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IN-SITU BIOASSAYS USING TRANSPLANTED MUSSELS: II. ASSESSING CONTAMINATED SEDIMENT AT A SUPERFUND SITE IN PUGET SOUND

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ABSTRACT: In a pilot study for the U.S. EPA, bioaccumulation and growth in mussels (*Mytilus trossulus)* were used to assess the bioavailability of contaminants and potentially adverse bioeffects in water overlying contaminated sediments. Caged juvenile and adult mussels were transplanted to 12 locations around a Superfund Site (Harbor Island), and a reference site (Carr Inlet) in Puget Sound, Washington. The exposure period was 82 days and animals were held 1 meter from the bottom. Accumulation of contaminants in adult mussel tissues was used to help identify the extent and magnitude of contamination in the overlying water. Reduced growth in juvenile mussels was used as an indicator of adverse bioeffects. Sites were ranked for these potentially adverse biological impacts. Juvenile mussel growth rates combined with toxicity-normalized sediment chemistry and toxicity-normalized tissue chemistry, provided a first-order approximation of potential biological effects associated with chemicals of concern in sediment and mussel tissues. Mussels from Harbor Island accumulated higher concentrations of contaminants and had significantly lower growth rates than the reference site mussels. Some differences were detected among the Harbor Island sites. Mussels from one site were much more contaminated than the others and, based on extremely low growth rates, exhibited the greatest potential for adverse biological effects. TBT and copper were the contaminants of greatest concern in both tissues and sediments; lead and zinc were an additional concern in sediments. A statistically significant inverse relationship was found between growth rate, toxicity-normalized tissue accumulation, and toxicity-normalized sediment contamination. Based on our results, we recommend the following integrated approach for evaluating contaminated sediments: (1) conduct *in-situ* bioassays, (2) estimate bioaccumulation in addition to bioeffects, and (3) evaluate water overlying the sediments.

KEYWORDS: mussel transplants, sediments, bioaccumulation, growth, Superfund, contamination, *in-situ* bioassay

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INTRODUCTION

Harbor Island is a 400-acre industrial island which was added to the Superfund National Priority List in 1983. It is located at the mouth of the Duwamish River and adjacent to Elliott Bay, in Seattle, Washington (Fig. 1). The Island was constructed with dredged sediments from the Duwamish River at the turn of the century. In 1982, the U.S. Environmental Protection Agency (EPA) completed a preliminary assessment to identify sources of industrial contaminants and the potential for human exposure. Potential sources of industrial contaminants included a secondary lead smelter, shipyards, metal recycling operations, foundries, a metal plating facility, ship and railroad cargo operations, and petroleum bulk fuel storage and transfer operations (Weston 1993).

Many studies have shown high concentrations of contaminants in both sediments and biota from the Harbor Island area, as well as associated adverse biological effects. Long and Chapman (1985) report that, among urban waterways in Puget Sound, the concentrations of metals and organic compounds were among the highest in the Duwamish Waterway. Adverse effects in sediment bioassays have been reported for amphipods, worms, and fish (Chapman et al. 1985). The Elliott Bay Action Program (EBAP) used sediment chemistry, laboratory bioassays, effects on benthic infauna, bioaccumulation potential, and persistence in the environment to assess contaminated sediment. This EBAP study identified PAHs, PCBs, and trace elements as the contaminants of concern (PTI and Tetra Tech 1988). There is strong evidence that contaminants in sediment are accumulated by, and are affecting fish in this area. For example, relative to other contaminated Puget Sound sites, the concentrations of PCBs in English sole and chlorinated hydrocarbons in juvenile Chinook salmon, have been among the highest in the vicinity of the Duwamish Waterway (Malins et al. 1987; McCain et al. 1990; Varanasi et al. 1993). Tissue accumulations at similar sediment concentrations have been associated with reduced immunocompetence and reproduction (Varanasi et al. 1993). The highest incidences of fin erosion and liver lesions have been repeatedly found in bottom-dwelling fish collected from the Duwamish River (Malins et al. 1987; Stein et al. 1992; Varanasi et al. 1992).

In 1992, EPA completed a Phase II Remedial Investigation (RI) for Harbor Island which included sampling sediments at 119 locations and analyzing them for priority pollutants. The objective of that study was to define the areas of sediment contamination which exceed the State of Washington sediment management standards (SMS) (Washington State Department of Ecology 1991) and identify those areas that require remediation. The SMS are based on the Apparent Effects Threshold (AET) concept that identifies sediment contaminant concentrations above which statistically significant biological effects are expected to occur in marine organisms (Barrick et al. 1989). This approach was developed using Puget Sound data. As in the EBAP study, the Phase II investigation concluded that sediments in a large number of areas around Harbor Island contained elevated concentrations of several contaminants. In contract to the EBAP data, cadmium concentrations frequently exceeded the state management standard. Tributyltin (TBT) contamination was widespread and usually exceeded reference area concentrations (Weston 1993), but TBT was not measured as part of EBAP.

As part of the Phase II Remedial Investigation, caged mussels were transplanted around Harbor Island to assess the potential for bioaccumulation and adverse biological effects. The value of biological testing in assessing contamination is well known and bioaccumulation studies using transplanted bivalves can demonstrate the extent to which chemicals have migrated into food webs (Lee et al. 1993). Laboratory bioassays have been used to identify toxic sediments at Superfund sites in Puget Sound (Chapman and Long 1983; Pastorok and Becker 1990). The importance of laboratory bioassays and integrated approaches have been

emphasized in the Sediment Quality Triad, which includes sediment chemistry, laboratory bioassays, and benthic community assemblages (Long and Chapman 1985). The use of *in-situ* bioassays is gaining wider acceptance as a potentially powerful tool because it combines the control of laboratory studies with the realism of field evaluations (Salazar and Salazar this volume), and the evaluation of sediments must ultimately be based on an ability to predict or measure biological effects at specific sites (Long and Chapman 1985; U.S. EPA 1992).

Our primary objective was to evaluate the utility of transplanted mussels in augmenting standard approaches to ranking contaminated sediments for regulatory purposes. In this paper we propose holistic assessments by combining mussel growth rates with estimates of potential sediment and tissue toxicity; thereby expanding sediment evaluations to include the overlying water. We also answer the following: 1) Did mussels accumulate the contaminants of concern associated with the sediments?; 2) Did the stations differ with respect to bioaccumulation and growth in mussels?, 3) Was there a relationship between sediment contamination, bioaccumulation, and mussel growth?, and 4) Which sites and which contaminants are of most concern?

EXPERIMENTAL METHODS

Mussel Deployment & Retrieval

Juvenile and adult mussels (*Mytilus trossulus*) were collected from the Penn Cove Mussel Farm, Coupeville, Washington (Fig. 1). Mussels were separated from the grow-out lines by carefully cutting the byssal threads. Since previous studies have shown that excessive handling can reduce mussel survival and growth rate, extreme care was taken to avoid injury that could stress the test animals (Salazar and Salazar this volume). Adult mussels in the 50 to 60 mm length size range were selected with sizing bars (i.e., a sorting device with two sets of fixed bars, the interval between representing the lower and upper acceptable size limits). This approach does not provide individual measurements. One hundred (100) adult mussels were selected at random and then placed into each of 33 bags of flexible vexar mesh tubing (Norplex Industries, Auburn, WA) approximately 1.5 m long. To control crowding and allow equal exposure of all individuals, each bag was subdivided into 10 clumps of 10 mussels each by constricting the mesh with plastic cable ties. Ample slack was provided within each clump to allow sufficient space for valve opening and growth during the test.

Juvenile mussels (24 to 30 mm length) were also selected by length, but individuals were measured with vernier calipers to the nearest 0.1 mm. In addition to recording length data, whole-animal wet-weight measurements (nearest 0.01 gm) were made for each juvenile mussel. Eighteen (18) juvenile mussels were placed into each of 24 cages. Cages were made of rigid plastic mesh cutlery trays (20 x 30 x 4.5 cm; Hutzler Manufacturing, Canaan, CT) modified to provide 18 separate compartments. We placed one individual per compartment to facilitate tracking survival and growth of individuals during the study. To ensure an equivalent distribution of juveniles among cages, the T_0 weight and length data were subjected to statistical analyses before deployment. No significant differences were found(" = 0.05). On a per-cage basis, mean whole-animal wet weights ranged from 1.46 to 1.61 g (overall range of individuals: 1.05 to 2.28 g), and mean length from 26.3 to 26.7 mm (overall range 23.9 to 29.7 mm).

After distribution to their respective cages, all adults and juveniles were tied to a pier and suspended in Penn Cove overnight. After this 12-hour holding period, animals were put into ice chests without seawater and transported to the 13 test sites: 12 sites around Harbor Island (E05, E18, K01, K09, N02, N06, N07, W06, W21, W31, W38,

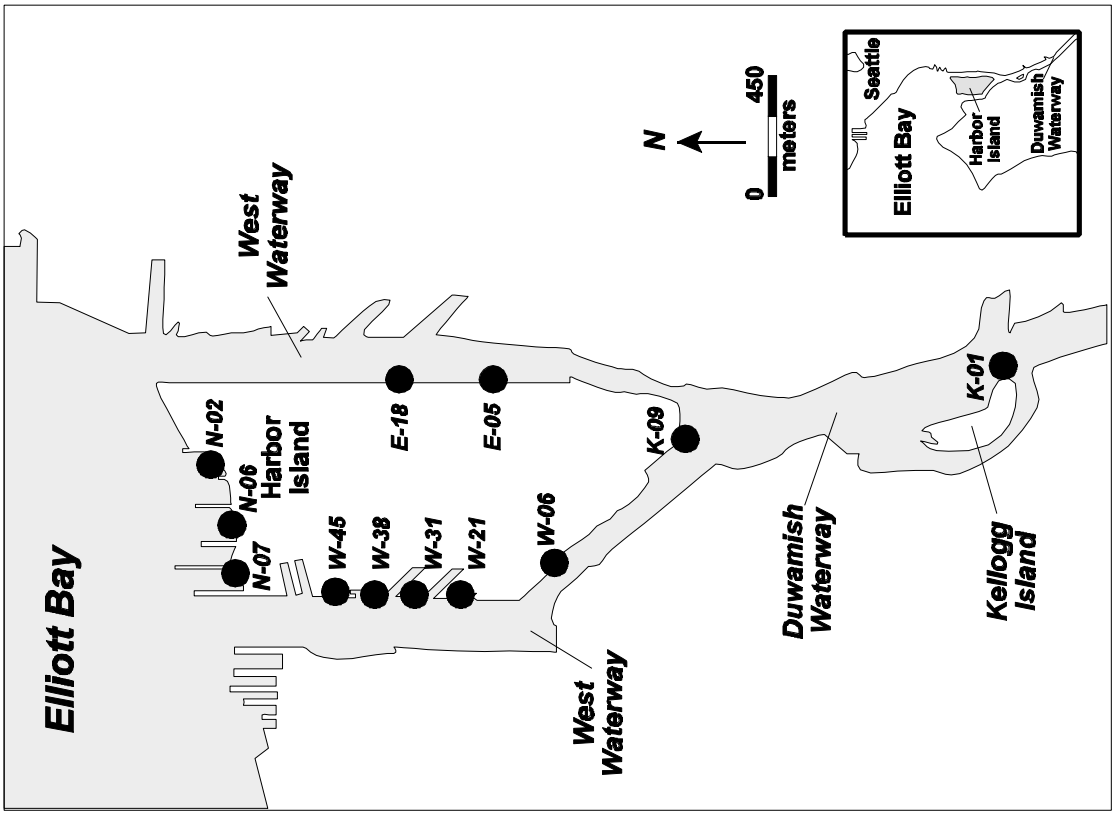

Fig. 2--The Harbor Island Superfund site showing the transplant sites, the Duwamish Waterway and Elliott Bay.

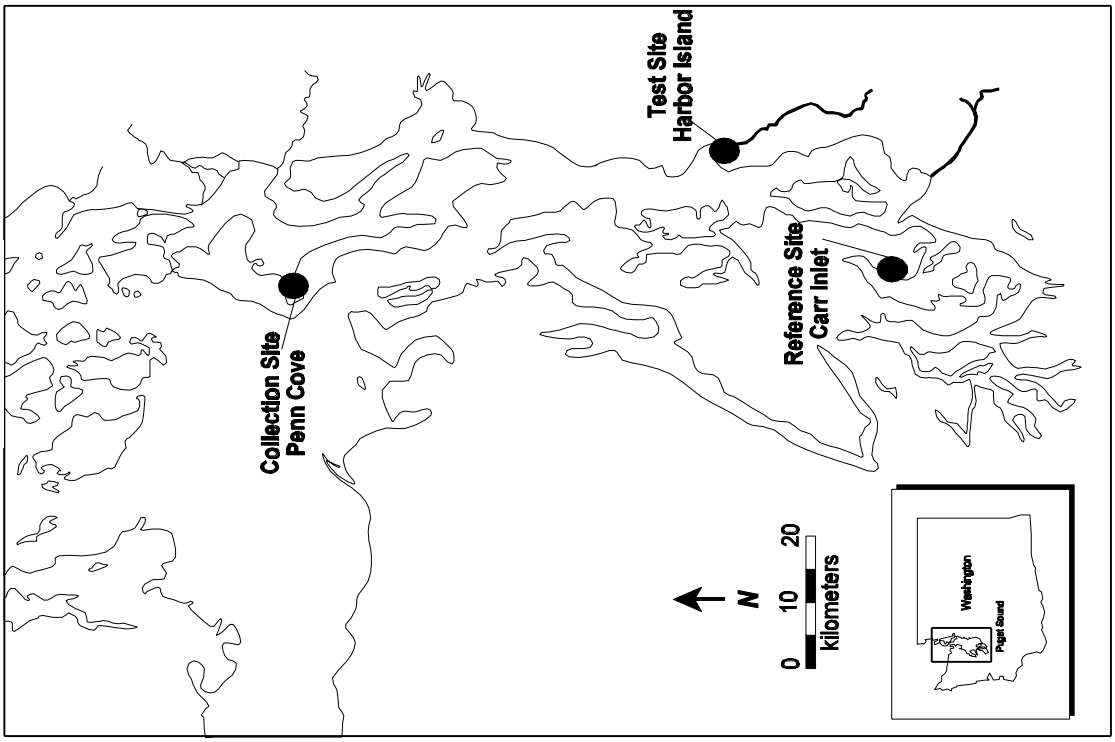

Fig. 1--The Puget Sound study area showing the Penn Cove collection site, the Carr Inlet reference Site, and the Harbor Island Superfund Site.

and W45) and the Carr Inlet (CAR) reference site (Fig. 2). Deployment site selections were based on the areal extent of elevated sediment contamination, as described in the RI, and the location of known contamination sources on Harbor Island. Using the convention of the RI, areas around the Island are designated as West (W) or East (E)Waterways, and North (N), or Kellogg Island (K). CAR was used as a reference site because this area is commonly used as a sediment reference site for other investigations in Puget Sound (Washington State Department of Ecology 1991). Test animals were out of seawater between three to six hours during deployment by divers. Around Harbor Island, mussels were deployed by divers approximately 1 m above the bottom in the vicinity of storm water outfalls, shipyards, and other industrial activities. The exposure period was 82 days (16 October 1991 to 7 January 1992). Two bags of 100 adult mussels (200 total) and one cage of juvenile mussels (18 total) were placed at most sites. Replication was used at the CAR reference site as well as K01 and K09 (the Harbor Island sites with the lowest concentrations of contaminants in sediments as measured in previous studies), and N07 (the Harbor Island site suspected of being the most contaminated). Three bags of 100 adult mussels (300 total) and three cages of 18 juvenile mussels (54 total) were transplanted at these sites. A total of 3000 adult mussels and 378 juvenile mussels were transplanted during this study.

Cages were either attached to nylon lines secured between adjacent pilings or to buoyed weights. At the end of the exposure period, mussels were retrieved by divers. While underwater and near the bottom, mussels were placed in plastic bags to isolate test animals from contamination in surface water as they were brought to the surface. Aboard the boat, mesh bags with adult mussels were opened and the mussels were sorted. Dead animals were discarded, and the number of survivors was recorded. Survivors, contained in plastic zip-lock bags with $\leq$ 100 animals per bag, were then placed in a large plastic trash can for transport. Cages with juvenile mussels were removed from the large plastic transfer bags and placed directly into the plastic trash can without any field processing. All adult and juvenile mussels were transported to the laboratory within 6 hours of collection. They were held overnight at the laboratory in a constant temperature room (4°C) without seawater, until processing the next day.

Adult mussel processing consisted of removing and homogenizing tissues according to the Puget Sound Estuary Protocols (Tetra Tech 1989). Each sample used for chemical analysis was a composite of tissues from 100 individuals. Tissue homogenates were frozen until analyzed. Adult mussel tissues were analyzed for trace metals, PAHs, pesticides, PCBs, and tributyltin (TBT). Analytical methods and detection limits are summarized in the sampling and analysis plan (Weston 1991). Contaminants of concern used in our ranking process were selected based on three criteria: (1) they were identified as contaminants of concern in previous studies of the Duwamish Waterway, Elliott Bay, and Harbor Island, (2) an ambient water quality criterion or equivalent existed for each contaminant of concern, and (3) there was at least a factor of two between the highest and lowest concentration in adult mussel tissues.

Juvenile mussel processing consisted of whole-animal length and weight measurements, and tissue collection. Juvenile mussel growth rates were estimated from changes in whole-animal wet weights and lengths. These measurements were made before and after deployment. Differences in growth were also estimated by comparing the final weights and lengths among sites at the end of the test.

RI & EBAP Sediment Data

To provide the best estimate of sediment contamination at each mussel deployment site, we calculated the mean concentration of contaminants using data for sampling stations located within a 150-m radius of each mussel deployment site. Both RI and EBAP (PTI and Tetra Tech 1988) sediment chemistry data were used in these calculations. We selected the same suite of contaminants that met our selection criteria for tissue contaminants of concern. Three to ten sediment sampling stations from either the EBAP or RI data sets fell within the 150-m radius for each mussel station. These means were used in normalization, ranking, and statistical analyses. Details regarding sediment sampling, handling, and analytical techniques are provided in Weston (1991, 1993).

Potential problems have recently been identified for the RI sediment data including: changes in detection limits among samples, low recovery of chemicals in some spiked samples, and the presence of high contaminant concentrations in the blanks. The cadmium and TBT data presented in the RI were inexplicably higher than previously reported values. This is particularly important because in many areas around Harbor Island the cleanup rankings are being driven by both cadmium and TBT. Although the concentration of cadmium in sediment was reported as an order of magnitude higher relative to previous studies, the tissue cadmium concentrations did not vary enough to meet our selection criteria, so we did not use cadmium in the rankings. We used TBT in the rankings because concentrations were elevated in both sediment and tissue.

Toxicity Normalization & Statistical Analysis

Growth in juvenile mussels (mg/wk/mussel) and bioaccumulation in adult mussel tissues (from the present study) were combined with previously collected sediment chemistry data and used in a preponderance-of-evidence approach to rank contaminated sites. Juvenile mussel growth rates, as estimated by increases in weight (mg/wk) were ranked from 1 (low) to 13 (high). We used growth rate based on weight rather than growth rate based on length, final weights, or final lengths because weight-growth measurements have been more informative in previous studies (Salazar and Salazar this volume). For replicated stations, significant differences in growth among stations were determined with an Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (Zar 1974; STATISTICA, StatSoft Inc., Tulsa, OK, 1991).

The tissue chemistry data from adult mussels were toxicity-normalized and ranked. To normalize the data, the concentrations of contaminants in tissues were divided by their respective Chronic Ambient Water Quality Criteria (AWQC) (U.S. EPA, 1986) to estimate relative potential toxicity. We chose these criteria because: (1) there are no tissue-based toxicity criteria, (2) concentrations of contaminants in mussel tissues have been related to water concentrations (Salazar and Salazar this volume), (3) there is a relationship between tissue concentrations and bioeffects in mussels (Salazar and Salazar this volume), and (4) there is some support for this procedure (Cook et al. 1992). In the tissue residue approach, maximum allowable tissue concentrations (MATCs) are estimated by multiplying chronic water quality criteria by bioconcentration factors (Cook et al. 1992), recognizing these MATCs may underestimate or overestimate measured tissue concentrations that elicit adverse effects (McKim and Schmieder, 1991; McCarty, 1991). Since there is no AWQC for TBT, we used the proposed State of California criterion (Water Resources Control Board 1991). Ensuing quotients for metals (arsenic, copper, lead, silver, and zinc), total PAHs (TPAHs = sum of Acenaphthylene, Ancenaphthene, Anthracene, Benzo(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Chrysene, Fluoranthene,

Fluorene, Ideno(1,2,3-cd)pyrene, Phenanthrene, and Pyrene), PCBs (sum of six congeners), and TBT were summed for each station. Stations were then ranked from lowest (1) to highest (13) using the summed quotients of these tissue contamination units. A tissue contamination unit >1 suggests potential adverse effects.

Relative sediment toxicity was estimated by dividing the mean contaminant concentrations at each station by their respective Effects Range - Median (ERM) concentration (Long and Morgan 1991; Long and MacDonald 1992). These ERM guidelines were developed using three basic methods to evaluate data gathered in a variety of geographic areas: equilibrium partitioning, sediment bioassays, and synoptic chemical and biological data from field surveys. The ERM is the concentration approximately midway in the range of reported values associated with biological effects. Since there was no published ERM for TBT, we used the Puget Sound Dredged Disposal Analysis (PSDDA 1989) screening guideline which is similar to that suggested by MacDonald (personal communication). We normalized the data because contamination is not a bioeffect and rankings based on absolute concentrations do not account for potential sediment toxicity. A potential sediment toxicity contamination unit > 1 indicates possible adverse effects. Potential sediment toxicity units for metals (arsenic, chromium, copper, lead, mercury, and zinc), total PAHs, total PCBs, and TBT were summed for each station and ranked from lowest (1) to highest (13).

Overall comparisons among stations based on sediment chemistry, tissue chemistry, and growth were made using ranks, scatterplots, and regression analyses. An overall rank was calculated for each station by summing the individual ranks for sediment chemistry, tissue chemistry, and growth. The relationship between growth, sediment toxicity, and tissue toxicity was examined using a three-dimensional scatterplot. The sediment data were log-transformed to spread out the data points along that axis. Various models of growth, as a function of potential sediment and tissue toxicity, were evaluated to determine which transformations would provide (1) improved normality and linearity, (2) the highest r^2 (greatest overall significance), and (3) significant independent contributions by toxicity-normalized sediment and tissue data. A multiple linear regression was performed and residuals were tested for normality and outliers. The correlation coefficient relating normalized tissue and sediment data was calculated (STATISTICA, StatSoft Inc., Tulsa, OK, 1991).

RESULTS

Sediment Chemistry

The concentrations of almost all contaminants were significantly higher in sediments around Harbor Island than in sediments from the Carr Inlet reference site (Table 1). The biggest differences occurred with TBT and lead where the most contaminated sites had concentrations that were factors of 920 and 230 above reference. Several of the least-contaminated Harbor Island sites were similar to CAR with respect to sediment contamination. The potential for toxicity associated with these contaminated sediments is shown in Table 2, where Station N07 has the highest ranking using ERM-normalized data. Station N07 had the greatest exceedances of the ERM for more contaminants than any other station and exhibited the greatest separation between both the other Harbor Island sites and the reference site. The ranking process was driven primarily by TBT. Other contaminants also contributed to potential sediment toxicity. PCBs, for example, were highest in the East Waterway stations (E05 and E18).

Table 1--Contaminant concentrations (mg/kg) in sediments from Harbor Island and the Reference Site in Carr Inlet (CAR). Also shown are highest and lowest Harbor Island values relative to CAR and the ERM used to create normalized concentrations in Table 2.

	As	Cr	Cu	Pb	Hg	Zn	TPAH	PCBs	TBT
CAR	2.4	40	18	1.3	0.035	42	3.9	0.033	0.02
E05	12	56	127	77	0.844	159	16	0.655	1.42
E18	12	76	119	96	0.442	187	9	0.638	1.16
K01	8	44	142	22	0.134	107	8	0.178	0.18
K09	10	81	85	34	0.128	117	11	0.296	0.21
N02	11	51	66	31	0.213	127	50	0.177	0.40
N06	23	92	275	88	0.679	280	28	0.094	5.21
N07	57	55	965	297	1.950	592	37	0.503	18.41
W06	9	47	90	60	0.331	121	8	0.148	0.62
W21	16	84	166	86	0.538	222	19	0.168	2.01
W31	22	84	276	261	0.582	362	20	0.209	3.37
W38	23	93	221	221	0.739	299	15	0.292	0.38
W45	24	89	228	194	0.888	281	15	0.280	1.07
High CAR	24	2	53	230	56	14	13	20	920
Low CAR	3	1	4	17	4	3	2	3	9
ERM	85	145	390	110	1.3	270	35	0.4	0.03

Table 2--ERM-normalized contaminant concentrations in sediments from Harbor Island and the Reference Site in Carr Inlet (CAR). TPAH and PCBs as in Table 1. R = overall rank; $\sum$NC = sum of normalized concentrations.

	As	Cr	Cu	Pb	Hg	Zn	TPAH	PCBs	TBT	$\sum$NC	R
CAR	0.03	0.28	0.05	0.01	0.03	0.16	0.11	0.08	0.67	1	1
K01	0.10	0.30	0.36	0.20	0.10	0.40	0.23	0.45	6	8	2
K09	0.12	0.56	0.22	0.31	0.10	0.43	0.32	0.74	7	10	3
N02	0.13	0.35	0.17	0.28	0.16	0.47	1.42	0.44	13	17	4
W38	0.27	0.64	0.57	2.01	0.57	1.11	0.43	0.73	13	19	5
W06	0.10	0.32	0.23	0.54	0.25	0.45	0.23	0.37	21	23	6
W45	0.29	0.61	0.59	1.76	0.68	1.04	0.43	0.70	36	42	7
E18	0.15	0.52	0.30	0.87	0.34	0.69	0.26	1.60	39	43	8
E05	0.14	0.39	0.32	0.70	0.65	0.59	0.45	1.64	47	52	9
W21	0.19	0.58	0.42	1.35	0.41	0.82	0.53	0.42	67	72	10
W31	0.26	0.58	0.71	2.37	0.45	1.34	0.57	0.52	112	119	11
N06	0.26	0.63	0.70	0.80	0.52	1.04	0.80	0.24	174	179	12
N07	0.67	0.38	2.48	2.70	1.50	2.19	1.05	1.26	614	626	13

Tissue Chemistry

The differences in tissue concentrations of contaminants among sites (Table 3) were not nearly as great as those for sediment, even following normalization (Table 4). The small differences observed were largely due to copper and TBT, but PCBs were also a factor. In general, mussels at the most contaminated sites accumulated more contaminants than the reference animals (note that arsenic and zinc were higher in reference mussels than those deployed at the least contaminated sites). As with sediment, station N07 had the highest concentrations of most contaminants as well as the highest ranking with AWQC-normalized data. Tissue concentrations of TBT and copper (both before and after normalization) were highest at N07. Adult mussels at the most contaminated sites had the greatest elevations above reference for PCBs (18 times higher) and TPAHs (17 times higher), but these contaminants were only considered to have a low toxicity potential based on our normalizations (Table 4). Normalized TPAHs were orders of magnitude below predicted toxicity thresholds. PCBs were highest in mussel tissues at E05, E18, and N07, but normalization suggested these concentrations were not potentially toxic. It was surprising that tissue concentrations of TBT in mussels transplanted to Harbor Island were only up to three times higher than reference mussels (Table 3), because sediment concentrations of TBT were up to three orders of magnitude higher than reference sediment concentrations (Table 1). After normalization (Table 4), tissues accumulated TBT at potentially toxic concentrations at all stations, even the reference site. The toxicity-normalized data suggest that mercury was a problem as well, but measurements were all at or below the limits of detection.

Mussel Growth

For mussels deployed at CAR, final whole-animal wet weights, growth rates expressed as mg/wk, final lengths, and growth rates expressed as mm/wk were significantly higher (" = 0.05) when compared with mussels deployed at the Harbor Island sites. Mean growth rates (mg/wk/mussel) for juvenile mussels at CAR were approximately 3 to 5 times higher than for mussels transplanted to any of the Harbor Island sites (Table 5). Mussels at N07 had the lowest growth rates. However, the Multiple Range Test did not detect significant differences in growth among any of the cages deployed around the Harbor Island sites when all sites were compared. Because the growth rates were not different among replicate cages, data were pooled for the four stations where replicate cages were deployed.

Analysis of pooled data for the four sites showed the same significant differences among sites in all four mussel metrics; i.e., final weights, final lengths, growth rate based on length, and growth rate based on weight ($n = 190$, $p < 0.001$). Duncan's New Multiple Range Test (Zar, 1974; STATISTICA, StatSoft Inc., Tulsa, OK, 1991) separated growth rates for these sites into the following three groups:

CAR > K01 = K09 > N07

Survival

Compared to most standard laboratory bioassays which run from four to 21 days, this 82-day exposure was a relatively long-term field experiment. Even so, survival was high and within standards commonly accepted for laboratory bioassays. Adult survival by station ranged from 72 to 88% with a mean of approximately 80%. Juvenile survival by station ranged from 78 to 100% with a mean of approximately 90%. This difference in survival between juvenile and adult mussels is statistically significant (" = 0.05). Since survival is not a sensitive measure of bioeffects, it was not surprising that there were no

Table 3--Contaminant concentrations (mg/kg ww) in tissues from Harbor Island and the Reference Site in Carr Inlet (CAR). Also shown are the highest and lowest Harbor Island values relative to CAR and the chronic ambient water quality criteria. TBT value is from the State of California proposed water quality criterion.

	As	Cr	Cu	Pb	Hg	Zn	TPAH	PCBs	TBT
CAR	1.71	0.15	0.92	0.072	0.04	17.2	0.036	0.0012	0.017
E05	0.54	0.27	0.93	0.232	0.04	16.9	0.134	0.0081	0.042
E18	0.98	0.22	0.97	0.112	0.04	14.7	0.121	0.0048	0.047
K01	1.67	0.17	1.07	0.176	0.04	17.4	0.165	0.0024	0.034
K09	1.10	0.16	1.01	0.172	0.04	16.0	0.118	0.0019	0.023
N02	1.34	0.18	1.09	0.132	0.04	18.0	0.227	0.0222	0.019
N06	1.20	0.23	1.18	0.155	0.04	17.8	0.169	0.0021	0.025
N07	1.52	0.23	3.00	0.186	0.04	22.1	0.210	0.0017	0.046
W06	1.75	0.20	1.17	0.252	0.04	16.4	0.289	0.0026	0.021
W21	1.69	0.23	1.46	0.269	0.04	18.5	0.597	0.0016	0.026
W31	0.67	0.26	1.31	0.298	0.04	20.0	0.279	0.0020	0.029
W38	1.47	0.25	1.35	0.521	0.04	20.6	0.196	0.0017	0.032
W45	1.25	0.20	1.11	0.329	0.04	20.0	0.379	0.0027	0.032
High CAR	1.0	1.9	3.3	7.2	1.0	1.3	16.6	18.0	2.8
Low CAR	0.3	1.1	1.0	1.6	1.0	0.9	3.3	1.3	1.1
AWQC	36	50	1.5	8.5	0.03	86	150	0.03	0.01

Table 4--AWQC-normalized contaminant concentrations in tissues from Harbor Island and the Reference Site in Carr Inlet (CAR).
R = overall rank; ∑NC = sum of normalized concentrations.

	As	Cr	Cu	Pb	Hg	Zn	TPAH	PCBs	TBT	∑NC	R
CAR	0.048	0.003	0.612	0.008	1.6	0.200	0.000	0.041	1.7	4.21	1
W06	0.049	0.004	0.780	0.030	1.6	0.191	0.002	0.088	2.1	4.84	2
K09	0.031	0.003	0.670	0.020	1.6	0.186	0.001	0.062	2.3	4.87	3
N06	0.033	0.005	0.787	0.018	1.6	0.207	0.001	0.071	2.5	5.22	4
N02	0.037	0.004	0.727	0.016	1.6	0.209	0.002	0.740	1.9	5.23	5
W21	0.047	0.005	0.973	0.032	1.6	0.215	0.004	0.054	2.6	5.53	6
W31	0.019	0.005	0.873	0.035	1.6	0.233	0.002	0.068	2.9	5.73	7
W45	0.035	0.004	0.740	0.039	1.6	0.233	0.003	0.091	3.2	5.94	8
K01	0.046	0.003	0.715	0.021	1.6	0.202	0.001	0.080	3.4	6.07	9
W38	0.041	0.005	0.900	0.061	1.6	0.240	0.001	0.057	3.2	6.10	10
E05	0.015	0.005	0.618	0.027	1.6	0.197	0.001	0.271	4.2	6.93	11
E18	0.027	0.004	0.647	0.013	1.6	0.171	0.001	0.159	4.7	7.32	12
N07	0.042	0.005	2.003	0.022	1.6	0.256	0.001	0.058	4.6	8.59	13

Table 5--Juvenile mussel growth data. Also shown are the highest and lowest Harbor Island values relative to the Reference Site in Carr Inlet (CAR). Initial n = 432; survival ranged from 78 to 100% ($\bar{x}$ = 90%).

Site	n	%Survival $\bar{x}$	T_0 Whole Animal Weight (g)	T_F Whole Animal Weight (g)	Growth Rate (mg/wk)	T_0 Length (mm)	T_F Length (mm)	Growth Rate (mm/wk)	Rank
CAR	54	83	1.49	4.74	276	26.5	39.2	1.09	1
W45	18	89	1.53	2.54	89	26.6	31.6	0.44	2
N02	18	94	1.50	2.51	84	26.6	32.0	0.45	3
K01	54	92	1.53	2.47	82	26.6	31.3	0.41	4
K09	54	94	1.52	2.47	82	26.5	31.3	0.42	5
W38	18	94	1.48	2.38	77	26.6	31.3	0.40	6
N06	18	94	1.51	2.39	76	26.6	30.9	0.36	7
W31	18	89	1.57	2.44	74	26.6	30.7	0.35	8
W06	18	94	1.49	2.34	71	26.4	30.6	0.37	9
E05	18	100	1.61	2.37	64	26.6	30.4	0.32	10
E18	18	94	1.53	2.30	64	26.5	30.1	0.30	11
W21	18	78	1.51	2.21	62	26.3	30.0	0.33	12
N07	54	81	1.50	2.19	59	26.5	29.6	0.26	13
CAR High				1.87	3.10		1.2	2.42	
CAR Low				2.16	4.68		1.3	4.19	

statistically significant differences in survival among sites. Unlike growth, where the highest growth rates were associated with low sediment and tissue contamination, no associations with contaminant concentrations in either tissue or sediment could be made for survival. Survival was actually lowest at the CAR reference site.

Overall Relationship between Bioaccumulation, Growth and Sediment

Based on the combined rankings for growth, normalized sediment toxicity, and normalized tissue toxicity (Table 6), the biggest difference was found between the CAR reference site and N07. CAR ranked first in terms of lowest normalized sediment and tissue contamination, and first in terms of highest growth rates. By contrast, N07 had the highest potential sediment and tissue toxicity, and lowest growth rate. There are two orders of magnitude difference in potential sediment toxicity (Table 2), a factor of two difference in potential tissue toxicity (Table 4), and a factor of five difference in juvenile mussel

Table 6--Overall ranks by station computed from sum of ranks for normalized sediment toxicity, normalized tissue toxicity, and growth rate (mg/wk/mussel).

Site	Sediment Rank	Tissue Rank	Growth Rank	$\sum$ Rank	Overall Rank
CAR	1	1	1	3	1
K09	3	3	5	11	2
N02	4	5	3	12	3
K01	2	9	4	15	4
W06	6	2	9	17	5
W45	7	8	2	17	6
W38	5	10	6	21	7
N06	12	4	7	23	8
W31	11	7	8	26	9
W21	10	6	12	28	10
E05	9	11	10	30	11
E18	8	12	11	31	12
N07	13	13	13	39	13

growth rate (Table 5). CAR and N07 are also separated from the other Harbor Island sites. It is difficult to separate the remaining Harbor Island sites based on the overall rankings. K09 and N02 appear to be two of the least potentially toxic sites, while E05, E18, and N07 are the most potentially toxic. Growth, normalized sediment toxicity, and normalized tissue toxicity are plotted in Figs. 3 and 4 to provide a graphical representation of separation among sites. Both figures show the largest differences attributable to normalized sediment contamination and the least difference attributable to normalized tissue contamination. Some stations with higher sediment contamination were lower in overall ranking due to a combination of higher growth rate and lower tissue contamination (Table 6).

The scatterplot of growth, normalized sediment, and tissue toxicity (Fig. 4) helps visualize the separation of stations into three major groups: CAR, N07, and the rest of the Harbor Island sites. As shown in all the previous data, the biggest difference among test sites by far, using any data set, normalized or non-normalized, is between CAR and N07. These sites also appeared different than the other Harbor Island sites. Sites E05 and E18 are potentially less toxic than N07, but more toxic than the remaining sites. This is based primarily on tissue and growth rankings with the sediment ranking having less impact. Mussels at E05 and E18 accumulated relatively high concentrations of PCBs.

Regression Analysis

The regression of potential sediment toxicity (inverse transformation, S^{-1}) and potential tissue toxicity (T) on juvenile mussel growth (inverse transformation, G^{-1}) is highly significant ($r^2 = 0.89$, $n = 13$, overall model $p < 0.0001$). This indicates that

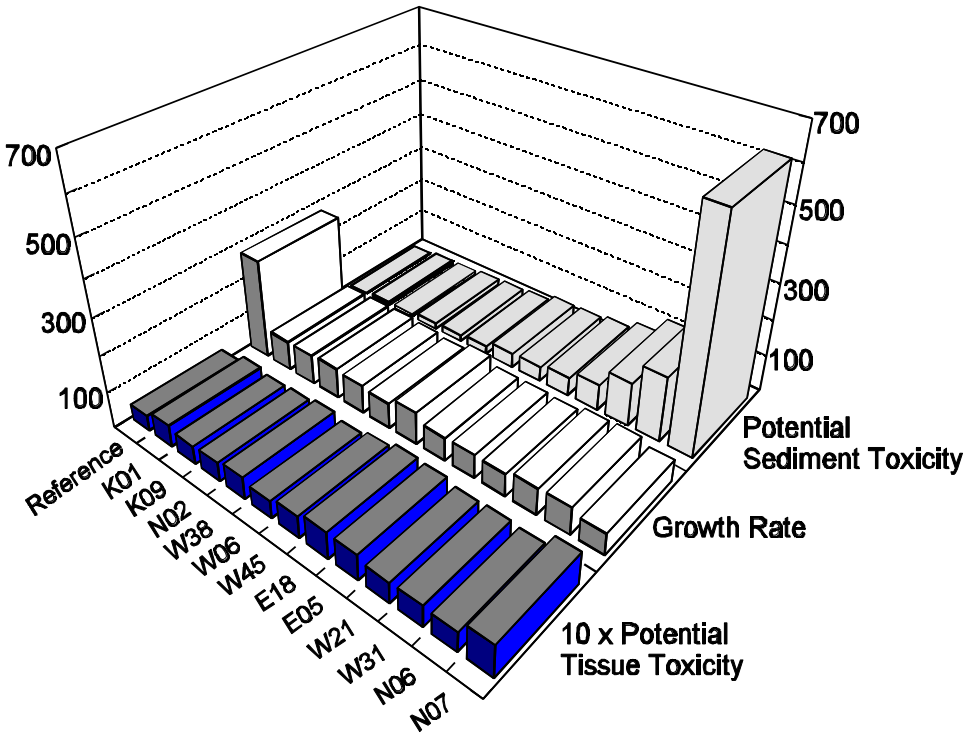

Fig. 3--Bar graph showing potential sediment and tissue toxicity and juvenile mussel growth rates (mg/wk/mussel). Stations are ranked in ascending sediment order.

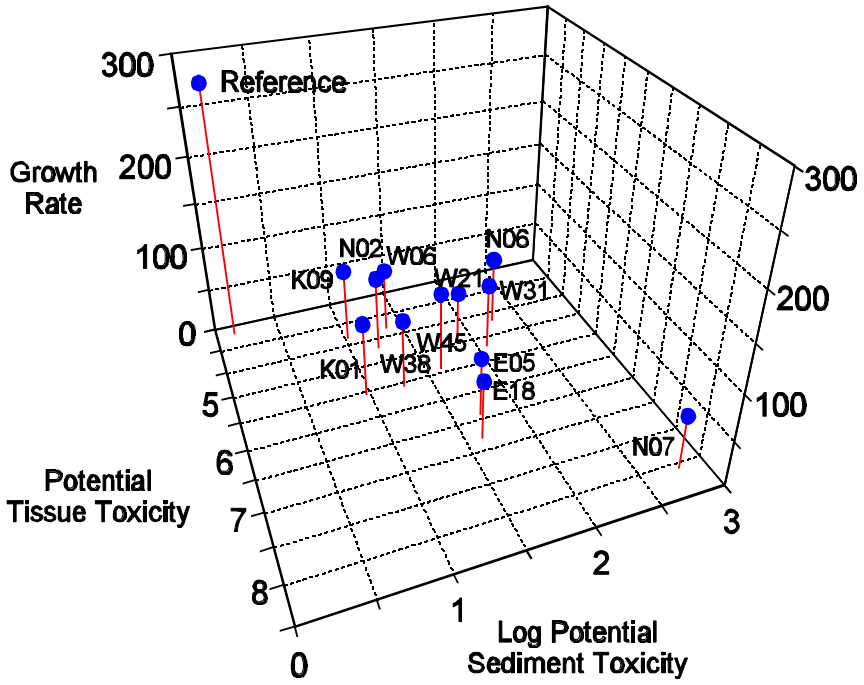

Fig. 4--Three dimensional scatterplot showing growth rate, potential tissue toxicity, and log potential sediment toxicity.

almost 90% of the observed variability in growth across stations could be accounted for by our estimates of potential sediment and tissue toxicity. The resulting equation is:

$$G^{-1} = 0.009 - 0.012(S^{-1}) + 0.00087(T) \quad (1)$$

The independent contributions of S^{-1} and T are also both significant ($p < 0.001$ and $p < 0.05$, respectively). The squared semipartial correlations, which indicate the proportion of total variability in G^{-1}, explained by these two parameters are 40% and 8%, respectively. These results suggest that potential sediment toxicity is more closely associated with growth than potential tissue toxicity. Potential tissue and sediment toxicity were significantly, but moderately correlated ($r^2 = 0.46$, $n = 13$, $p < 0.05$). A plot of residuals versus deleted residuals showed that the regression was influenced by CAR data, but not by N07 data. Without the CAR data, the overall regression analysis model was still significant ($r^2 = 0.53$, $n = 12$, $p < 0.05$) and the regression equation was very similar. However, the ability to account for variability in growth decreases to 53% and the independent contributions of S^{-1} and T are not significant ($p = 0.14$, $p = 0.08$). In addition, the proportion of total variability in G^{-1} explained by S^{-1} and T changed to 13% and 21%, respectively.

DISCUSSION

In this pilot study for EPA, we developed a first-order estimation of potential biological harm from contaminated sediments and tissues using direct measurements of animals exposed *in-situ*. We evaluated the utility of transplanted mussels to augment standard approaches to evaluate contaminated sediments. Mussels accumulated contaminants of concern associated with sediments, stations differed with respect to bioaccumulation and growth, there was a statistically significant relationship between sediment chemistry, tissue chemistry, and growth, and we identified the sites and contaminants of most concern. Based on these results, we recommend that standard approaches to evaluating contaminated sediments be augmented to include: (1) *in-situ* bioassays, (2) bioaccumulation in addition to bioeffects measurements, and (3) evaluations of water overlying the sediments. We used transplanted mussels to accomplish this, but any similar approach could be used.

Bioaccumulation

The tissue chemistry data demonstrate that there is a connection between contaminants in the sediments and contaminants in the water column around Harbor Island. Our results show that the biota in the water column are exposed to and can accumulate these contaminants. Similar results have been reported by others (Young et al. 1976; Widdows et al. 1990; Widdows and Donkin 1992). Even though we have not proven that the contaminants in the mussel tissues came from the sediment (the experiment was not designed to show this) nor investigated the relative contribution of dissolved versus particulate-associated contaminants, we have shown a significant correlation between contaminants in the sediments and contaminants in the tissues. Although it is unclear if bottom sediments or land-based activities were the primary source of contaminants measured in mussel tissues, the accumulation was from one of these sources. Source control has greatly reduced or eliminated contamination from storm drains and many other land-based activities on Harbor Island. This circumstantial evidence combined with the strong statistical relationships suggests that contaminated sediments were the source of the contaminants measured in mussel tissues.

It is well established that suspended particulates adsorb a number of contaminants and that this process reduces the bioavailability of those contaminants to organisms that do not utilize those particles as food. Mussels have been used to estimate potentially bioavailable

contaminants because of their ability to filter seawater for dissolved, phytoplankton-associated, and sediment-sorbed contamination (Hermsen et al. 1994; Ekelund et al. 1987; Widdows and Donkin 1992; Salazar 1986). One of the reasons that many of these sediment-sorbed contaminants are available to mussels is that mussels are able to utilize the organic coatings on these particles as food (Kiorboe et al. 1981). The presence of suspended sediments in laboratory bioassays has been shown to enhance the bioaccumulation of TBT (Waldock and Thain 1983; Laughlin et al. 1986) and other chemicals (Hermsen et al. 1994; Ekelund et al. 1987).

Because the mussels in our study were not depurated after field exposure and retrieval, bioaccumulation may have been overestimated. Previous investigators have shown that the mussel gut can contain significant amounts of sediment-sorbed contaminants (Lobel et al. 1991; Metcalfe-Smith et al. 1992; Robinson et al. 1993). It is not clear to what extent this may have biased our results, but the limited data available suggests that this may not be a problem for all of the contaminants measured in our study. Furthermore, even if the contaminants were not bioavailable and had not been incorporated into mussel tissues, the transplanted animals served as effective accumulators of contaminated, suspended sediment. Unless the contaminants were adsorbed on particles to be rejected as pseudofeces, these accumulated contaminants could be available. Nevertheless, the significant correlation between growth and tissue accumulation suggests that the contaminants were available to mussels and that this measurement may be more appropriate for predicting bioeffects than measuring contaminants in sediment.

Growth

We used juvenile mussel growth as an indicator of water column effects associated with contaminated sediments. As in previous studies, our results (Table 5) show that growth is a more sensitive indicator of biological effects than survival and that growth based on increase in weight is more discriminating than growth based on increase in length (Salazar and Salazar this volume). For the replicated sites, juvenile mussel growth could discriminate bioeffects between the control site and some contaminated sites around Harbor Island (Table 2). This suggests that increased replication could help discriminate differences among other Harbor Island sites.

It is possible that growth rates around Harbor Island could be significantly lower than those in Carr Inlet because of differences in the natural physical and chemical factors that affect mussel growth rates such as temperature, salinity, current speed, etc. Similarly, factors such as sediment grain size, diet, and pre-exposure conditions can affect the results of laboratory bioassays. However, despite the influence of other factors that could affect growth, we are convinced that contaminants played a major role in affecting growth since the highest growth rates were associated with the lowest toxicity-normalized sediment and tissue contamination, and vice versa. In addition, after controlling for sediment toxicity, higher growth rates were significantly ($p < 0.05$) associated with lower concentrations of contaminants (Equation 1).

Since mussel growth is such an important component of this assessment procedure and there are so many natural factors affecting growth, these factors should be more thoroughly evaluated in future studies. To evaluate site-specific effects, *in situ* measurements of appropriate natural factors should be included. To help explain the effects of these factors on growth, laboratory bioassays controlling these factors should also be included.

Normalizing and Combining Sediment and Tissue Concentrations

There are inherent problems in any scheme used to estimate the combined potential toxicity attributable to a suite of contaminants. Despite this limitation, we have demonstrated the utility of producing a single estimate of potential tissue or sediment toxicity for comparison with mussel growth rates. The extent to which this approach may have biased the results is unknown, because the toxicity of our selected contaminants may not be additive. We attempted to weight contaminants on the basis of available and biologically relevant factors. Because the ERMs include sediment chemistry, community effects, laboratory bioassays, and equilibrium partitioning, they are one of the most integrative guidelines that can be used to normalize sediment concentrations. Further, the ERM is only the 50th percentile (median) effects level and as such provides a midpoint estimate of the potential for toxicity. We did not feel it was reasonable to use elevations above reference to rank tissue chemistry data (as previously reported in the remedial investigation [Weston 1993]) because the concentration of several contaminants in tissues from our reference site sometimes exceeded those from the Harbor Island site. Without relating these exceedances to biological effects, one could develop erroneous concerns about the contaminants that might reasonably be associated with various sites. Moving the basis for evaluating effects of contaminants from elevation-above-reference values to toxicity-based values should improve sediment quality assessments. Shifting from estimating toxicity with established criteria to actual site-specific bioeffects measured *in-situ* should provide the necessary field validation for laboratory bioassays.

We agree with previous investigators in suggesting that more carefully designed studies are needed to relate bioaccumulation and toxicity (Cook et al. 1992; McKim and Schmieder 1991). Dose-response relationships for TBT, copper, and zinc based on field exposures of mussels in San Diego Bay (Salazar and Salazar this volume) suggest that the concentrations of these contaminants accumulated in adult mussels at the most contaminated sites around Harbor Island were not high enough to adversely affect mussel growth rates. This suggests that: (1) Dividing by the AWQC may have been reasonable to rank potential tissue toxicity, but dose-response data provide the best indicators; (2) It is important to develop these relationships from measuring dose and response in the same species and the same individuals (we used adults for bioaccumulation and juveniles for growth in this Puget Sound study); and (3) There are differences between juveniles and adults in bioaccumulation, growth, survival, and dose-response that should be considered in any assessment of this type (Salazar and Salazar this volume; data from this EPA pilot study not presented). A better normalization might be achieved by using a bioconcentration factor, along with a mussel-specific water quality criterion, to estimate maximum allowable tissue concentrations (MATC). Dividing the mussel tissue concentrations by this MATC could more closely approximate tissue toxicity.

Site-Specific Differences

Important information can be gained from our results that regulators could use in prioritizing cleanup. The highest sediment contamination, tissue contamination, and the lowest mussel growth rates were all found at N07 and justify a ranking of highest concern. Other work supports this conclusion. Previous studies have demonstrated high sediment toxicity around Harbor Island and near N07 in particular. For example, N07 is the only site where four different evaluation criteria were not met (i.e., less than 50% of reference abundance for polychaetes, crustaceans, and molluscs, and failure of the amphipod bioassays [Weston 1993]). In two separate laboratory bioassay studies with *Neanthes arenaceodentata* lasting 63 and 108 days, juvenile growth and reproductive success were significantly reduced in exposures to

sediment from site N07 when compared to a Carr Inlet Control (Johns and Ginn 1990; Johns et al. 1991).

Improving Assessment Tools

This EPA pilot study conducted in Puget Sound has demonstrated how mussel transplants can supplement the chemical analysis of sediments to prioritize contaminated sites and emphasize the importance of biologically-based criteria. The concept that direct measurements of bioavailability and bioeffects provide information beyond routine analysis of contaminated sediments has been repeatedly emphasized by others. For example, the U.S. Army Corps of Engineers added a bioeffects component to their regulatory program in 1977 when they included a requirement for laboratory bioassays of dredge material for disposal (U.S. EPA 1990). This approach was improved with the development of a Sediment Quality Triad for the evaluation of contaminated sediments that combined bulk sediment chemistry, laboratory bioassays and assessment of benthic community structure (Chapman et al. 1985). Recently, the State of Washington adopted a regulatory program based on the Apparent Effects Threshold which combines bulk sediment chemistry with effects from laboratory bioassays and benthic effects measurements (Washington State Department of Ecology 1991; Barrick et al. 1988, 1989). The approach proposed here and tested in this pilot project can be used to supplement these other approaches.

We have shown how replication at some of the sites increased the discriminating power of the approach in detecting differences among sites. With replication, we were able to detect a statistically significant difference in juvenile mussel growth where sites were only separated by 23 mg/wk (K01=K09 > N07; see Table 5). Further, any statistically significant difference in growth could have effects at the population level (Bayne et al. 1985). Just as we have shown how increased replication can improve the ability to detect differences among sites based on growth, additional transplants at various depths and horizontal distances from suspected sources can identify and locate sources on a finer scale. Because the mussel transplant approach provides a great deal of experimental control, it could be used more intensively around Harbor Island to clarify pathways and exposure routes of contaminants. By transplanting mussels at various depths and distances from suspected sources (sediments, outfalls or landfills) the location of sources can be more clearly identified. This gradient approach, used in San Diego Bay showed that ship hulls rather than sediment were the primary of copper and TBT in the water column. The details of this approach are provided in a companion article in this volume (Salazar and Salazar) and elsewhere (Salazar and Salazar 1991, 1994).

Assessments of water overlying contaminated sediments have been conducted previously. Several other studies have transplanted bivalves near contaminated sediment and made various measurements, such as bioaccumulation and bioeffects (Widdows and Donkin 1992; Roesijadi et al. 1984). For example, in addition to the San Diego Bay study mentioned above, results from mussels transplants in the southern California Bight demonstrated that DDT in bottom sediment was the source of DDT in mussel tissues (Young et al. 1976). In Bermuda, TBT and PAHs in sediment were shown to accumulate in mussel tissues and cause reductions in scope for growth (Widdows et al. 1990). We believe, ours is the first study to use synoptic measurements of bioaccumulation and bioeffects in transplanted mussels to assess contaminated sediments in Puget Sound or at any EPA Superfund site.

CONCLUSIONS

By using transplanted mussels, we demonstrated that combining mussel growth, tissue chemistry, and sediment chemistry was effective in differentiating areas of contamination and potential bioeffects. Our limited synoptic measurements of mussel growth rates and tissue chemistry could only distinguish between the most contaminated and the least contaminated sites. However, we were able to show an association between toxicity-normalized tissue and sediment concentrations. There was a statistically significant relationship between mussel growth and both tissue and sediment chemistry, and mussels accumulated many of the contaminants measured in sediments.

Our original purpose was to determine the utility of measuring bioaccumulation and growth in transplanted mussels as a supplement to standard approaches used to evaluate contaminated sediments. When we attempted to rank the sediment and tissue concentrations, we were dissatisfied with approaches such as elevations above reference, so we normalized for toxicity. The normalization and ranking system presented here should be regarded as preliminary. We encourage continuing efforts to develop a method for normalizing based on toxicity.

More importantly, our results could be used as part of an ecological risk assessment. Assessments of sediment toxicity could benefit greatly from (1) using *in-situ* bioassays, (2) evaluating overlying water, and (3) measuring bioaccumulation and bioeffects. In the context of environmental toxicology and risk assessment, we believe that the sediment compartment should be viewed as a dynamic, interactive system. As such, sediments should be evaluated for potential bioaccumulation and bioeffects in the ecosystem. We have provided one approach that could be used for such an holistic assessment.

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