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A SEDIMENT-CONTACT BIOASSAY WITH PHOTOBACTERIUM PHOSPHOREUM

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Management Perspective

The sediment contact Photobacterium bioassay was developed to determine the spatial variation in toxicity of Hamilton Harbour sediments. The bioassay used the same bacterium as the Microtox bioassay, but the assay was changed to measure the toxicity of the particles, rather than the elutriates. When sediments contain hydrophobic contaminants (i.e. PCB's), the new bioassay is more sensitive than the original Microtox bioassay.

Earlier experiments by the Department of Fisheries with Chironomus or Pontoporeia were confirmed indicating that the sediments of Hamilton Harbour are acutely toxic. Moreover, our bioassays were able to resolve the spatial variation in toxicity in much more detail than earlier studies (48 vs. 4 sites). The speed of the new Photobacterium bioassay will be very useful for any screening analysis, i.e., it will be very suitable for studying the chemical treatment of harbour sediments.

The new Photobacterium bioassay confirms the results of earlier NWRI bioassays of harbour sediments with Daphnia magna. Sediment samples from the northern and western areas of the harbour have no detectable acute toxicity. Both assays indicate that the most toxic sediments in the harbour are near the discharge pipes of the steel mills; the Photobacterium test indicates that the sediments near the steel mills are greater than a hundred times more toxic than sediment samples from Windermere Basin.

PERSPECTIVE DE GESTION

La méthode de biodosage des sédiments par contact avec les photobactéries a été mise au point pour déterminer la variation spatiale de la toxicité des sédiments du port de Hamilton. La méthode fait appel à la même bactérie que l'essai Microtox, sauf qu'elle a été modifiée pour mesurer la toxicité de particules plutôt que celle d'éluats. Dans le cas de sédiments renfermant des contaminants hydrophobes (c.-à-d. des BPC), la nouvelle méthode de biodosage est beaucoup plus sensible que l'essai Microtox initial.

Cette méthode nous a permis de confirmer les résultats obtenus antérieurement par le ministère des Pêches avec Chironomus ou Pontoporeia, qui indiquaient que les sédiments du port de Hamilton étaient de toxicité aiguë. En outre, l'application de notre méthode a permis d'établir la variation spatiale de la toxicité de façon plus détaillée que les études antérieures (48 sites contre 4 sites). La rapidité d'exécution de cette nouvelle méthode permettra son utilisation comme méthode de contrôle, et elle pourra servir à étudier le traitement chimique des sédiments du port.

La méthode confirme aussi les résultats de biodosages antérieurs réalisés par l'INRE avec Daphnia magna. Les échantillons de sédiments provenant des secteurs nord et ouest du port ne donnent aucun signe de toxicité aiguë. Les deux méthodes de biodosage indiquent que les sédiments les plus toxiques sont localisés près des canalisations de décharge des aciéries; la nouvelle méthode avec Photobacterium phosphoreum révèle que les sédiments aux abords des aciéries sont au-delà de cent fois plus toxiques que les sédiments obtenus du bassin Windermere.

A Sediment-Contact Bioassay with Photobacterium phosphoreum

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Abstract

A new, rapid test is reported for the toxicity screening of sediments using inhibition of Photobacterium phosphoreum. The bacteria are placed in direct contact with the sediment and the change in luminescence of the Photobacterium is used to determine the toxicity of the sediment relative to a control site. This sediment-contact bioassay appears to be more sensitive to hydrophobic contaminants such as a polychlorinated biphenyl than the standard sediment elutriate test with the Microtox bioassay.

Keywords Photobacterium, sediments, toxicity, bioassay

ABRÉGÉ

Une nouvelle méthode rapide, basée sur l'inhibition de Photobacterium phosphoreum, a été mise au point pour contrôler la toxicité de sédiments. La méthode consiste en la mise en contact direct des bactéries et des sédiments, le changement de luminescence des photobactéries permettant la détermination de la toxicité des sédiments relativement à une zone témoin. Cette méthode de biodosage de sédiments par contact direct semble plus sensible à la présence de contaminants hydrophobes, tels les biphényles polychlorés, que le biodosage Microtox usuel permettant de mesurer la toxicité d'éluats de sédiments.

MOTS CLÉS : Photobacterium, sédiments, toxicité, biodosage.

Introduction

The majority of the techniques for studying sediment toxicity use aqueous extracts (elutriates) or a solvent extract of the sediment [1-6]. Some tests use organisms grown directly in the sediment [1,5] but these methods are time consuming.

The change in the bacterial luminescence when Photobacterium is exposed to toxic chemicals can be used as an indication of organic and metallic toxicity [7-9]. Since the bacterial luminescent pathway is a branch of the electron-transport chain, the luminescent measurement assesses the flow of electrons in the respiratory chain and the metabolic state of the cell (10). These bacteria are now widely used in the Microtox test since this screening test is rapid and relatively inexpensive. Microtox is a bioassay marketed by Beckman which includes the freeze-dried marine bacteria, salt solution, photometer and methods. This test, however, cannot be used directly on sediment, since the sediment particles absorb the light given off by the bacteria. The standard method devised to avoid this problem is to use solvent or acid extraction to remove chemicals adhering to the sediment particles for bioassays [3,11].

This study reports a simple, rapid and inexpensive method of determining the relative toxicity of sediments using the decrease in luminescence of Photobacterium when exposed directly to untreated sediment. In brief, this method involves adding a small quantity of Photobacterium culture to a suspension of the test sediment; after a 15-min incubation, the mixture is centrifuged

and the activity of the bacteria in the supernatant is measured using a Beckman Microtox photometer. The results of this screening procedure were compared to the response of Photobacterium in centrifuged sediment elutriates.

METHODS

The research site was Hamilton Harbor (43° 17'N, 79° 50'W), a heavily industrialized harbor at the extreme western end of Lake Ontario [12,13]. The harbor receives the wastes of 500,000 people and many factories, including Canada's two largest steel mills. Some of the harbor sediments are heavily contaminated with lead (320±200 μg/g), and zinc (3110±2000) [12]. Approximately 20 hectares of sediment contain over 200 μg/g of polyaromatic nuclear hydrocarbons (PAH's) and another 200 hectares contain over 50 μg/g of PAH's. The sediments can be either aerobic or anaerobic and they vary from being primarily sand to organic muds.

All 48 samples were collected with a Mini-Ponar dredge. The locations of sample stations were determined with a Mini-Ranger positioning system. To determine the relative toxicity of the samples, a sediment sample (30 L) from the northwest portion of the harbor (station 46, Figure 1) was used as a control in each screening test. The control station is located four and nine km, respectively, from the industrial and municipal discharges, and receives sediment from Grindstone Creek and Cootes Paradise (areas with low population density, either residential or agricultural [12,13]). The control sample was kept in a 4°C walk-in incubator

for the duration of the experiments (30 d) in a covered polyethylene container. The control sample remained oxidized for over six months. Other samples were analyzed within a week of collection and were maintained at 4°C between analyses.

The Photobacterium culture used in the sediment-contact test was prepared by adding a vial of the freeze-dried bacteria as used in the Microtox test, to 50 mL of sterilized culture medium. It was prepared by adding 66 g of Difco Photobacterium broth to 1 L of distilled water heated to boiling, subdivided and autoclaved. To maintain a fresh culture, 1-2 mL of the most recently inoculated culture was aseptically transferred the day before testing to 50 mL of freshly sterilized culture medium and shaken for 18 h. A maximum time of one week was allowed between inoculations. An active culture suitable for use in the tests was luminescent to the unaided eye in a dark room. If it did not glow, it was either shaken longer or more of the culture was used in the test.

The quantity of culture required to give sufficient light emission was determined for each set of analyses by adding 10 μL of the culture to 1.0 mL of 2.0% NaCl and measuring the light emission using a Beckman 2055 Microtox apparatus. If the luminescence was not sufficiently high to allow a reading of 100, more culture was added. The quantity of Photobacterium culture used in the actual tests was usually 50 μL.

For the screening tests, 0.25 mL of the sediment (control and up to five samples) was transferred into each of two centrifuge tubes for every sample; 4.75 mL distilled water was added followed

by 0.5 mL 22% NaCl (to bring the salt concentration to 2%). Each sediment sample, including the control, was analyzed in duplicate and the results averaged. The sediment was measured by volume, as this was the most convenient method of transferring the sediment into the tubes. After mixing well on a vortex mixer, 50 µL (or as needed) of the Photobacterium culture were added to the sediment with an Eppendorf digital pipette, the contents were again mixed using the vortex mixer, and the mixtures allowed to stand for 15 minutes. After centrifuging for 10 minutes at 700 g (setting 3 on the IEC clinical centrifuge using a 12 tube angled head), 1.0 mL of the supernatant from each tube was transferred to the Microtox cuvettes using an Eppendorf pipette.

The light emission from the Photobacterium was measured using the Microtox apparatus set at room temperature. A set of six samples can be analyzed in 30 minutes. The average light output from the duplicate samples was calculated as a percentage of the control. A high percentage would indicate low toxicity (relative to the control) and a low percentage would indicate high toxicity. The same control station (46) was used for the elutriates.

A ^{14}C-labelled Photobacterium culture was used as an internal standard to determine the proportion of bacteria removed by centrifugation. Sediments from 15 of the 48 stations were studied with a ^{14}C-tracer. The culture was prepared by adding 0.5 µCi of ^{14}C-labelled sodium acetate per mL of culture medium and inoculating it with approximately 20 µL/mL of the Photobacterium culture. After shaking at 200 rpm for 18 h, the bacteria were centrifuged

at 1400 g (setting 6 on an IEC clinical centrifuge using a 12 tube angled head) for 15 minutes. The cells were resuspended in fresh culture medium and shaken at 200 rpm for an additional two hours prior to use. This ^{14}C-labelled Photobacterium culture was then used in the toxicity tests. After the light emission of a sample was measured, it was added to 10.0 mL of scintillation solution (ACS II) to enable measurement of the radioactivity. The ^{14}C-activity in each sample was measured using a United Technologies series 4000 liquid scintillation counter.

For the standard Microtox tests, aqueous elutriates were obtained by centrifuging the sediment directly at 5875 g for 20 minutes with a Sorvall GSA rotor. The Microtox tests were conducted following normal procedures [7-9], except that dilution studies were not routinely performed, since in most cases the decrease in bacterial activity was very slight. To determine if the toxicity of sediment elutriates was associated with colloids, some elutriates were pretreated prior to Microtox analysis by filtration through Nuclepore membrane filters with a range of pore sizes.

The direct-contact bioassay with Photobacterium may be capable of measuring hydrophobic contaminants better than an elutriate bioassay with the same bacterium. To test this hypothesis these two procedures were used on the control sediment that was spiked with either zinc chloride or polychlorinated biphenyl congener 194 (PCB-194). The concentrations of Zn and PCB used were 6.12 mg/g and 0.12 mg/g respectively. The sediment samples were diluted with

unspiked control sediment so that the final concentrations of spiked sediment were 0, 20, 40, 60, 80 or 100%.

RESULTS

The results of testing the Photobacterium using the sediment-contact method for 48 different sites in Hamilton Harbor are summarized in Table 1. The toxicity of Hamilton Harbor sediments was characterized using the sediment-contact Photobacterium method and the following scale :

Range of Activity	Toxicity
<1%	severe
1 - 20%	high
20 - 40%	intermediate
40 - 80%	low
>80%	very low

In general, the most toxic sites are found along the southern shore near municipal storm water and industrial discharge pipes (Figure 2). Sediments from the western and northern parts of the harbor have a relatively low toxicity.

The ^{14}C-labelled Photobacterium was used as an internal standard to determine if the differences in light emission from the Photobacterium exposed to the sediment were caused by the bacteria being spun down with the sediment particles at different rates during the centrifugation step. Although approximately two-thirds of the ^{14}C-bacteria were removed by the centrifugation, the

percentage of bacteria remaining in suspension was acceptably constant (mean and coefficient of variation were 31±4.3%, n=23, Table 2). The coefficient of variation for ^{14}C-activity remaining in suspension after centrifugation of bacteria and sediment was approximately 14%.

The variation in bacterial concentration in the supernatant is not enough to account for the wide differences observed in the light emission from the Photobacterium after contact with the sediments (Table 1). With replicated analyses the worst possible interpretation would be to misclassify the toxicity by one class, i.e., intermediate toxicity for low toxicity. The bioassay is strongest when used to map relative toxicity or to screen for severely toxic samples. In eight of the nine severely toxic samples, the samples were still severely toxic after a 50% dilution with uncontaminated sediment.

The variation in sample toxicity between duplicate analyses is also greater than the variation in the number of bacteria removed by centrifugation. In 23 of 28 samples with photoactivity processed with ^{14}C, the coefficient of variation of photoactivity was at least twice as high as the coefficient of variation for the centrifugation of bacteria. The variation in the distribution of toxics appears to be greater than the variation in handling the bacteria.

Absorption due to color in the media was less significant with the sediment-contact assay than with sediment elutriates. The amount of colour varied greatly. In general, the color correction

in the sediment-contact assay was insignificant. The greatest correction was 16%. The elutriates required as much as a 33% correction for color.

Filtration of sediment elutriates through different pore size filters reduced the toxicity of sediment elutriates relative to an elutriate sample prepared with low speed centrifugation (Table 3). The variations in toxicity indicated that most of the toxic materials were bound to sediment particles greater than 8 μm that were not removed by centrifugation.

Sediments from the control site that were spiked with PCB-194 were more toxic when analyzed with the direct contact Photobacterium bioassay than with an elutriate bioassay with the same bacterium (Figure 3). By comparison, sediments that were spiked with zinc chloride were equally toxic in both bioassays (Figure 3).

DISCUSSION

The greatest advantage of the sediment-contact Photobacterium method is that the toxicity of the entire sediment is measured. Very little of many hydrophobic toxins such as PCBs and polyaromatic hydrocarbons is extracted when a sediment elutriate is prepared [14]. Hamilton Harbor sediments contain organic and inorganic contaminants, some of which are soluble and some of which are insoluble in water. The spatial distribution and reactivity of these contaminants is poorly resolved; thus, the direct-contact bioassay is more appropriate for these than an elutriate bioassay.

To use the sediment-contact Photobacterium method at a new site may require some modifications of sample preparation. For Hamilton Harbor sediments, a volume of 0.25 mL was optimal. A greater volume often resulted in a colored supernatant, which would require correcting for colour absorption. Using less sediment decreased the toxicity effect and increased the measuring error. Different centrifugation speeds and times were also tried; lower speeds left a turbid suspension with too much sediment and higher speeds removed too many bacteria. Longer incubations of 30 and 45 minutes were also tried but longer incubations produced similar responses with no advantage over the shortest incubation.

Researchers at other research sites must be cautious in selecting a relatively constant reference sample. The Hamilton Harbor reference sample remained non-toxic to Photobacterium, Hexagenia and Daphnia magna for a year. The pH, Eh, and color of this sediment also remained constant. The toxicity and geochemistry of contaminated sediments in Hamilton Harbor changes with extended storage (>week). One strength of this assay is its ability to quickly screen toxicity and the samples should be processed quickly.

Sediments from a new environment should be calibrated using ^{14}C-labelled bacteria or another internal standard, i.e., counting culture plates or stained bacteria. Use of an internal standard will ensure that the toxic effects being measured are due to chemicals in the sample and are not a result of varying numbers of Photobacterium in the supernatant.

The spatial variability of the sediment toxicity in Hamilton Harbor as determined by the sediment-contact Photobacterium method is consistent with earlier bioassay studies [11, 12]. However, these studies could only determine the toxicity of three or four sites. Most bioassays with whole sediment used Chironomus or Pontoporeia, and are time consuming and unsuitable for either a screening test or resolution of the spatial variation in toxicity. The sediment-contact Photobacterium method is a good tool to quickly screen for toxic sediments in Hamilton Harbor and it should be useful in other sites.

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Table 1. Effect of Sediments on Activity of *Photobacterium*

Site	(% *Photobacterium* activity relative to control) mean (n=3)	coefficient of variation
1	90	36
2	42	44
3	3	93
4	4.4	31
5	91	2
6	3	9
7	29	99
8	18	85
9	0.2	100
10	57	23
11	9.6	5
12	12	35
13	190	45
14	8.3	93
15	90	36
16	11	101
17	0	0
18	3.1	67
19	12	94
20	9.2	42
21	0.6	-
22	0.6	100
23	0	0
24	115	48
25	19	43
26	0	0
27	0	0
28	4.8	37
29	1.8	65
30	0.1	80
31	1.4	21
32	372	10
33	1.8	65
34	0.2	95
35	35	67
36	88	35
37	4.6	52
38	151	0
39	64	1
40	31	96
41	0.5	20
42	64	56
43	96	14
44	97	38
45	82	38
46	100	0
47	122	20
48	102	5

Table 2. *Photobacterium* relative luminescence and radioactivity in supernatant

Run	Sample	% Activity[1]	$\%^{14}C$
1	10 μL culture		100
	water	646	96
	27	0.2	36
	30	44	35
	35	46	28
	42	101	37
	46	100	39
2	3	28	24
	27	0.9	29
	30	11	28
	35	60	25
	42	104	32
	46	100	32
3	4	59	29
	9	35	31
	19	93	32
	26	0.5	31
	44	98	32
	46	100	33
4	22	1.4	27
	29	21	28
	33	12	25
	36	149	41
	40	69	31
	46	100	29

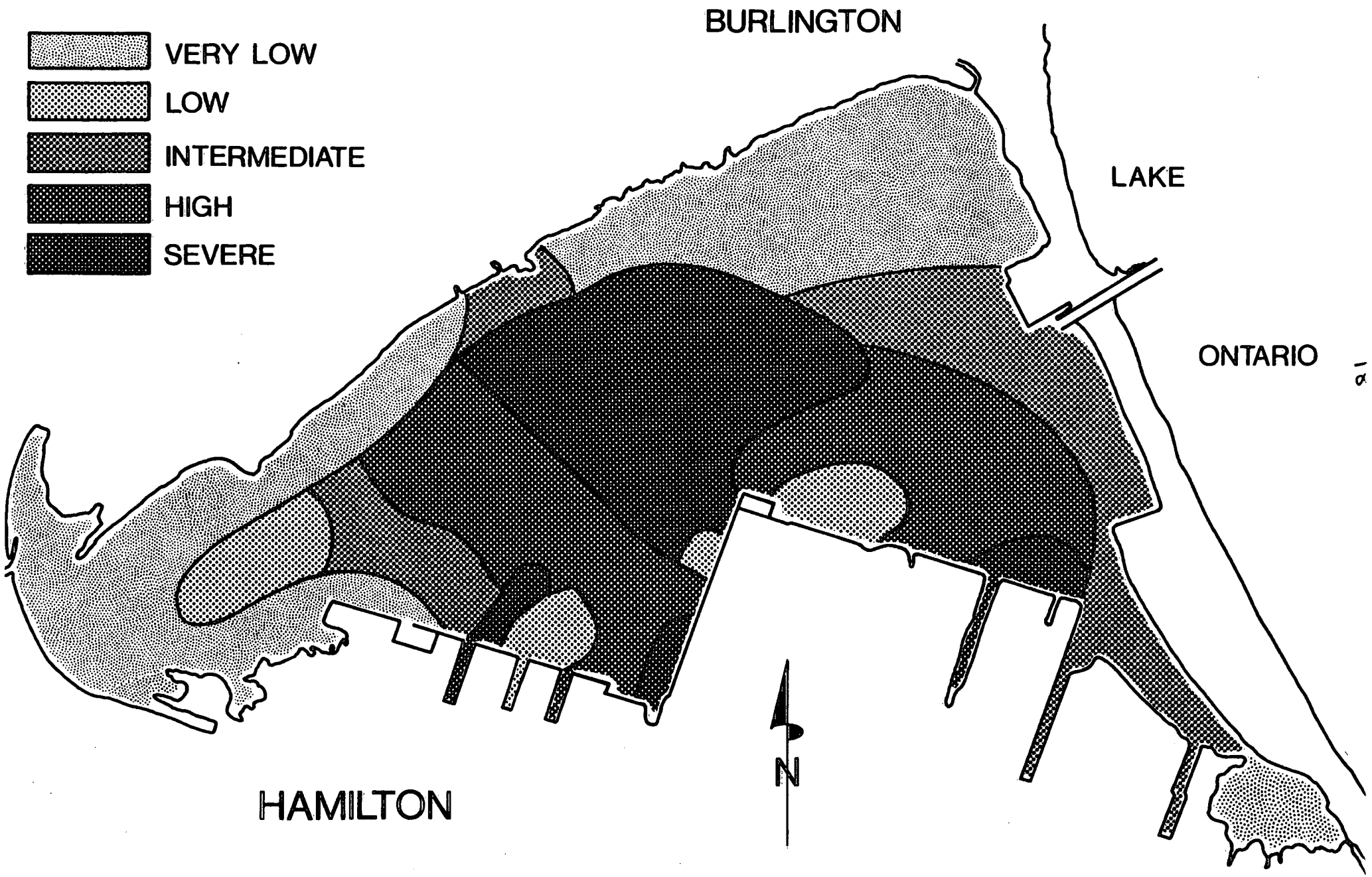
[1] relative to site 46

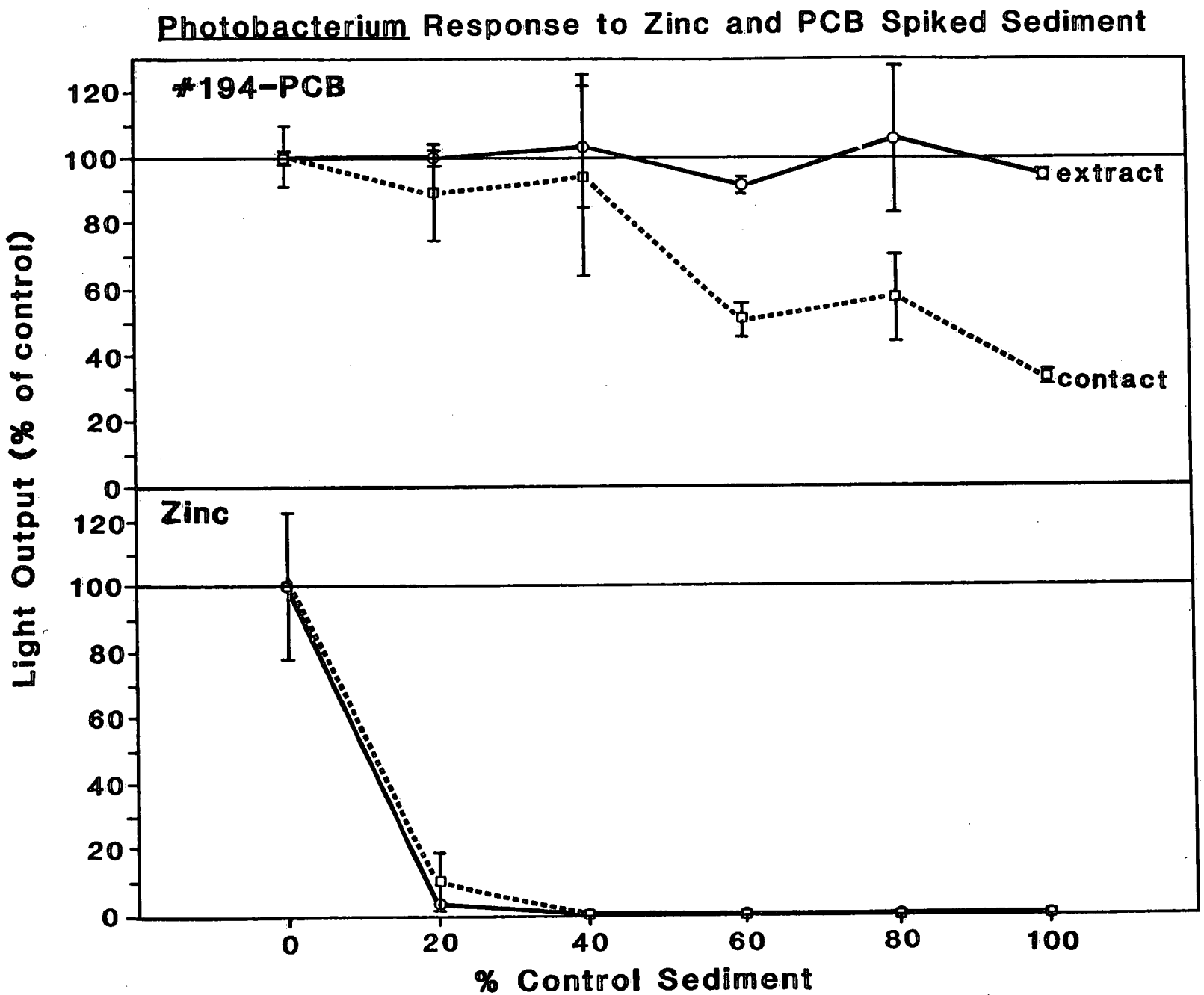
Table 3. Microtox tests on sediment elutriates obtained by different methods

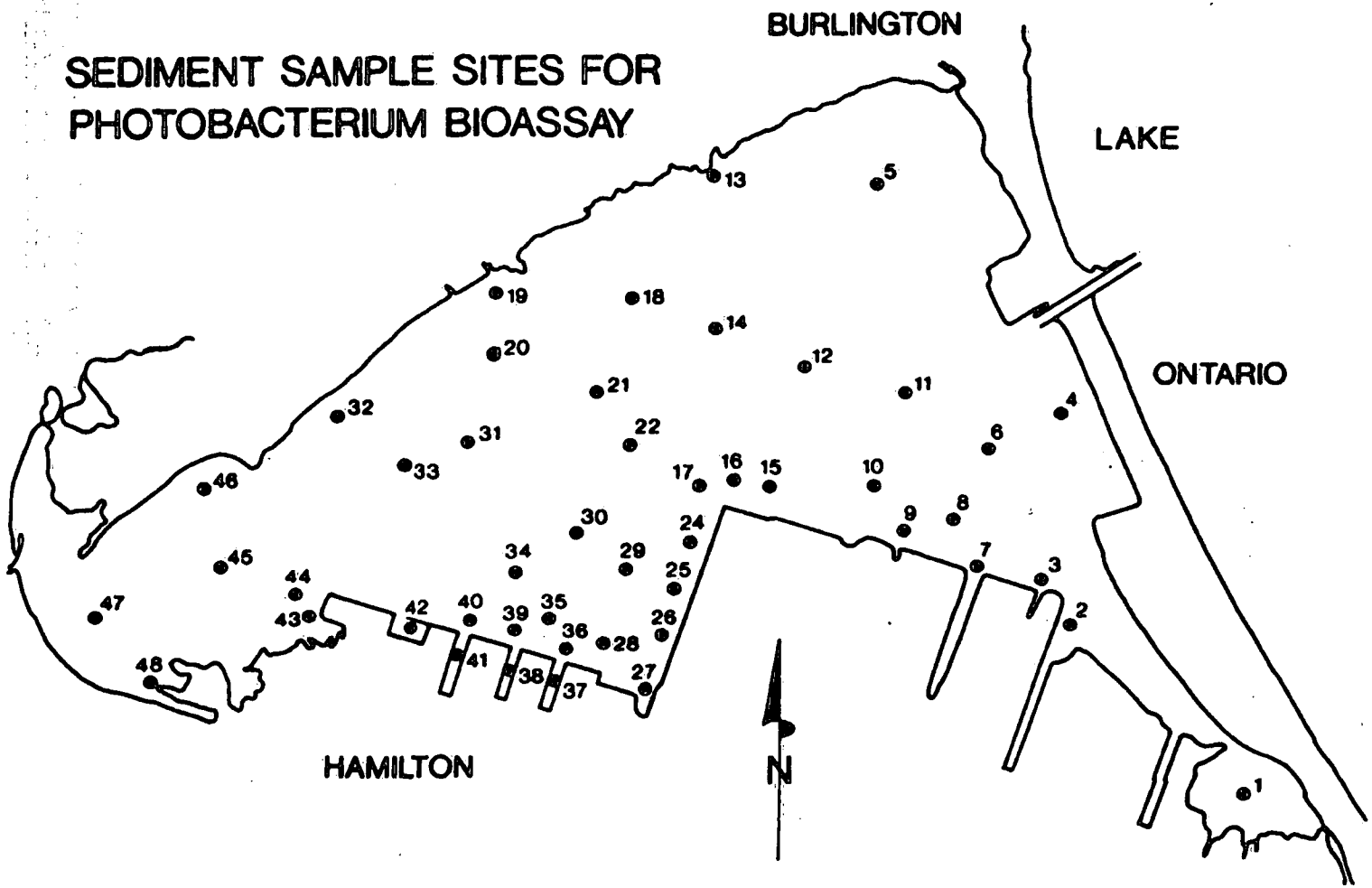
Treatment	Γ1
8 μm filter[2]	0.54
3 μm filter	0.18
1 μm filter	0.05
0.4 μm filter	0.16
0.1 μm filter	0.07
centrifuged[3]	9.24

[1] Γ is the ratio of light emitted to the light remaining. A value of Γ=1 represents a sample in which 50% of the light output was suppressed; higher values indicate greater suppression and hence greater toxicity. References 7-8 discussion Γ in more detail.
[2] filtered under low pressure -50 Kpa.
[3] centrifuged at approximately 1000 rpm (700 g).

TOXICITY OF HARBOR SEDIMENTS TO PHOTOBACTERIUM




Photobacterium Response to Zinc and PCB Spiked Sediment
#194-PCB
extract
contact
Zinc
Light Output (% of control)
120
100
80
60
40
20
0
0
20
40
60
80
100
% Control Sediment


SEDIMENT SAMPLE SITES FOR
PHOTOBACTERIUM BIOASSAY
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LAKE
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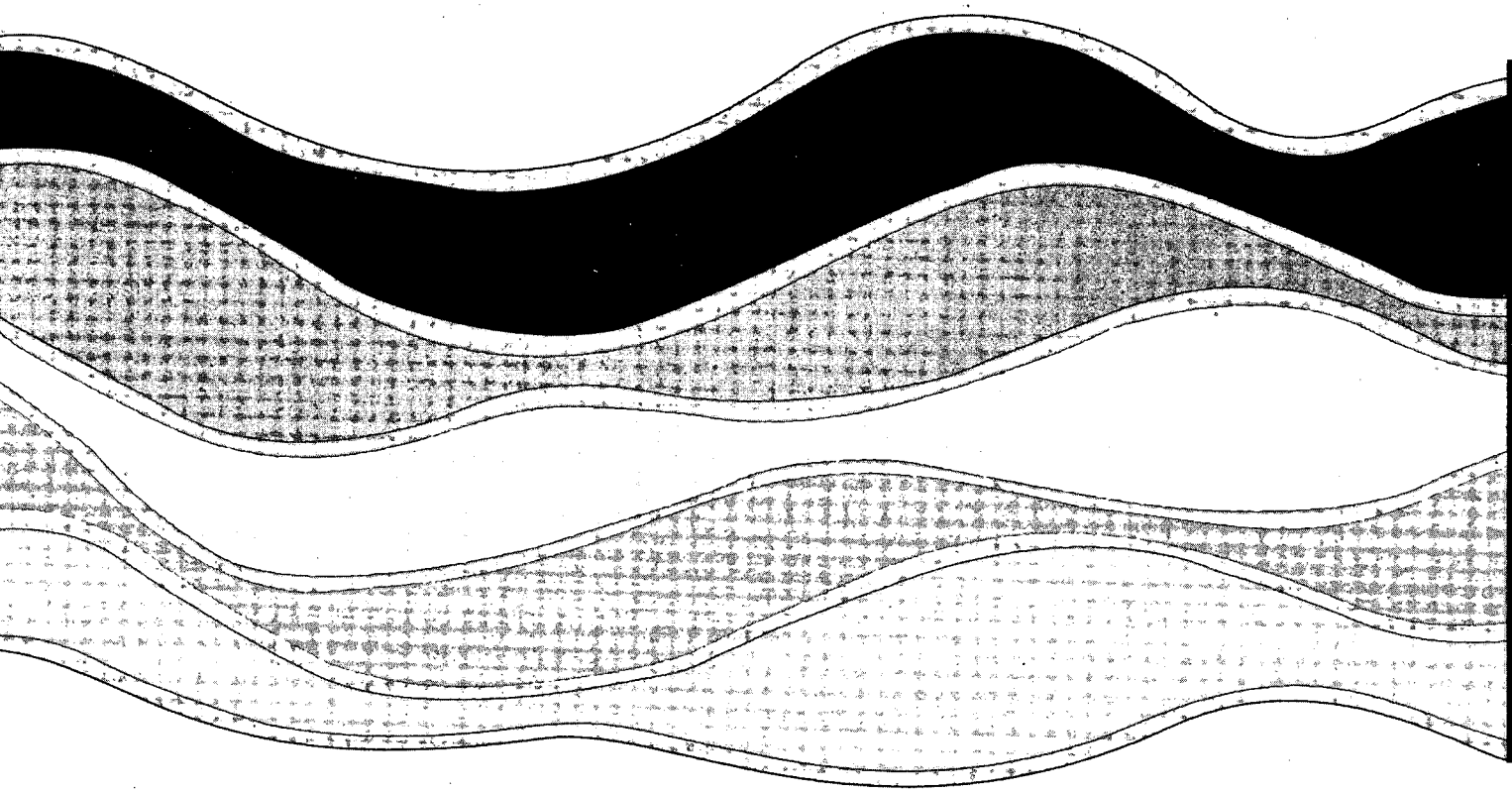

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