Dredging Research

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Assessing significance of contaminant bioaccumulation: A biological-effects-based approach

Adapted from ERCD/TN EEDP-01-48 by Elke Briuer, APR, U.S. Army Engineer Research and Development Center (ERDC); authored by Dr. Jeffery A. Steevens, ERDC, and Dr. Peter F. Landrum, National Oceanic and Atmospheric Administration (NOAA)

Dredging activities are federally regulated under the Clean Water Act 404b1 and the Marine Protection Research and Sanctuaries Act 103. These acts require biological evaluations to determine suitability of dredged material sediment for placement in open water. Included are assessments of biological effects resulting from presence of chemical contaminants, as well as the extent of bioaccumulation of contaminants. Specifically, 40 CFR § 227.6 requires that bioassay results on the solid phase of the dredged material do not show significant mortality or sublethal effects, and that no significant undesirable effects caused by chronic toxicity or bioaccumulation of contaminants of concern will be found.

Currently used methods

Environmental Protection Agency and U.S. Army Corps of Engineers guidance can be found in the *Inland*- and the *Ocean Testing Manuals* (*http://www.wes.army.mil/el/dots/guidance.html*). These documents outline a tiered process for sediment evaluation, using background and historical data, analytical chemistry, screening methods, toxicity and bioaccumulation tests, and risk assessment. Biological tests to assess toxicity and bioaccumulation of contaminants in dredged sediments apply at the third tier. To measure acute toxicity, two different species of organisms are exposed to the sediment for a period of 10 days with testing endpoints including survival and growth of the organisms. Concurrently, 28-day bioaccumulation tests are conducted with two different species of organisms to assess the bioavailability and exposure an organism may receive to contaminants present in the sediment. Test results determine the suitability of the dredged sediments for aquatic placement.

Next, the toxicity and bioaccumulation results from the proposed dredging site and a reference site are compared. Sediments proposed for dredging are considered to have an unacceptable potential for adverse effects if statistical analysis reveals significant toxicity compared to the reference site and the difference is a magnitude of at least 10 percent. Results of the bioaccumulation test are compared for statistical significance and then interpreted based on several factors listed in the guidance manuals. These factors include

- Toxicological importance of the chemical
- Potential for biomagnification
- Magnitude of bioaccumulation compared to reference
- The number of chemicals that were observed in the test organisms.

Often, these factors are used subjectively to make regulatory decisions when the bioaccumulation test did not provide clear evidence regarding the potential for adverse effects associated with the sediment. Chronic, long-term tests may be used to gain a better understanding of the potential for effects of the contaminants in the sediment proposed for dredging. Currently several such tests are available, including the EPA's *Hyalella azteca* 42-day test and *Chironomus tentans* life-cycle test and the *Leptocheirus plumulosus* 28-day test.

Acute toxicity tests evaluate adverse effects associated with

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short-term exposure. These effects are measured as dramatic differences in mortality associated with exposure to the sediment. These acute toxic responses may underpredict the "real" long-term biological effects associated with a contaminant present in sediment. Chronic toxicity tests address the long-term exposure and associated effects (i.e., changes in growth and reproduction) but must be conducted for 28 days or longer. These tests typically cost more than $5,000 per sample due to long exposure duration and personnel time. Bioaccumulation tests address the requirement to assess contaminant bioavailability and potential for the contaminants to bioaccumulate in organisms. Bioaccumulation tests have additional cost for expensive analysis of small quantities of tissue for a large number of contaminants. Furthermore, the interpretation of bioaccumulation data is subjective and small differences in tissue concentrations are difficult to interpret as outlined in Technical Note EEDP-01-41 *http://www.wes.army.mil/el/dots/eedptn.html*.

Alternative approach to improve assessment of dredged material

An alternative approach to evaluate chronic toxicity and the significance of contaminant bioaccumulation in dredged material assessments was developed. Potential approaches were examined and experimental progress of a project focusing on an effects-based approach to assess bioaccumulation was undertaken. The approach was chosen from three existing approaches that aim to improve the quality of bioaccumulation assessment and the potential effects associated with contaminant exposure. These three approaches are:

- To include multiple organisms in a single test chamber so as to reduce the efforts associated with a toxicity test
- To combine the chronic toxicity test and bioaccumulation test
- To employ an effects-based bioaccumulation test where the organism is exposed to a "challenge" chemical during a traditional bioaccumulation test.

Because the challenge method provides information about the amount and toxicological significance of contaminant bioaccumulation without the need for extensive analytical chemistry, this method can be used as an effects-based screen for bioaccumulation testing. Most of the time and cost associated with bioaccumulation testing is due to the extensive chemistry performed on tissue samples at the conclusion of the exposure. Use of the challenge in coordination with Tier III toxicity testing could eliminate the need for bioaccumulation testing and its associated costs for a measurable portion of test sediments. In addition, the challenge method offers the means to reduce the uncertainty associated with a chemical-specific bioaccumulation assessment. The challenge method, once validated, would provide an effects-based measure of the level of contamination experienced by the test organisms and account for the bioavailability of all organic contaminants in the test sediment. Development of the effects-based approach has the potential for substantial cost and time savings as well as improved assessments of the risks associated with contaminant bioaccumulation.

Effects-based bioaccumulation test

Research focusing on the critical body residue (CBR) approach has demonstrated that toxicity in aquatic organisms is observed when the load of bioaccumulated contaminants exceeds specific body residues. Assuming that the toxicants act additively, body residue can be used to assess the significance of contaminant concentrations in an organism's tissues during a bioaccumulation test. CBR theory and dose additivity predict that for surviving test organisms challenged during or at the conclusion of a bioassay with a known chemical, the amount of challenge chemical required to produce a toxic response would be proportional to the total load of non-polar organic contaminants that organism acquired. The closer the contaminant concentration in the tissue of an organism is to the toxicity threshold, the smaller the amount of challenge chemical required to produce an effect from the challenge. Given that such a relationship between toxicity and residue levels exists, an effects-based method for determining how close an organism is to a body burden toxicity threshold following exposure to contaminated sediment could be developed. The proximity to a body residue threshold could be determined using a toxicological challenge. This challenge would be applied to test organisms when exposure and bioaccumulation are insufficient to produce toxicity. Such a challenge could be implemented during or after a chronic exposure (i.e., toxicity or bioaccumulation tests) to determine the biological significance of the contaminants accumulated by the organisms in test sediment compared to the reference sediment. This effects-based challenge simultaneously answers two questions:

- Did organisms bioaccumulate biologically significant amounts of non-polar organic contaminants?
- What is the potential for adverse effects from that bioaccumulation?

The fact that these questions are addressed without the need to analyze tissues for a wide range of contaminants means that this method can be used as a cost-effective screen for both potential effects and bioaccumulation.

Several approaches could be considered to deliver the challenge chemical to aquatic organisms exposed in sediment. These approaches include delivery of the challenge chemical by

- Spiking the sediment prior to conducting the test
- Adding the chemical to the overlying water during the test

- Delivering food laced with the challenge chemical to the experimental chamber
- Removing the organisms at the end of the experiment and exposing them for less than 48 hours to the challenge chemical.

Challenge compounds have been delivered through water-only and spiked-sediment exposures. In addition, recent studies have demonstrated that even very water-insoluble compounds such as hexachlorobiphenyl can be delivered in toxic concentrations through dietary exposure to aquatic organisms, resulting in mortality and chronic toxicity. The difference in delivery methods can have significant consequences on the rate and extent of challenge chemical delivery to the organism. Therefore, the choice of delivery methods will be determined based on the specific question addressed and whether acute or chronic effects are the endpoint of the challenge.

Current LEDO investigations

Long-term Effects of Dredging Operations research is focused on proof of concept, development, and validation of the approach described above. In the first year, the project focused on identifying and toxicologically assessing a potential challenge chemical using two model organisms. The proof of concept was through determination of a CBR for the model compound and demonstration that the model organisms would respond at concentrations predicted by chemicals acting through non-polar narcosis. Toxicant delivery methods were also assessed.

The effects-based approach to assess bioaccumulation requires the identification and toxicity characterization of a challenge chemical. Although it is nearly impossible to find a compound that fits all criteria necessary for valid results, pentachlorobenzene (PCBZ) was chosen for the initial evaluation of the effects-based approach since it was important that the compound be available in a radiolabeled form. Use of a radiolabeled compound allowed early developmental work to proceed during the compilation of the PCBZ analytical methodology.

Two aquatic organisms were selected to develop the effects-based approach for bioaccumulation. The freshwater amphipod, *Hyalella azteca*, is routinely used for assessing the toxicity of dredged materials for aquatic disposal. *Leptocheirus plumulosus*, an estuarine amphipod, was chosen for comparison purposes to assess the bioaccumulation challenge approach in a saltwater environment. Initial studies assessed the challenge chemical and included two 10-day water-only PCBZ uptake and toxicity experiments and a 28-day PCBZ uptake and toxicity experiment. These studies served to characterize the uptake (K_u) and elimination (K_e) of the challenge chemical as well as to establish the tissue concentration at which toxicological effects occur (survival for 10-day, and growth for 28-day studies).

Results of 28-day experiment. A 28-day experiment was conducted to assess the chronic toxicity and to characterize the uptake and elimination of ^{14}C-PCBZ. No significant mortality was observed at any of the doses ranging from 10 to 100 µg/L. The water concentration varied substantially on a daily basis but the time-weighted average was fairly constant (Fig. 1).

Toxicokinetics of PCBZ were determined from the initial doses using all of the water data and were nearly constant across the doses. This resulted in proportional accumulation as the dose in the water increased. The only observed toxicity in the 28-day study was a decrease in growth rate compared to the control (Fig. 2). The growth rate based on body residue was not as strong a relationship as that based on the concentration in the water due largely to the variability in determining the body residue concentration of individual *Hyalella* (Table 1).

Results of 10-day experiment. Two 10-day mortality studies were performed in a manner similar to that of the 28-day study except all of the water was exchanged daily by moving the organisms to fresh exposure media. Two measures of body residue effects were used to define the toxicity of PCBZ to *H. azteca*. A significant change in body residue with increasing

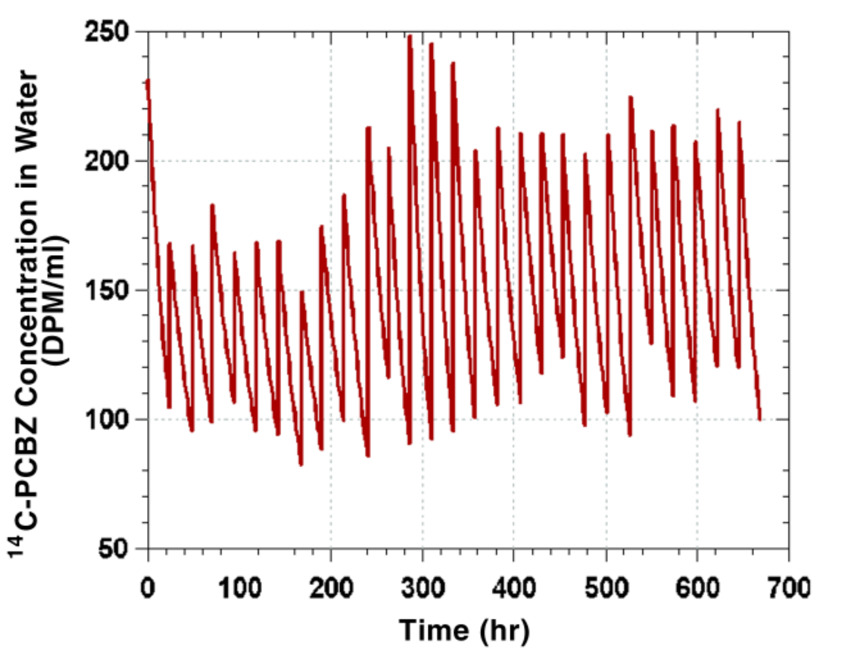


Figure 1. Daily PCBZ concentration in water for 10-µg/L concentration over the 28-day exposure period

Table 1
Toxicokinetics of PCBZ in *H. azteca* Determined in a 28-day Bioassay

Concentration (µg/L)	k_u (mL/g/hr)	k_e (hr)
10	44.8 ± 13.1	0.023 ± 0.007
20	57.1 ± 10.3	0.022 ± 0.005
40	43.6 ± 15.3	0.023 ± 0.009
60	40.7 ± 16.0	0.019 ± 0.009
100	35.3 ± 11.0	0.016 ± 0.006

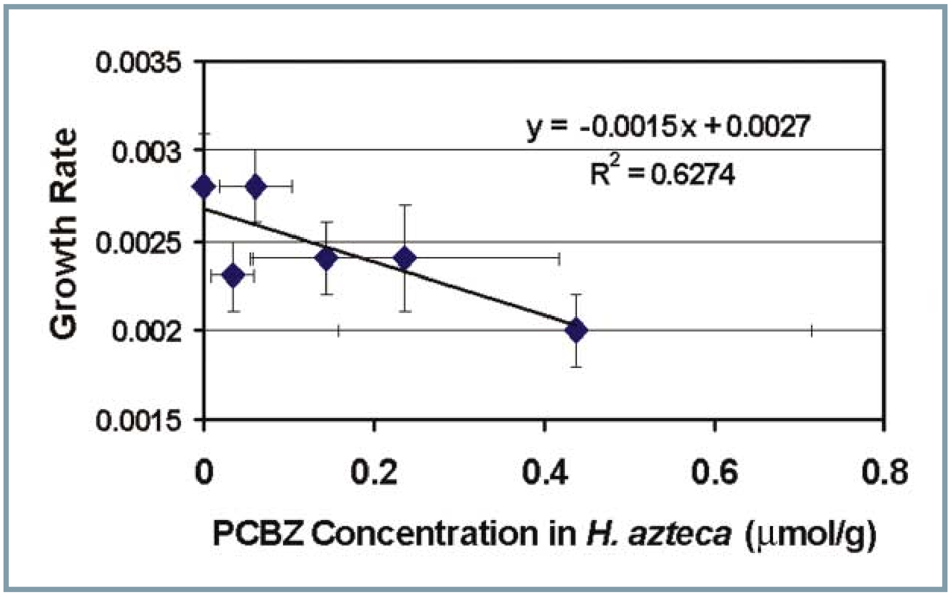


Figure 2. Reduction in growth rate as a function of PCBZ in H. azteca

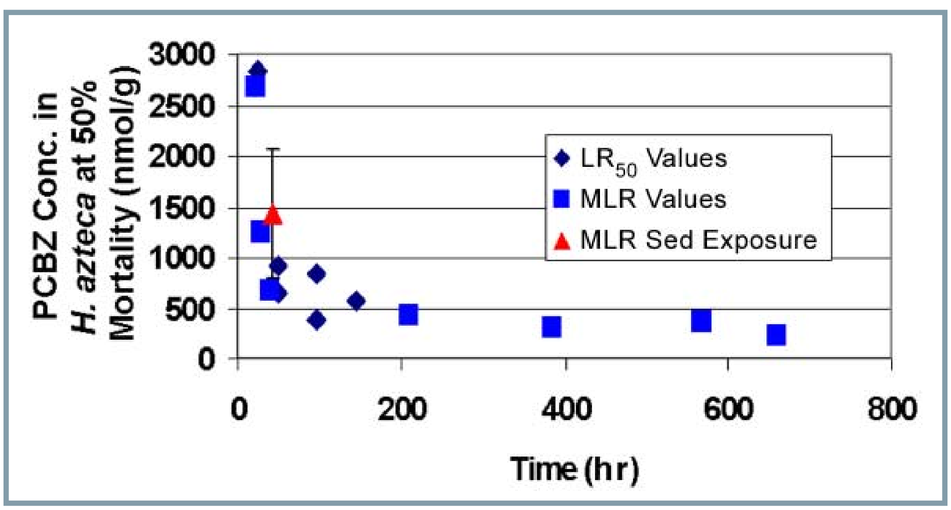


Figure 3. Body residue producing 50-percent mortality with increasing duration of exposure

duration of exposure was observed (Fig. 3).

Estimates of the body residue associated with 50-percent mortality, whether estimated as residue in tissues corresponding to 50-percent mortality (LR_{50}) values or mean lethal residue in dead organisms (MLR) values corresponding to the LT_{50}, yield essentially identical results for the same duration of exposure. Thus, the residue required to produce a given percent mortality in a population is the same whether determined from the PCBZ concentration in live or dead organisms. This is consistent with a dose response relationship where a portion of the population responds while another portion does not for a fixed dose; e.g., at the LD_{50}, 50 percent of the population dies while the other 50 percent survives. This is critical to the challenge approach since in most tests concentrations will be determined in live organisms. In addition to testing *H. azteca* in water-only exposures, it was important to demonstrate that the challenge approach would work when organisms were in sediment. In a simple demonstration, *H. azteca* were allowed to burrow into sediment and then the challenge chemical was added to the water and 75 percent of the water was exchanged daily. Since there was only one dose, the time to 50-percent mortality (LT_{50}) was determined and the MLR was measured. The resulting toxic dose on a body residue basis was the same as that observed in water-only exposures.

Context of bioaccumulation testing: Interpretation of CBR toxicity data

Finally, to use the toxicity information for the purposes of a challenge it is necessary to have a predictive model for the time-dependent data. The data in Figure 3 were fit to a damage assessment model that was previously developed at the Great Lakes Environmental Research Laboratory, National Oceanic and

Atmospheric Administration. The model assumes first-order kinetics for the accumulation and loss of the contaminant from the organism. This is appropriate for exposures to aqueous solutions where the compound is not biotransformed by the organism as is expected for PCBZ. This is coupled to a damage repair model that assumes damage and repair in the organism are first-order processes. In this case, the damage accrues with increasing concentration of contaminant in the organism and damage is repaired in response to increasing damage up to a critical level where the organism can no longer function. While it is likely that this damage assessment model is somewhat simplistic, it provides a first approach to evaluate the results of the 10-day and 28-day studies. If there is a critical damage level (D_L) that produces 50-percent mortality then the LR_{50} has the following relationship:

$$LR_{50}(t) = \frac{D_L / k_a}{\dfrac{1}{\left(1 - e^{-k_e \cdot t}\right)} \times \left(\dfrac{e^{-k_r \cdot t} - e^{-k_e \cdot t}}{k_r - k_e} + \dfrac{1 - e^{-k_r \cdot t}}{k_r} \right)}$$

Where k_r is the rate of damage repair, k_a is the rate of damage accrual, and k_e is the elimination rate constant.

Fitting the mortality data generated above provides us with a relationship that describes the data with a coefficient of determination of 0.80. With this relationship, the challenge exposure can be conducted for the duration that is required to produce a toxic response and the observed data can be compared to the data generated in the absence of a competing contaminant.

Sufficient data have been generated that PCBZ can now be tested in *H. azteca* for its use as a challenge chemical when the amphipods are exposed to other compounds. The one complication to the use of PCBZ found during these studies is the volatility of the compound as demonstrated in the water concentration variation. While the development of this potential compound will continue for the next year, efforts to find a more suitable compound will be made.

More information about the research can be found online in LEDO ERDC/TN EEDP-01-48 available at *www.wes.army.mil/el/dots/eedptn.html*. The authors may be contacted as follows: Dr. Jeffery A. Steevens, phone: (601) 634-4199, e-mail: *Jeffery.A.Steevens@erdc.usace.army.mil*, or Dr. Peter F. Landrum at the Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration, e-mail: *landrum@glerl.noaa.gov*.

Dredging Calendar – 2002

April 16 – Environmental Windows Workshop, "Achieving Dredging Decisions that Balance Economic and Environmental Concerns," Vicksburg, MS. **POC:** *www.wrsc.usace.army.mil/pianc*

April 17-19 – PIANC 100th Anniversary Meeting, "New Era for Water Transportation," Vicksburg, MS. **POC:** *www.wrsc.usace.army.mil/pianc*

May 5-8 – ASCE: Dredging '02, Orlando, FL. **POC:** *www.asce.org/conferences/dredging02*

May 7-9 – Battelle and U.S. EPA. 25th Annual Conference on Analysis of Pollutants in the Environment, Norfolk, VA, Sheraton Norfolk Waterside Hotel. **POC:** *www.battelle.org/conferences/Pollutants/*

May 13-16 – WEFTEC Asia Pacific 2002, Kuala Lumpur, Malaysia. **POC:** *weftecasiapacific@wef.org*

June 12 -15 – Western Dredging Association WEDA XXII, Texas A&M 34th Annual Dredging Seminar and Exposition, Omni Interlocken Resort, Denver, CO. **POC:** FAX 360-750-1445, mail WEDA, POB 5797, Vancouver, WA 98668-5797.

July 9-11 – EPA and U.S. Army Corps of Engineers, Dredged Material Assessment and Management Seminar, Crowne Plaza Union Square, San Francisco, CA. **POC:** online, *www.wes.army.mil/el/dots/training.html*, FAX 601-634-3528, e-mail *Billie.H.Skinner@erdc.usace.army.mil*

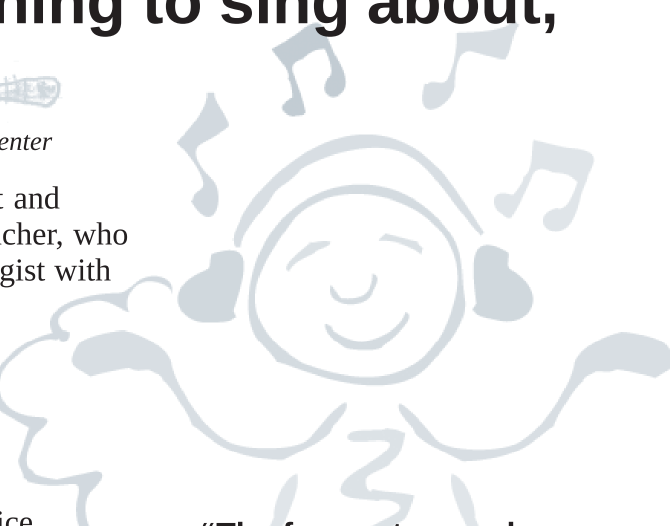
September 22-27 – American Association of Port Authorities Annual Convention, Palm Beach, FL. **POC:** *www.aapa2002.com*

September 22-26 – PIANC 30th International Navigation Congress, Sydney, Australia, **POC:** *www.pianc-aipcn.org/pi200.html*, e-mail *pianc2002@tourhosts.com.au*

November 16-22 – SETAC (Society of Environmental Toxicology and Chemistry) North America 23rd Annual Meeting, Salt Lake City, UT. *Achieving Global Environmental Quality: Integrating Science & Management*. **POC:** *www.setac.org/meet.html*

Science education something to sing about, say 5th graders

by Elke Briuer, APR, U.S. Army Engineer Research and Development Center



During the week 11 to 15 November 2001, the 22nd Annual Meeting of the Society of Environmental Toxicity and Chemistry took place in Baltimore, Md. This enormous get-together of the world's best toxicologists and chemists attracted students and teachers, research personnel, and industry, among others. A large exhibit hall served to bring together attendees by showcasing technology, serving evening snacks, and providing an Internet Café setting. Daily, a luncheon speaker addressed a huge crowd in the auditorium, one of them EPA Administrator, Govenor Christie Whitman.

Among all these actvities, it may have been easy to miss one evening's entertainment that earned accolades: Forty-five 5th graders from North Glen Elementary School in nearby Glen Burnie performed a "Science Opera" for SETAC members.

As it turned out, the musical entertainment was well-attended and the two behind-the-scene characters in this event were pleased with their success. "The kids were so nervous and antsy before the performance, but once it started, they just did great, getting a standing ovation," said Alfreda (Freda) Gibson. Gibson, a research biologist from the U.S. Army Engineer Research and Development Center, Vicksburg, Miss., and Dr. Don Waite, an environmental scientist and conservatory music teacher, who also is a research biologist with Environment Canada, conducted the SETAC education committee's science project during this annual meeting. Dr. Beth Magee, U.S. Fish and Wildlife Service, who was co-chairman of the SETAC meeting, collected invertebrates from the Chesapeake Bay for use in a "Pour-A-Pond" classroom demonstration.

The program flyer "Singing about science" states, "Teach a child to sing about the environment and adults will listen. Teach children to sing about the world and their eyes willl open to its wonders." It sums up the reason why Waite sings and writes songs about the environment. SETAC presents science in the ancient oral traditions of early societies, through music, drama, and art. Gibson and Waite spent Thursday and Friday before the meeting at the school, teaching the children about science in general, working on a hands-on, pour-a-pond project, teaching songs about the various flora and fauna found around Chesapeake Bay, encouraging the children to create their opera props and costumes, and teaching them how to spell such words as bioaccumulation, polychaetes, and vertebrates. Thank-you letters received by Gibson and Waite prove their teaching

"The fun part was when we all sat down and Don was playing the guitar.

success, as these words appear in easily recognizable form on the list of things the children wrote about as most memorable.

But what they liked best can be summed up in their own words. "I'd like to thank you for coming to our school and showing us cool experiments," writes Atia Burgos. Brandy Johnson said "thank you for making those beautiful worm costumes," and Patrick Swann said, "Freda, one thing I liked was you made good songs and you spent most of your time helping all the 5th graders when we needed help the most." Almost every letter referred to Waite's guitar performance. "The fun part was when we all sat down and Don was playing the guitar. Thank you," wrote Aude Harison. Brandie Starlings wrote, "I have the best day of my life in school. I did not know that school could be so mush(sic) fun. Thanks a million..."

More information about this program is available from Waite at (306) 780-6438 or Gibson at (601) 634-4027. Artwork by 5th graders from North Glen Elementary School.

Dear Don and Freda,

I'd like to thank you for coming to our school and showing us cool experiments. The three things that really stuck out in my mind was, the game in the play, the bioaccumulation thing when the fish get the toxic chemicals from the sediment gang, then the crabs ate the fish, which gave the crabs the toxic chemicals and the fish ate the crabs, the fish got the toxic chemicals, then the Captians crew ate the fish and got the toxic chemicals, and then the whole crew shouted "And that's bioaccumulation!" The second thing that I'll never forget ... the ... is when we ... polychaets, ...

Dear Don and Freda,

I'd really like to thank you both for playing songs on the guitar, those songs were really good. The three I remeber are. First, the pond we made I remeber because we got to ... old the different inverbrates ... the spoons. Secondly, when ... taught about bioacculmation ... d how it forms. For example ... all of the toxic chemicals ... the Chespake Bay. Thirdly, ... thing I really liked about ... time you were here was ... you played songs on the

Sincerley,
Cecilia

Polchaet

Dear Don and Freda,

I'd really like to thank you. First, I learned bioaccumulation is that some toxic runs into the water. the worms eat it, then the crabs eat the worms then the crab has the toxic and the fish comes and ate the crab now the fish has the toxic in it. Next, I learned that when erosion and biocumulation meet it will be a explosion. Finally, I like when we got to pick up crabs and other animals.

Sincerely,
Chad Boulden

"I have the best day of my life in school. I did not know that school could be so mush(sic) fun. Thanks a million..."

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Technical Brief Now Available

PIANC's Technical Brief about the management of contaminated dredged material within the navigation community was conceived and published as part of the PIANC Project on Contaminated Dredged Material with the support of the PIANC Environmental Commission. For a copy, contact Ms. Billie Skinner, FAX 601-634-3528, e-mail *Billie.H.Skinner@erdc.usace.army.mil*, or by mail, USAERCD, ATTN: Ms. Billie Skinner, EM-D, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199.

Dredging Research

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