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Method for the Preliminary Assessment of Aquatic Contamination Sites Using Sediment Extract Toxicity Tests

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Aquatic sediments, particularly those of high organic content, tend toward a higher affinity for many organic and inorganic contaminants. Depending upon the ambient physical and chemical conditions (e.g. temperature, pH, O_2, redox potential) at a particular site, the sorption to sediments can affect the persistence, toxicity, uptake and transport of contaminants (Breteler and Saksa 1985; Burton et al. 1987; Landrum et al. 1987; Lewis and McIntosh 1986; Malueg et al. 1984; Nebeker et al. 1986; Pritchard et al. 1986; Seelye et al. 1982; Wolfe et al. 1986). In general, contaminants incorporated into sediments are more persistent, less mobile, and occur at higher concentrations than those in the overlying waters. The method of toxicity assessment described herein utilizes the affinity of contaminants for sediments to develop and refine sampling strategies used in the preliminary assessment of site contamination by evaluation of the presence or absence of extractable toxic components in aquatic sediments collected in different areas of the site.

Big Lake Sissabagama is a relatively deep, rocky lake located in northwestern Wisconsin (see Fig. 1). The surrounding watershed is primarily forest and sphagnum bog. Most residences on the lake are seasonal and lake use is usually light recreational. Between the mid 1960's to the present, a cranberry farm has operated in the southeast region of the lake. In accordance with a 1867 Wisconsin state legislation, commonly referred to as "the Cranberry Law", water may be pumped from a surface water, used for irrigation, harvesting and frost-protection of cranberries, then released back into that same waterbody. This process is suspected in the transport of materials applied to farms into some lakes. In recent years, several organic pesticides have been reported to occur in water and sediments near the effluent of a cranberry farm (Larson 1986; Wisconsin Department of Natural Resourcese, unpublished). The possible occurrence of at

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least 12 pesticides or metabolites based upon use, mobility and persistence has been projected. No other information is available on the extent of potential pesticide contamination over the lake as a whole. Because of the large number of potential contaminants, the uncertainty about lowest detectable limits for each, especially in organic sediments, and the high cost of analytical services, it was determined that a screening procedure should be developed to assess the lake as a whole to determine what contaminants were most likely to occur and where. The method described identifies sediment samples containing solvent-extractable components which are toxic to the cladoceran, Daphnia magna. To date no subsequent site analyses have been completed.

MATERIALS AND METHODS

Sediment samples were collected at 9 sites on Big Lake Sissabagama on October 4, 1986. Superficial sediment was collected by dredge to a maximum depth of 2-3 cm and placed in polyethylene sample bags. Bags were placed on ice and returned to the laboratory for subsequent analysis.

A known volume of each sediment (250-500 cm^3) was extracted with two - 50 mL aliquots of analytical grade petroleum ether. Approximately 2 g of reagent grade NaCl was added to each sediment sample. Extractions were conducted in 1-L Erlenmeyer flasks. Samples were shaken vigorously for 15 min. and ether was decanted off. After the second extraction, samples were frozen at -30° C after which any remaining ether was decanted off. Extracts were combined and evaporated under mild air flow to dryness, and residues were redissolved in analytical grade acetone. Acetone solutions were all brought up to an equal volume (25 mL at 25° C) and stored in the dark at -30° C until used in toxicity tests. Subsamples of each sediment were retained and % organic content of each was estimated by combustion.

Cladocerans (Daphnia magna) used in acute toxicity tests were from cultures maintained at the Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, Superior, WI. Reconstituted hard water (Table 1) was used as both the culture water and dilution water in toxicity tests. Gravid females were isolated in 250 mL Erlenmeyer flasks containing 200 mL of reconstituted water for 24 hrs, after which offspring were separated by pipette. Only early instar (<24 hr. old) neonates of D. magna were used in toxicity tests.

Test chambers were 100 mL beakers. Appropriate volumes of each acetone extract were transferred to test chambers containing 3-5 mL dilution water. Such treatment was thought to reduce adsorption of residues to glass. Acetone was then evaporated under gentle air flow for 15 min. Each treatment was run in duplicate or triplicate. Both acetone and non-acetone controls were run. Acetone controls consisted of an evaporated

Table 1. Quantities of reagent-grade chemicals used to prepare reconstituted hard water and water qualities (ASTM 1980).

Chemical or Quality	mg/L
$NaHCO_3$	192
$CaSO_4$	120
$MgSO_4$	120
KCl	8.0
pH	7.8-8.0
hardness	160-180
alkalinity	110-120

* Standard pH units.

ether/acetone volume equal to the highest extract volume tested. Control survival at 48 hr. in all tests was 100%.

After addition of the acetone extract, dilution water volume was brought up to 80 mL, test solutions were stirred by a glass rod and allowed to equilibrate for at least 15 min. Ten neonates were then transferred to each test chamber. No food was provided for the duration of the tests. Tests were conducted at 20° C and under constant illumination. Dissolved oxygen concentrations were measured in each treatment at 48 hr. All DO levels were greater than 60% saturation. Neonate survival and behavior was observed at 24 and 48 hr. End-points were affected neonates (organisms unable to maintain themselves in the water column, yet still motile, in addition to mortalities) and mortality (defined as those neonates not responding to physical prodding). Tests were ended after 48 hrs.

Volumes of sediment extracted varied, therefore the volume of extracts used in each treatment was normalized to a sediment volume of 1,000 cm^3 by the following equation: vol. of extract used (uL) x vol. of sediment extracted (cm^3)/1,000 cm^3 = normalized vol. (uL). In those tests which produced sufficient effects, a trimmed Spearman-Karber method was used to determine 48-hr EC and LC50s and their confidence limits.

RESULTS AND DISCUSSION

Locations of the 9 sample sites, along with their respective 48-hr EC/LC50s are found on Fig. 1. Five sediment extracts resulted in no observed effects at the highest extract volume tested. These volumes were 1,000 uL/80 mL for sites 4, 6 and 8; and 1,500 uL/80 mL for sites 7 and 9. Extract of sediment from site 5 resulted in 40% affected neonates and 20% mortality at 1,200 uL/80 mL. Extracts of sediments from sites 1, 2 and 3 produced sufficient effects and mortalities to calculate 48-hr EC and LC50 values (Table 2). The % organic content estimate for each sediment and the depth at each site is in Table 3.

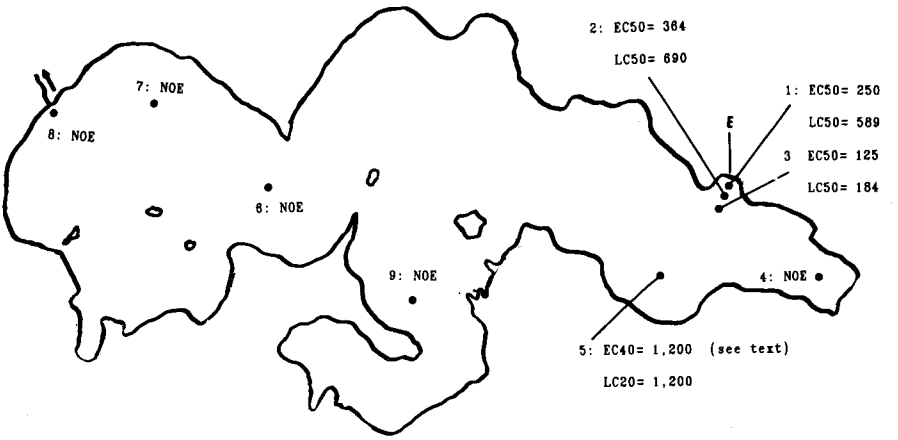

Figure 1. Locations of sample sites (numbered) and their respective 48-hour EC and LC50s (in uL/80 mL). NOE = no observed effect at highest concentration tested (refer to text). E indicates the location of the farm effluent.

Table 3. Percent organic content estimate of sediment samples and depth at each site.

Site	% Organic	Depth (ft)
1	27.1	2
2	23.8	6
3	35.6	18
4	39.7	22
5	49.1	32
6	39.4	23
7	44.4	24
8	0.6	3
9	43.2	18

This method for site evaluation appeared to be effective and cost-efficient as a basis to begin a primary assessment of the site. The assumption is made that sites with sediments containing ether-extractable toxic components have the highest probability of pesticide contamination, therefore these sites should be emphasized in subsequent site assessments. This method

Table 2. Results of toxicity tests on sediment extracts from sites 1, 2, and 3. Results are means of duplicate or triplicate exposures.

Extract Concentration[a] (uL/80 mL)	Site 1		Site 2		Site 3	
	% Affected	% Mortality	% Affected	% Mortality	% Affected	% Mortality
25	-[b]	-	-	-	0	0
37.5	-	-	-	-	0	0
50	0	0	0	0	19	0
75	0	0	6	0	-	-
100	25	0	13	0	-	-
125	-	-	-	-	40	10
250	60	20	40	0	90	70
500	73	27	40	0	100	100
750	80	50	50	70	-	-
1,000	100	80	100	100	-	-

[a] Normalized for volume of sediment extracted.

[b] - = This concentration was not tested.

does not address the question as to whether adverse effects due to these toxic components may or may not be occurring in the environment.

The obvious benefits of this methodology over other sediment toxicity methods (e.g., Burton and Lanza 1985; LeBlanc and Surprenant 1985; Swartz et al. 1985) are a) tests can be conducted following established protocol, such as ASTM (1980); b) tests can be conducted with existing equipment and personnel in laboratories conducting static toxicity tests. The use of alternative extractants or methods, such as fractionation (Lopez-Avila et al. 1985) for other specific classes of chemicals may be effective in further characterizing contaminants. As an example, acid extractions may be appropriate for sites where non-organic heavy metal or metaloid (e.g., Se) contamination is suspected. An additional modification to the use of a soft dilution water may also be warranted. The sensitivity of this method will be enhanced if factors of water chemistry (e.g., hardness, pH) of the dilution water do not mitigate toxicity.

This protocol appears to be most appropriate for contaminants with a high acute toxicity, especially to aquatic invertebrates. Certainly many pesticides and heavy metals would be included in this category. Modifications to assess toxicity to fishes are possible, but would require extractions from a much greater volume of sediment to accommodate the larger volume test chambers, which may be cumbersome. At present it is not envisioned that this method could easily be adapted for use in a flow-through exposure system, again primarily due to the large volume of extract which may be required.

For use in toxicity tests with D. magna, and presumably other small aquatic invertebrates, a final extract volume of 25 mL appeared to be the most acceptable. Larger volumes, thereby being more dilute, required larger doses of the extract to test chambers and more time for evaporation of the acetone. As the final extract volume was reduced, the ease with which aliquots of extractant used in lower concentrations could be measured was also reduced. The solutions in these smaller volumes also became turbid as materials reached their solubility limits in the acetone and precipitated, especially in extractions from high-organic sediments. No precipitation was observed in any extracts at a final volume of 25 mL, although sediment organic content was as high as 49.1%. Use of alternative extractants may require re-evaluation of appropriate final extract volume.

Data interpretation will require the expertise of several fields of environmental science including but not limited to toxicology, environmental chemistry, and limnology. And, as is hopefully self-evident, a thorough evaluation of any other available data on the ambient conditions of the site, its watershed, land-use, flow patterns, historical data, etc., should be of value in data interpretation. In the case described, acutely

toxic components in sediments occurred at sites 1,2, and 3 (and possibly 5). Relative toxicity (based on 48-hr. LC50) of these sediment extracts were 3 > 2 >1. The depths at sites 1, 2, and 3 were 2 ft, 6 ft, and 18 ft, respectively. It could be predicted that ambient conditions for sediments in deeper waters are more amiable to the persistence of organic contaminants. These conditions include low light, lower temperatures, and low dissolved oxygen levels (both winter and summer anoxia are common on Big Lake Sissabagama according to historical records). Therefore, it could be predicted that at sites 1 and 2, only highly recalcitrant compounds and/or metabolites would occur. At site 3, more labile compounds may occur and persist, as well as recalcitrant species. The relative order of toxicity of sediment extracts from site 1, 2 and 3 provides additional support for the inference that parent materials, presumably more toxic than their degradation products (with notable exceptions), are more likely to occur at site 3, where ambient conditions would retard degradation.

Any additional sample collection and/or subsequent analyses would appear to be most effective in the general areas encompassed by sites 1, 2 and 3 (and possibly 5). A list of candidate compounds, both parent materials and likely metabolites should be assessed to determine the likelihood of recalcitrance under ambient conditions, and chemical analyses might proceed based upon the logic presented above. In the case of Big Lake Sissabagama, this would translate into a reduction in samples analyzed from 9 to 3 or 4, and would also reduce the number of specific chemicals assessed in each sample. If an adequate quantity of sediment were collected, and the excess has been retained under proper storage conditions, initial chemical analyses could potentially proceed without further on-site collections. Ideally, the assessment of sediment extract toxicity could reduce costs by localizing and prioritizing sampling and analysis strategies.

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