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June 12, 1995

Dennis J. Suszkowski, Ph.D., Science Director
Hudson River Foundation
40 West 20th Street, 9th Floor
New York, New York 10011

Re : Final Report for HRF Graduate Fellowship grant # GF/02/93
- SUNY internal grant # 431-4883A

Fellow: William G. Wallace
Advisor: Dr. Glenn Lopez

Dear Dr. Suszkowski:

Inclosed is the final report for the Hudson River Graduate Fellowship entitled "Acclimation and Adaptation to Pollutants: Effects on Metal Trophic Transfer" awarded to William Wallace (1993-1994). The goal of this research was to determine whether conditions to which prey, the oligochaete *Limnodrilus hoffmeisteri*, are exposed to the toxic metal Cd influence Cd trophic transfer to a predator, the grass shrimp *Palaemonetes pugio*. This research was broken down into nine sections: (1) determine how acute Cd exposure influences the subcellular distribution of Cd in worms and the subsequent trophic transfer of Cd to grass shrimp, (2) determine how worms obtain Cd from the environment (sediment vs. pore water), (3) collect worms and sediment cores along the Cd contamination gradient in Foundry Cove, (4) determine if worms from different sites along the contamination gradient accumulate Cd differently, (5) determine differences in Cd resistance in worms from different sites, (6) measure time dependent uptake and loss of Cd from resistant and nonresistant worms, (7) determine the subcellular distribution of Cd in worms from different sites, (8) determine the trophic transfer of Cd form worms at different sites to shrimp, and (9) isolate Cd-binding proteins (metallothioneins) from Cd exposed grass shrimp (preliminary data). The research conducted under this fellowship has already resulted in one publication (submitted to Estuaries) and at least two others will follow. If there are any questions or comments please contact us (516-632-8755).

Respectfully yours,

William G. Wallace

Glenn Lopez

1) Acute Cd exposure, subcellular Cd distribution and Cd trophic transfer

These experiments were designed to determine whether concentration and duration of Cd exposure influences the subcellular Cd distribution of nonresistant oligochaetes and the subsequent trophic transfer of Cd to grass shrimp. Nonresistant worms collected from South Cove were exposed for one or six weeks to one of three Cd concentrations (0.5, 47, or 140 μg Cd l^{-1}; including ^{109}Cd as a tracer). After the exposure period subcellular Cd distributions in worms were determined through homogenization and differential centrifugation (Klerks 1987; Wallace 1992a). Feeding experiments with these worms and grass shrimp were also conducted (Wallace 1992a).

Cd exposure conditions had direct effects on oligochaete subcellular Cd distribution and Cd trophic transfer to shrimp (Fig. 1). The amount of Cd bound to oligochaete cytosol increased with increased exposure concentration and exposure duration. There was a 1:1 relationship between the amount of Cd bound to worm cytosol and Cd absorbed by shrimp. This research was conducted under a 1992 Tibor T. Polgar Summer Fellowship granted by the NYS DEC and the Hudson River Foundation and is the first chapter of my doctoral dissertation (Wallace 1992b). A manuscript based on this study has been submitted to Estuaries (Wallace and Lopez 1995).

2) Uptake of Cd from the environment (sediment vs. pore water)

The purpose of this study was to determine the pathway by which oligochaetes obtain Cd from the environment. This experiment also helped identify the most appropriate method of exposing worms to Cd in subsequent experiments. In these studies microcosms were constructed which allowed partitioning of Cd uptake from sediment verses pore water (Fig 2a). The microcosm contained a layer of ^{109}Cd-labeled sediment topped with a layer of water. Other modifications provided for the collection of pore water (a syringe needle at the bottom) and fecal pellets (a layer of cotton and a sheet of "Ouchless" Band-Aid material). Each microcosm received two worms, one of which had its anterior end ablated. This worm would only absorb Cd from pore water, whereas the other would be exposed to Cd in the pore water as well as through the ingestion of Cd-labled sediment. The importance of the ingestion of Cd-labled sediment verses absorption of Cd from pore water for the uptake of Cd could then be determined by difference. Over the course of two weeks fecal pellets were collected and analyzed for ^{109}Cd. At the end of the exposure period, worms were extracted from the sediment, cleaned and assayed for ^{109}Cd.

After 13 days no worm had absorbed Cd from either source (Fig. 2b). The lack of Cd uptake in ablated worms was not surprising as no ^{109}Cd was ever detected in pore water. The lack of Cd absorption in worms feeding on sediment however was surprising. After 13 days worms deposited an average of ~3000 cpm of ^{109}Cd-labeled fecal pellets; the maximum was 10000 cpm. If worms absorbed Cd from the sediment with an efficiency of 1% they would have had a ^{109}Cd body burden of ~30 cpm. The results from this study seem to indicted that the absorption of Cd from the ingestion of Cd contaminated sediment is not an important pathway for Cd absorption. The importance of pore water Cd remains an open question.

3) Field sampling and determination of Cd depth profiles

In October of 1993 sediment samples (containing oligochaetes) and sediment cores were collected at three stations along the contamination gradient in Foundry Cove (Fig. 3). Oligochaetes from these sites are being maintained in laboratory cultures at MSRC. Sediment cores were sectioned at 2 cm intervals and were processed for total Cd, exchangeable Cd and pore water Cd (Tessier et al. 1979). Foundry Cove station 1 (FC 1) had the highest surface (0-2 cm) Cd concentrations (2000 μg Cd g dry wt.$^{-1}$), while FC 2 and FC 3 had lower concentrations, 430 and 240 μg Cd g dry wt.$^{-1}$ respectively. Samples of exchangeable Cd and pore water Cd are stored and are awaiting analysis.

4) Cd accumulation across the contamination gradient

This purpose of this experiment was to determine if oligochaetes from different sites along the contamination gradient of Foundry Cove accumulate Cd differently. In this study worms from all three Foundry Cove station (FC 1, FC 2 and FC 3), as well as South Cove worms (SC) were exposed for seven days to ^{109}Cd in solution. At the end of the exposure period worms were weighed and concentration factors (CF) were calculated.

Cd accumulation in oligochaetes depended upon site of collection and increased with sediment Cd exposure (Fig. 4). Differences in Cd accumulation are more apparent if medians are considered (dots embedded in bars), as the means of FC 2 and FC 3 were heavily influenced by a few high values. These results demonstrate that worms inhabiting increasing more polluted sites are more efficient at accumulating Cd. Site specific differences in Cd accumulation may reflect differences in Cd resistance (see section 5), as well as genetic selection of Cd accumulation and detoxification systems (Klerks and Levinton 1989; Klerks and Bartholomew 1991).

(5) Cd resistance in oligochaetes from different sites

The purpose of this experiment was to determine differences in Cd resistance between oligochaetes collected from the most polluted (FC 1) and control sites (SC). Toxicity bioassays consisted of exposing worms to 8.9 μM Cd and determining survival times (Klerks 1987). Worms from FC 1 survived significantly longer ($P<0.001$) than SC worms with mean survival times (mean of the first 50% to die) of 26.3 and 14.7 hours, respectively. Worms from FC 1 are most likely genetically resistant to Cd, as work by Klerks and Levinton (1989) has shown that increased Cd resistance in this oligochaete is an inherited trait. Toxicity bioassays with worms from FC 2 and FC 3 will be conducted in the near future.

6) Uptake and loss of Cd from resistant and nonresistant worms

The purpose of this experiment was to determine the kinetics of Cd uptake and loss by resistant (FC 1) and nonresistant (SC) oligochaetes. Worms were exposed to ^{109}Cd through solution for 25 days and were then allowed to depurate the metal for another 25 days. Every few

days worms were removed from the labeling solution or the sediment, cleaned and assayed for ^{109}Cd.

SC worms accumulated Cd linearly with time, while Cd uptake by FC 1 worms decreased with time (Fig. 5a). The decreasing accumulation of Cd in FC 1 worms is most likely due to a "shutting off" of Cd detoxification systems (i.e., synthesis of metallothioneins) which were functioning while they were in the contaminated sediment (2000µg Cd g dry wt.$^{-1}$) of the laboratory culture. The labeling solution only had a spike of ^{109}Cd (1 ng ^{109}Cd l^{-1}) and the Cd already present in the water (~ 0.5 µg Cd l^{-1}; R. Young unpub.). This meant that FC 1 worms were put into a "cleaner" environment when they were removed from the culture and put in the labeling solution. Worms from both populations depurated the accumulated metal at similar rates.

An interesting aspect of this work was that FC 1 and SC worms lost 45% and 20% of their respective tissue weight after remaining out of sediment for 25 days (Fig. 5b - day 0 to 25). Both sets of worms regained this weight after feeding on sediment during the depuration period (Fig. 5b - day 25 to 50).

7) Subcellular Cd distributions in worms along the contamination gradient

This experiment was conducted to determine if oligochaetes from different sites along the Cd contamination gradient store Cd differently. Worms used to determine the accumulation of Cd across sites were used in these studies (see section 4). Worm subcellular Cd distributions were obtained by homogenization, differential centrifugation and tissue digestion techniques (Klerks 1987; Silverman et al. 1983; Bebianno and Langston 1992; Wallace 1992a).

Oligochaetes from FC 1 had ~30% of their Cd in metal-rich granules (MRG), compared to 2% in SC worms (Fig. 6a). Microscopic examination of these MRG fractions revealed striking differences between FC 1 and SC worms. Numerous MRG were isolated from FC 1 worms; they closely resembled those photographed by Klerks and Bartholomew (1991) in thin sections of Foundry Cove worms. This fraction from SC worms contained very few MRG. Worms from FC 1 only had 11% of their Cd in the cytosol; SC worms had 56%. Both populations had ~ 65% of the Cd in cytosol bound to a heat-stable metallothionein (MT) fraction (Fig. 6b). There was substantial loading of ^{109}Cd (DPM/g wet wt.) in the MRG fraction of FC 1 worms; SC worms had virtually no ^{109}Cd in this fraction (Fig. 6c). SC worms had most of their absorbed ^{109}Cd bound to the cytosol. Cd subcellular distributions for FC 2 and FC 3 worms will be obtained in the near future.

8) Cd Trophic transfer along the contamination gradient

The purpose of this experiment was to determine if the trophic transfer of Cd from oligochaetes to grass shrimp differed along the contamination gradient. Grass shrimp were fed other ^{109}Cd contaminated worms obtained from the Cd accumulation studies (see section 4) and, over the course of a few days, were periodically assayed for ^{109}Cd body burdens. Cd absorption efficiencies were then calculated by curve stripping techniques.

Shrimp fed FC 1 and SC worms lost ingested Cd in a 2-component curve; an initial rapid lose (production of fecal material) and a slow gradual loss (physiological turnover) (Fig. 7). The

production of radiolabeled fecal material in both sets of shrimp was most rapid in the first 12 hr; by 24 hr shrimp had egested all ^{109}Cd labeled fecal material. Shrimp fed worms from FC 1 absorbed only 21% of the ingested Cd, while shrimp fed SC worms absorbed 76%. Both sets of shrimp had similar rates of Cd physiological loss (~.07% hr^{-1}).

The reason for the drastic difference in the absorption of Cd between shrimp fed FC 1 and SC worms resides in differences in the worms' subcellular Cd partitioning. Referring to Fig. 6a, FC 1 and SC worms had 28% and 70% of their respective total Cd body burdens in subcellular fractions (intracellular and cytosol) that contained the most bioavailable Cd (Wallace 1992a; Wallace 1992b; Wallace and Lopez 1995). These percentages agree well with shrimp Cd absorption efficiency data (21% for FC 1 and 76% for SC). The following mass balance equations use Cd concentrations of oligochaetes collected in the field (nmoles Cd/g worm dry wt.) (Klerks 1987) and absorption efficiencies (AE%) obtained in this study to predict Cd trophic transfer (nmoles Cd absorbed/g worm dry wt. ingested):

	Oligochaete Cd concentration in (Klerks 1987)		AE% (this study)		Predicted Cd trophic transfer (to shrimp)
South Cove (sed. Cd @ 20ppm)	305 (17)[a]	*	76%	=	231 (4.7)
Foundry Cove (sed. Cd @ 7000ppm)	5178	*	21% (3.6)	=	1087

[a]values in parenthesis are multiples needed to attain comparative value.

Even though there is a 17 fold difference in Cd body burdens between Foundry Cove and South Cove worms, there is only a 4.7 fold difference in the amount of worm Cd absorbed by shrimp. This drastic reduction in the bioavailability of Cd is a direct result of Cd resistant worms sequestering absorbed Cd into MRG. Feeding experiments with worms from FC 2 and FC 3 will be conducted in the near future. Results from these additional studies will complement the current findings.

9) Isolation of metallothioneins from grass shrimp (Preliminary Data)

This portion of my research was directed towards attaining the skills and techniques necessary to isolate metallothioneins from grass shrimp ingesting Cd contaminated oligochaetes. This work was conducted under the close supervision of Dr. Marius Brouwer at the Duke Marine laboratory in Beaufort NC (summer 1994). Grass shrimp were exposed to Cd in solution (1.6 mg Cd l^{-1} for 48 hr), then homogenized and centrifuged (Howard and Hacker 1990). Shrimp metallothioneins were then isolated from a heat-denatured cytosol using ion-exchange chromatography (Brouwer et al. 1992). Three distinct Cd peaks (I, II, III) were obtained from ion-exchange chromatography (Fig. 8). The first (I) and second (II) peaks may be from the same

Cd-binding protein or two distinct isoforms. Peak III is most likely glutathione (M. Brouwer per. comm.).

Conclusions

The research conducted through this fellowship has shown that the subcellular Cd distribution of prey (oligochaetes) plays a key role in controlling the trophic transfer of Cd to a predator (grass shrimp). What is most significant is that the evolution of Cd resistance in *Limnodrilus hoffmeisteri* has altered the normal cycling of Cd by reducing the bioavailability of this toxic metal. The storage of Cd by nonresistant worms into proteins (metallothioneins) increases Cd trophic transfer, while the formation of insoluble metal-rich granules by resistant worms reduces Cd trophic transfer. Subcellular distributions of Cd in worms from the other two Foundry Cove stations (FC 2 and FC 3) will be obtained in the near future. Feeding experiments with these worms and grass shrimp will also be conducted. Results form these studies may identify a level in sediment contamination that induces the formation of metal-rich granules. Predators feeding in sediment below this level may absorb more Cd than those feeding in sediment above it due to differences in the preys' subcellular Cd distribution. In additional studies I will investigate behavior (alterations in feeding behavior) as well as physiological (induction of metal-binding proteins) manifestations of Cd toxicity in shrimp fed Cd contaminated oligochaetes. This final leg of my research will complete a model linking molecular aspects of metal resistance in prey to direct consequences in a predator. This model will extend beyond Cd, oligochaetes and grass shrimp to include other predator/prey relationships in which toxic metals are sequestered and detoxified by similar mechanisms (i.e., Cu, Zn, Pb, Co, Ni, Cr, Mn, Ag and Hg) (Roesijadi 1980; Brown 1982).

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Figure 1. Relationship between Cd bound to oligochaete cytosol and Cd absorbed by shrimp ingesting the worms. Oligochaetes were collected from South Cove and were exposed for either one or six weeks to one of three Cd concentrations (0.5, 47 or 140 µg Cd l^{-1}).

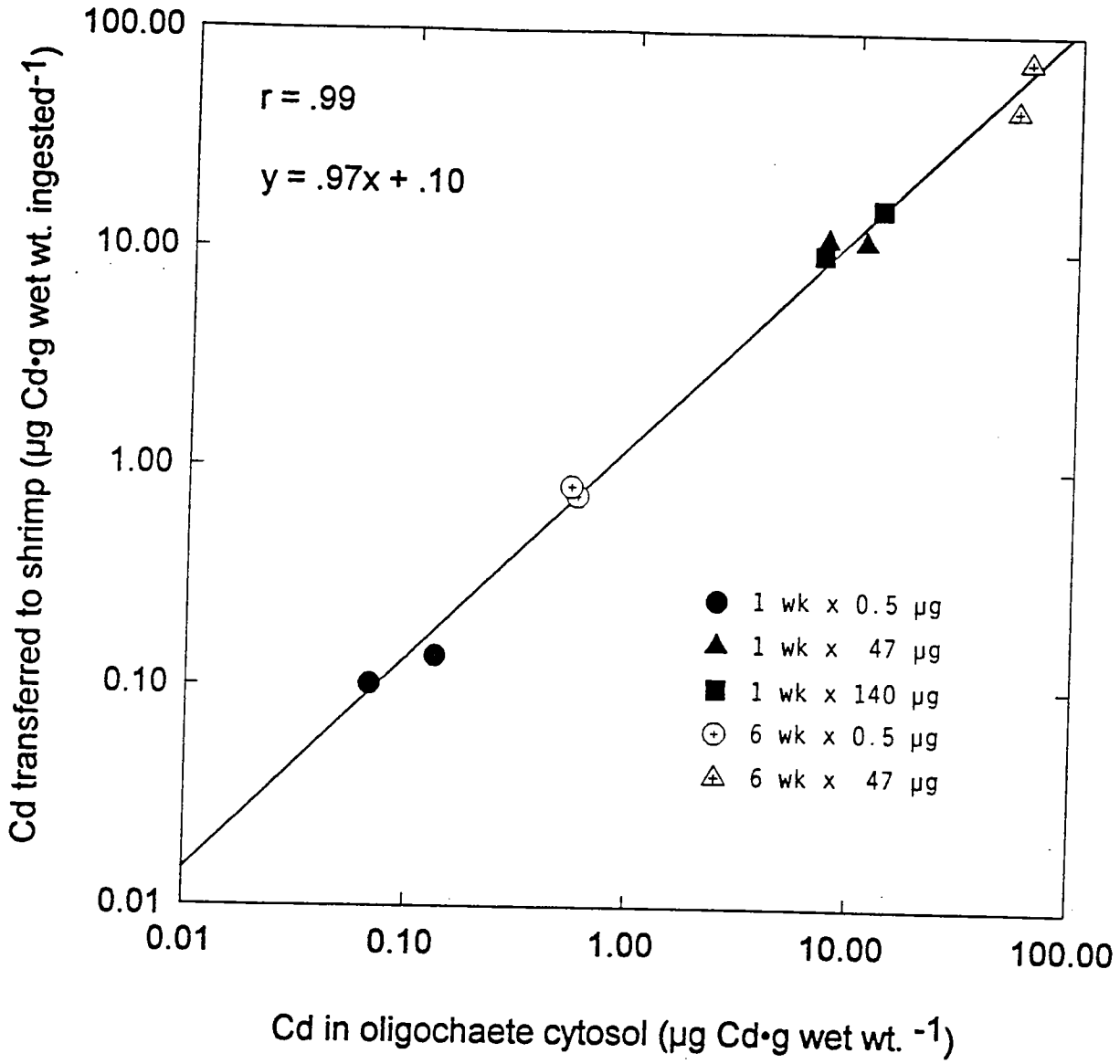
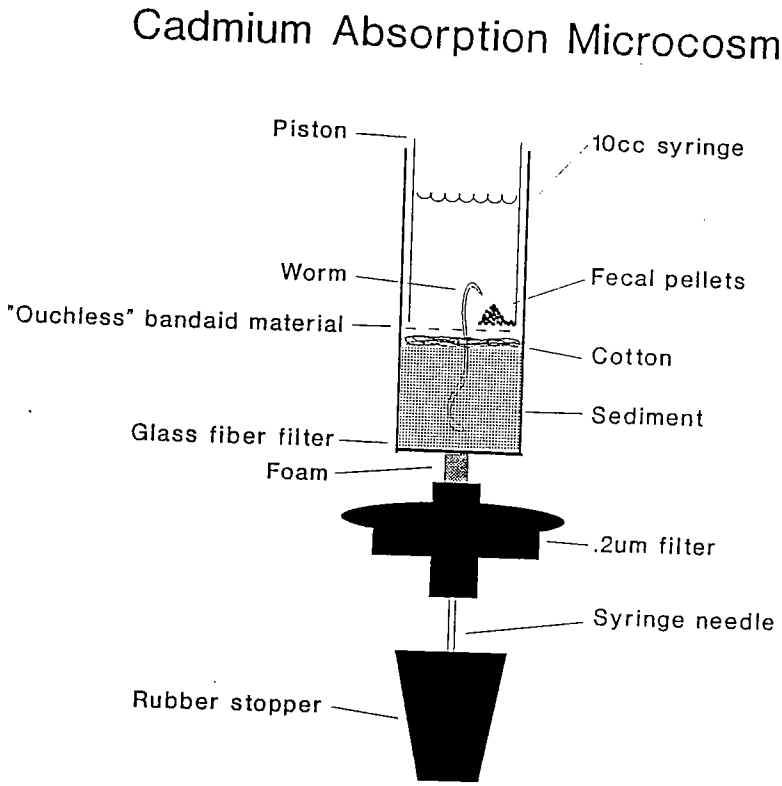


Figure 2. (a) Microcosm for partitioning ^{109}Cd uptake from the ingestion of ^{109}Cd contaminated sediment and exposure via pore water, and (b) production of ^{109}C-labeled fecal pellets by worms ingesting ^{109}Cd-labeled sediment.

a



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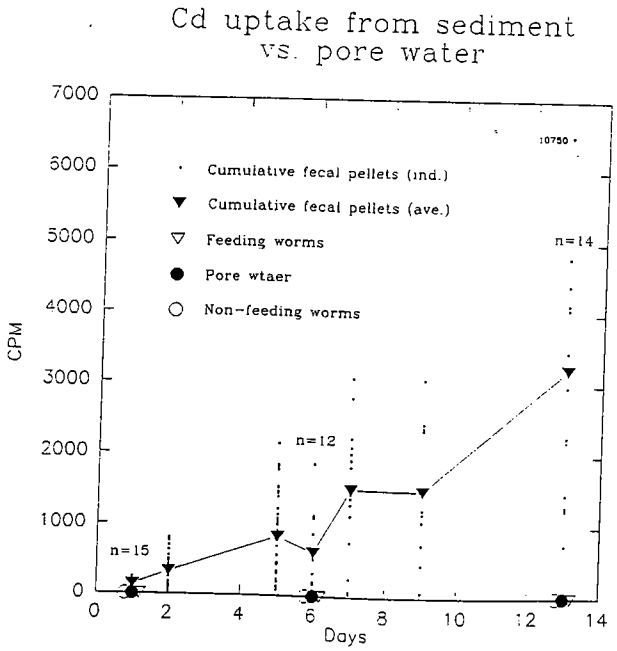

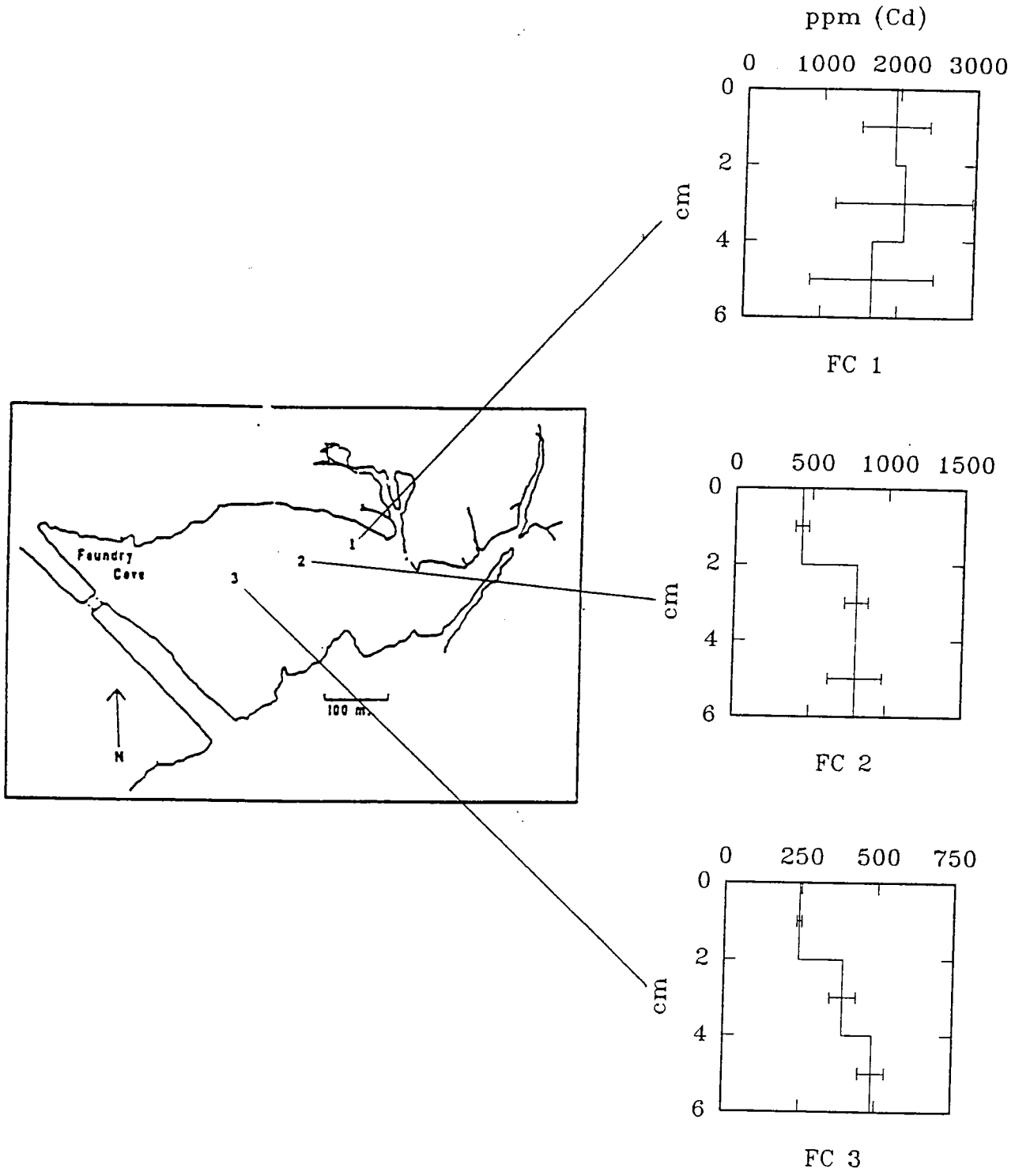

Figure 3. Field sites and Cd depth profiles for stations within Foundry Cove, NY.

Figure 4. Accumulation of Cd, given as concentration factors (CF), for oligochaetes collected along the Cd contamination gradient of Foundry Cove, NY. Worms were exposed to ^{109}Cd for seven days.

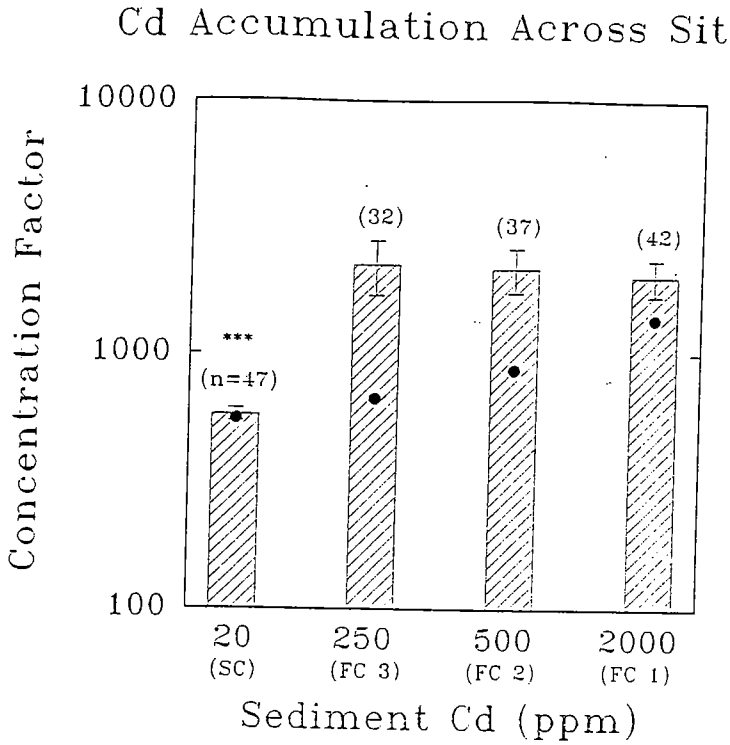

Figure 5. (a) Uptake and loss of ^{109}Cd and (b) weight change with time for worms from South Cove and Foundry Cove 1. Worms were exposed for 25 days to ^{109}Cd through solution and were allowed to depurate Cd for another 25 days. Arrows indicate point at which worms were removed from the labeling solution and put into clean sediment and water.

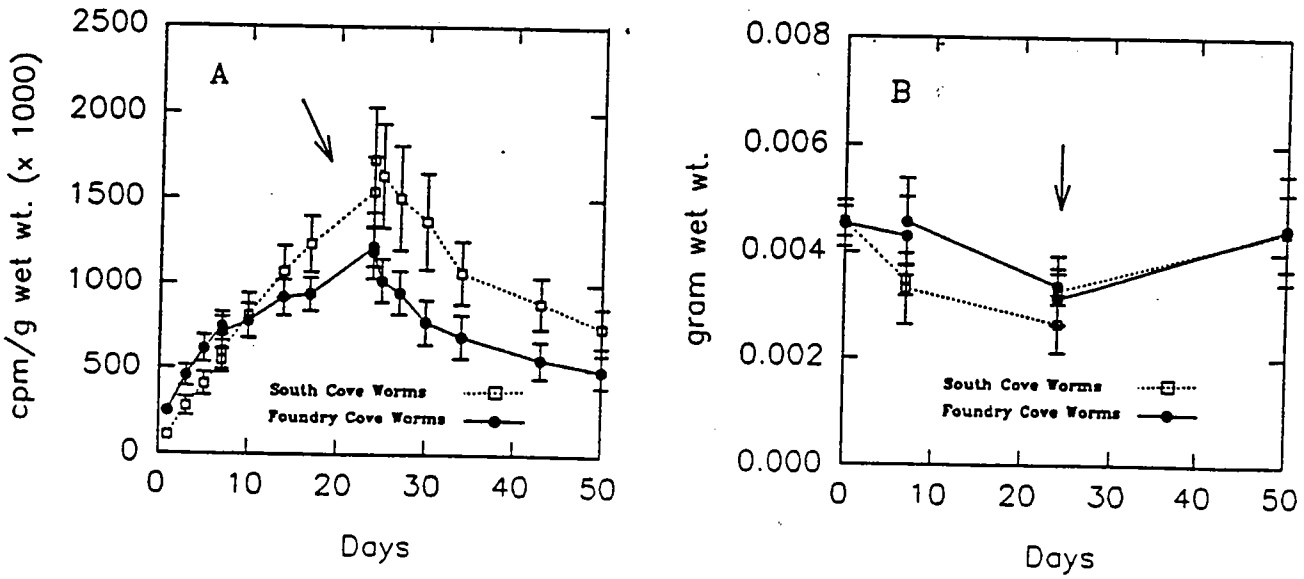

Figure 6. (a) Percent of oligochaete ^{109}Cd body burden in each of four subcellular fractions (MRG -metal-rich granules; Tissue; Intra.-intracellular; cytosol), (b) percent of cytosolic ^{109}Cd in heat-stable (metallothioneins -MT) and non-heat stable proteins and (c) DPM/g wet wt. in each of the four subcellular fractions. Foundry Cove 1 (FC 1) and South Cove (SC) worms were exposed for seven days to ^{109}Cd through solution. Subcellular distributions were obtained through homogenization, centrifugation and tissue digestion techniques.

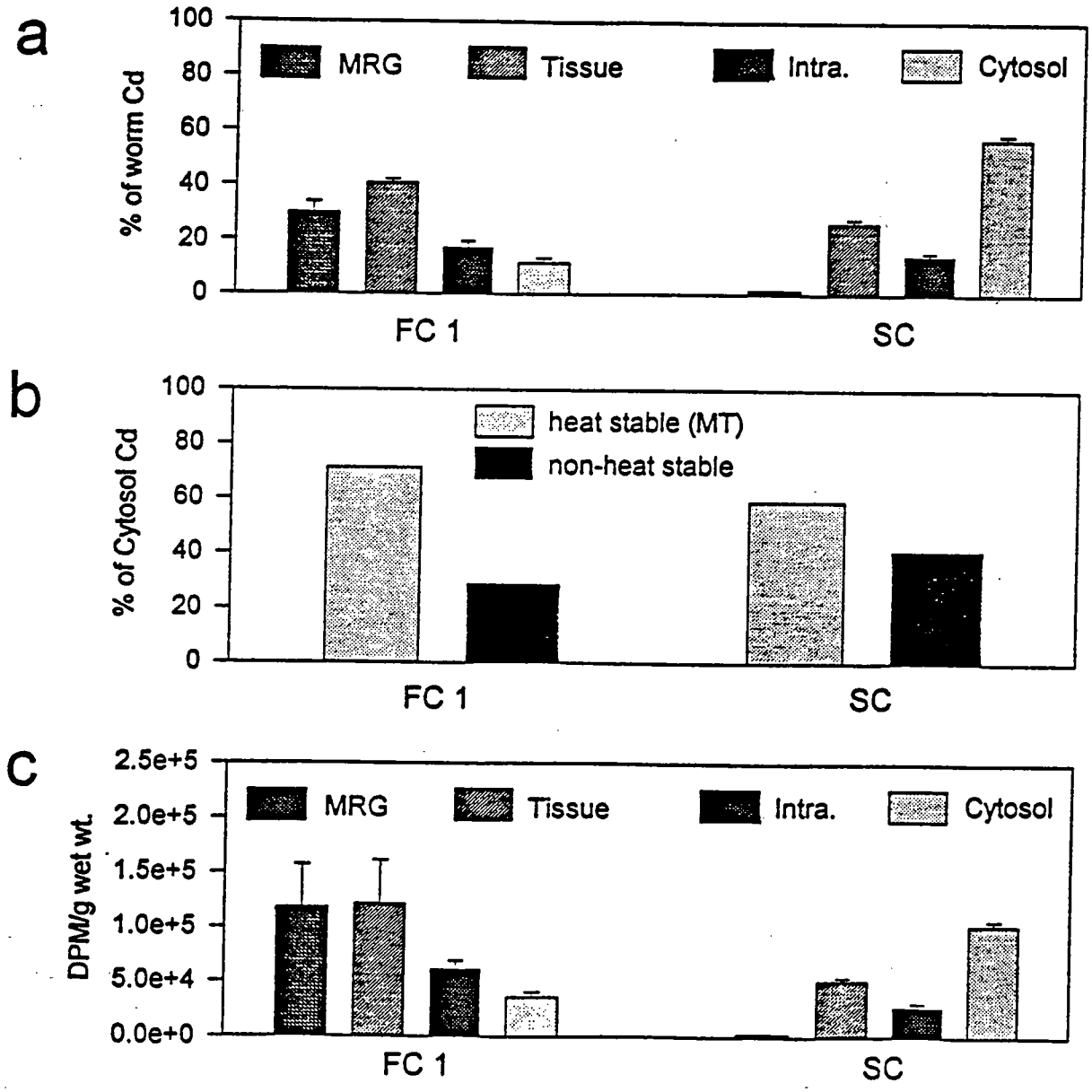

Figure 7. Time dependent retention and egestion of ^{109}Cd (percent) for grass shrimp fed Cd contaminated South Cove (SC) or Foundry Cove 1 (FC 1) oligochaetes. Regression equations are for the physiological loss components of the retention curves and give the loss rate, in percent ^{109}Cd lost per hr (slope), and absorption efficiency (AE%) (y-intercept) for shrimp fed SC or FC 1 worms. Regressions were fit to retention curves following the production of radiolabeled feces (> 24 hr).

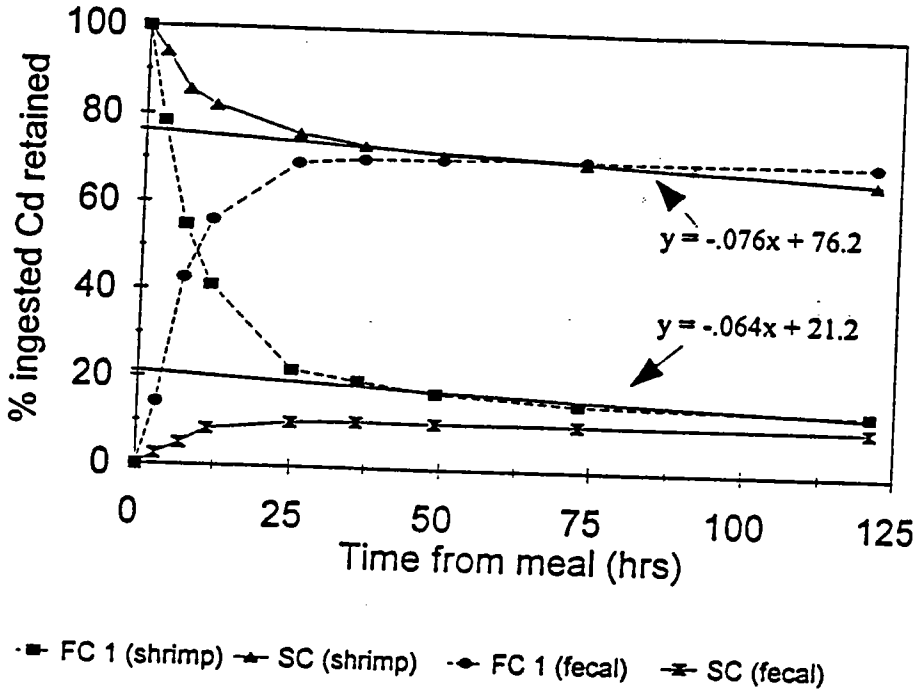

Figure 8. DEAE-cellulose ion-exchange chromatography of grass shrimp, *Palaemonetes pugio*, Cd-binding proteins.