organisms exposed to different sediments.

Based on ANOVA/Duncan's Range Test for metal/Fe ratios, *Palaemonetes* were enriched with respect to the sediment in Cr, Cu, Ni, Pb, and Zn using data from any of the three extractants, and enriched in Mn with respect to Conc HNO₃ and 1 N HNO₃ extracted levels. *Mercenaria* were enriched in Pb and Zn with respect to all three ex-

tractants and in Mn with respect to the Conc HNO₃ extract data (Tables 5 and 6).

Metal/Fe ratios are used to eliminate the effect of ingested (or other) sediment when determining enrichment or bioaccumulation. A potential problem with this method is that Fe may also be enriched or bioaccumulated. The normally high amounts of sediment Fe in relation to other metals usually prevents this type of problem from occurring, i.e., the amount of Fe taken up by organisms in relation to its sediment concentration is much greater than for any other metal (Table 2). Unpublished research by this author suggests that the use of metal/Al data might be more informative, but additional research is necessary.

Summary

Uptake of metals by *Palaemonetes pugio* and *Mercenaria mercenaria* varied little as a function of the metal concentrations in sediments to which they were exposed including the reference sediment. Only three instances of bioaccumulation were noted and based on these results and the criteria of the Implementation Manual (EPA and COE 1976), the test site sediments should be acceptable for ocean disposal. Since metal uptake did not vary as a function of sediment metal concentration, regression analyses of tissue concentrations against sediment extracted levels showed no correlations. It is postulated that either the bioavailabilities of metals are similar for all sediments or the organisms are able to regulate accumulation of metals from these test sediments.

All extractants removed variable amounts of metals from the sediments with the extracted concentrations in the order of Conc HNO₃ + H₂O₂ > 1 N HNO₃ > DTPA. The Conc HNO₃ and DTPA were better discriminators between sediments than was 1 N HNO₃. The order of extracted metal con-

Table 5. Mean and standard deviation of metal/Fe ratios for sediment extractions and tissue concentrations. Each mean is an average of six replications

| SITE | Sediment extraction | | | Tissue concentration | |
|-----------|-----------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|
| | DTPA† | 1 N HNO ₃ | Conc HNO ₃ | Shrimp | Clam |
| CR | | | | | |
| Reference | .0153 ± .0119 ^{b*} | .0011 ± .0002 ^b | .0011 ± .0002 ^a | .6472 ± .6070 ^b | .0299 ± .0206 ^a |
| d | .0048 ± .0022 ^a | .0006 ± .0001 ^a | .0014 ± .0001 ^b | .3376 ± .2399 ^{ab} | .0154 ± .0173 ^a |
| e | .0016 ± .0000 ^a | .0006 ± .0001 ^a | .0013 ± .0001 ^b | .2061 ± .1673 ^a | .0096 ± .0150 ^a |
| h | .0024 ± .0006 ^a | .0009 ± .0001 ^{ab} | .0013 ± .0001 ^b | .0887 ± .1426 ^a | .0084 ± .0131 ^a |
| p | .0141 ± .0010 ^b | .0016 ± .0006 ^c | .0014 ± .0001 ^b | .0962 ± .0819 ^a | .0493 ± .0867 ^a |
| CU | | | | | |
| Reference | .0078 ± .0086 ^a | .0006 ± .0002 ^a | .0004 ± .0002 ^a | 3.2727 ± .9287 ^a | .1084 ± .0190 ^a |
| d | .0056 ± .0009 ^a | .0007 ± .0001 ^{ab} | .0004 ± .0000 ^a | 2.6562 ± 1.0495 ^a | .0821 ± .0138 ^a |
| e | .0077 ± .0005 ^a | .0010 ± .0003 ^b | .0004 ± .0000 ^a | 2.6832 ± 1.1215 ^a | .0936 ± .0312 ^a |
| h | .0155 ± .0005 ^a | .0018 ± .0002 ^c | .0006 ± .0000 ^b | 2.6977 ± 1.1444 ^a | .0966 ± .0093 ^a |
| p | .0055 ± .0051 ^b | .0017 ± .0005 ^c | .0008 ± .0000 ^c | 2.9719 ± 1.3895 ^a | .1404 ± .0294 ^b |
| MN | | | | | |
| Reference | .3521 ± .0889 ^c | .0344 ± .0043 ^c | .0153 ± .0016 ^c | .2221 ± .1256 ^b | .1161 ± .0525 ^b |
| d | .9382 ± .0415 ^d | .0320 ± .0047 ^{bc} | .0152 ± .0012 ^c | .1265 ± .0686 ^{ab} | .0788 ± .0375 ^{ab} |
| e | .2468 ± .0052 ^b | .0259 ± .0096 ^{ab} | .0111 ± .0004 ^b | .1712 ± .1000 ^{ab} | .0990 ± .0124 ^{ab} |
| h | .2054 ± .0063 ^b | .0279 ± .0014 ^{abc} | .0102 ± .0003 ^b | .1227 ± .0271 ^{ab} | .0960 ± .0174 ^{ab} |
| p | .0533 ± .0024 ^a | .0216 ± .0065 ^a | .0078 ± .0002 ^a | .1123 ± .0362 ^a | .0712 ± .0265 ^a |
| NI | | | | | |
| Reference | .0373 ± .0153 ^b | .0002 ± .0004 ^a | .0004 ± .0002 ^a | .4389 ± .1659 ^a | .0527 ± .0402 ^a |
| d | .0207 ± .0031 ^a | .0006 ± .0001 ^{ab} | .0009 ± .0001 ^c | .3520 ± .3186 ^a | .0621 ± .0253 ^a |
| e | .0115 ± .0011 ^a | .0007 ± .0002 ^{ab} | .0007 ± .0001 ^b | .4393 ± .2449 ^a | .0596 ± .0273 ^a |
| h | .0128 ± .0014 ^a | .0011 ± .0001 ^b | .0008 ± .0000 ^{bc} | .2595 ± .2625 ^a | .0473 ± .0213 ^a |
| p | .0299 ± .0058 ^b | .0036 ± .0010 ^c | .0013 ± .0002 ^d | .2078 ± .2359 ^a | .0743 ± .0410 ^a |
| PB | | | | | |
| Reference | .0282 ± .0036 ^d | .0046 ± .0019 ^b | .0014 ± .0005 ^b | .1053 ± .0528 ^a | .1578 ± .0695 ^{ab} |
| d | .0066 ± .0003 ^a | .0011 ± .0002 ^a | .0005 ± .0000 ^a | .0895 ± .0677 ^a | .0830 ± .0527 ^{ab} |
| e | .0159 ± .0003 ^c | .0016 ± .0005 ^a | .0006 ± .0000 ^a | .1110 ± .1148 ^a | .0825 ± .0425 ^{ab} |
| h | .0279 ± .0032 ^d | .0027 ± .0003 ^{ab} | .0007 ± .0000 ^a | .0952 ± .0341 ^a | .0689 ± .0621 ^a |
| p | .0124 ± .0009 ^b | .0109 ± .0032 ^c | .0031 ± .0003 ^c | .1651 ± .0804 ^a | .1704 ± .1185 ^b |
| ZN | | | | | |
| Reference | .0201 ± .0087 ^a | .0054 ± .0010 ^b | .0022 ± .0002 ^b | 1.8076 ± .6184 ^a | .6401 ± .0466 ^b |
| d | .0460 ± .0022 ^c | .0024 ± .0003 ^a | .0025 ± .0002 ^c | 1.3426 ± .5504 ^a | .5170 ± .0646 ^a |
| e | .0401 ± .0012 ^b | .0047 ± .0016 ^b | .0026 ± .0001 ^c | 1.2380 ± .3655 ^a | .6268 ± .0897 ^{ab} |
| h | .0966 ± .0032 ^c | .0095 ± .0011 ^c | .0034 ± .0001 ^d | 1.1540 ± .5482 ^a | .6689 ± .0965 ^b |
| p | .0703 ± .0038 ^d | .0045 ± .0012 ^b | .0020 ± .0000 ^a | 1.4507 ± .6708 ^a | .8311 ± .1502 ^c |

* Within a column, for each metal, means with the same letters are not significantly different at the 95% confidence level using the Duncan's Range Test.

† Diethylenetriamine pentaacetic acid

Table 6. Mean and standard deviation of metal/Fe ratios for the averages of all replications by extraction method and tissue concentration. Each mean is an average of 30 replications

| | Cr | Cu | Mn | Ni | Pb | Zn |
|-----------------------|-----------------------------|------------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------|
| DTPA† | .0076 ± .0078 ^{a*} | .0084 ± .0056 ^a | .3591 ± .3129 ^d | .0224 ± .0123 ^a | .0182 ± .0089 ^a | .0546 ± .0272 ^a |
| 1 N HNO ₃ | .0010 ± .0005 ^a | .0012 ± .0006 ^a | .0284 ± .0072 ^{ab} | .0012 ± .0013 ^a | .0042 ± .0039 ^a | .0053 ± .0026 ^a |
| Conc HNO ₃ | .0013 ± .0002 ^a | .0005 ± .0002 ^a | .0019 ± .0031 ^a | .0008 ± .0003 ^a | .0013 ± .0010 ^a | .0025 ± .0005 ^a |
| Shrimp | .2752 ± .3566 ^b | 2.8563 ± 1.0828 ^b | .1509 ± .0857 ^c | .3385 ± .2512 ^b | .1132 ± .0749 ^b | 1.3986 ± .5693 ^b |
| Clams | .0225 ± .0417 ^a | .1042 ± .0289 ^a | .0922 ± .0343 ^{bc} | .0592 ± .0312 ^a | .1125 ± .0812 ^b | .6568 ± .1363 ^c |

* Within a column, for each metal, means with the same letters are not significantly different at the 95% confidence level using the Duncan's Range Test.

† Diethylenetriamine pentaacetic acid

centrations was generally Reference Site < Site P < Site E < Site D < Site H.

Using metal/Fe ratios, *Palaemonetes* were enriched with respect to the sediment in Cr, Cu, Ni, Pb, and Zn using data from either of the three extractants and *Mercenaria* were enriched in Pb and Zn utilizing levels from any of the three extractants.

Acknowledgments. A portion of this research was conducted under Research Contract DACW65-31-C-0051. Thanks are given to Mr. Britt McMillan for his help with computer analyses. Mr. Jim Melchor of the Norfolk District and Dr. Richard Peddicord of U.S. Army Engineer Waterways Experiment Station made many helpful suggestions in editing the manuscript. Special thanks go to Dr. Anna Rule for reviewing and editing.

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Manuscript received March 21, 1985 and in revised form June 15, 1985.

