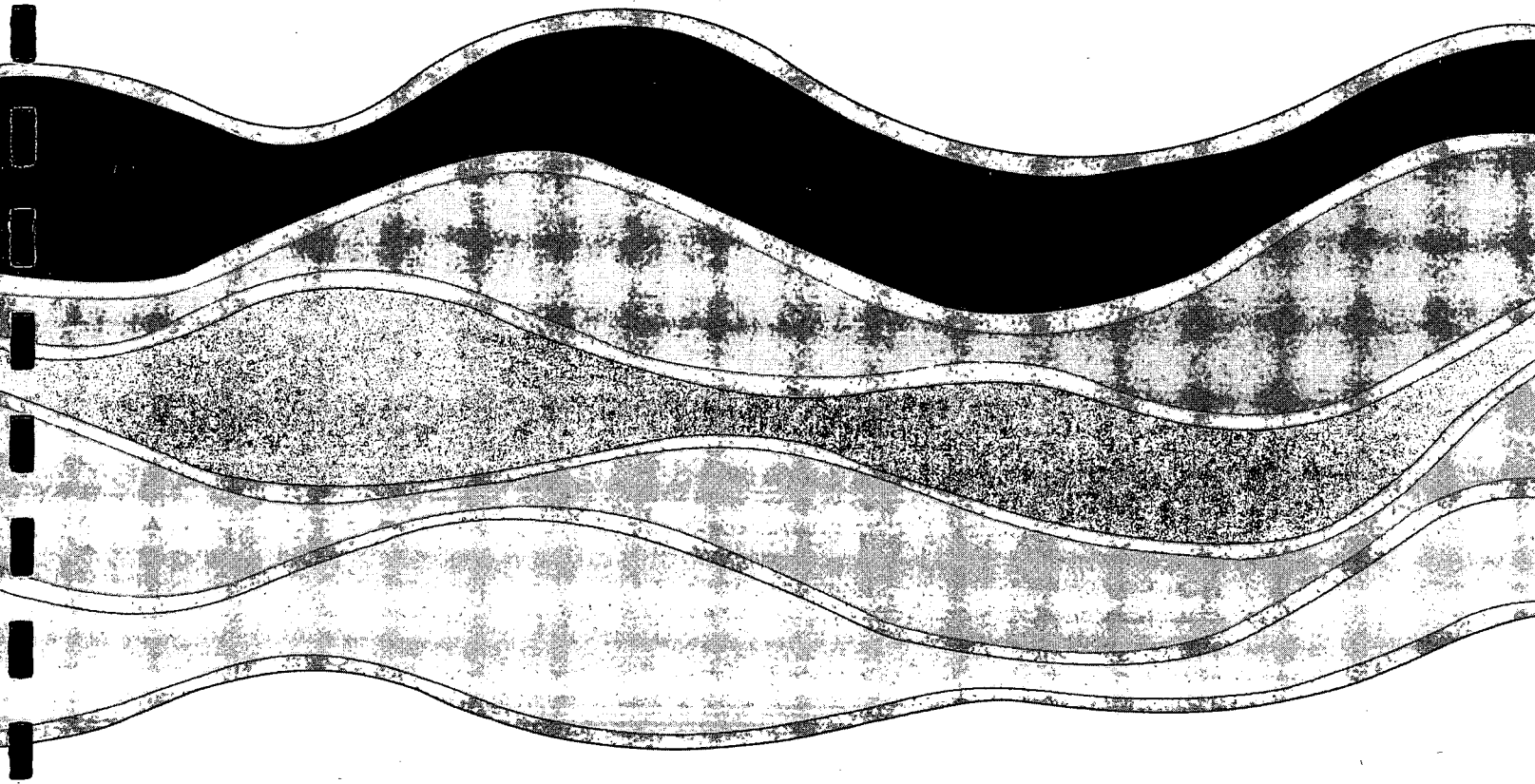

DIRECT SEDIMENT TOXICITY TESTING PROCEDURE (DSTTP)

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NWRI Contribution No. 91-90

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MANAGEMENT PERSPECTIVE

Toxicity screening of sediments, suspended sediments, soils and solid wastes is becoming increasingly important in environmental studies. To date toxicity testing of sediments, suspended sediments, soils and solid wastes has almost always been performed on exudates, water and/or solvent extracts. These procedures are often complicated, labour-intensive and expensive. The results are often questionable as to whether they are truly representatives of the real or available chemicals in the samples. This report describes a simple, quick and inexpensive qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) which can be used to test solid phase samples without changing the original identities of the samples. This DSTTP does not require special instrumentation and can be easily used under field conditions.

SOMMAIRE À L'INTENTION DE LA DIRECTION

La recherche de produits toxiques dans les sédiments, les sédiments en suspension, les sols et les déchets solides est de plus en plus importante dans les études environnementales. Jusqu'à maintenant, cette recherche a presque toujours été effectuée sur des exsudats, des extraits dans l'eau et (ou) dans des solvants. Les méthodes sont souvent compliquées, longues et coûteuses. On se demande souvent si les résultats sont réellement représentatifs des produits chimiques vraiment présents ou disponibles dans les échantillons. Le présent rapport décrit une méthode qualitative et semi-quantitative simple, rapide, directe et peu coûteuse de recherche de produits toxiques dans les sédiments qui peut être utilisée pour analyser des échantillons solides sans modifier leurs caractères originaux. Cette méthode n'exige pas d'appareils spéciaux et elle peut être utilisée facilement sur le terrain.

ABSTRACT

A qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) using the Toxi-Chromotest kit was developed. This DSTTP has advantages over many other toxicity bioassays in that it is simple, quick, and inexpensive. It does not require any instrumentation and can be easily applied under field conditions. The DSTTP measures the available toxicants of the test sediments, suspended sediments, soil and any other solid wastes without altering the original characteristics of the samples as occurs in extraction and concentration procedures.

RÉSUMÉ

Une méthode qualitative et semi-quantitative directe de recherche de produits toxiques dans des sédiments, au moyen de la trousse Toxi-Chromotest, a été mise au point. Cette méthode présente plusieurs avantages par rapport à de nombreuses autres méthodes biologiques de mesure de la toxicité : elle est simple, rapide et peu coûteuse. Elle ne requiert aucun appareil et elle peut être facilement appliquée sur le terrain. Cette méthode mesure les produits toxiques disponibles dans les sédiments à l'étude, les sédiments en suspension, les sols et dans tout autre déchet solide sans modifier les propriétés caractéristiques des échantillons tels qu'ils sont obtenus par des méthodes d'extraction et de concentration.

INTRODUCTION

Aquatic toxicity tests using microorganisms to detect toxic contaminants in the environment have been carried out for many years. Microorganisms are used as indicators for the presence of toxicants due to their simplicity, speed, sensitivity, replaceability and relatively low cost (Bauer *et al.*,1981; Blaise *et al.*,1986; Kwan and Dutka,1991; Kwan *et al.*,1990; Kwan,1989). Over the last 10 - 15 years toxicity screening tests have been extended to test sediments, suspended sediments, soils and solid wastes. In the routine toxicity screening of sediments, suspended sediments, soils and solid wastes using variety of bioassay tests, it is often difficult to detect the presence of toxicants due to their low concentrations. To compensate for these low levels of toxicants extraction and concentration procedures are routinely used (Atkinson *et al.* 1985; Schiewe *et al.* 1985; Kwan and Dutka, 1988; Brouwer *et al.*, 1990; Kwan and Dutka, 1991). Milli-Q water, organic solvents and/or combinations of organic solvents are routinely used in laboratories to extract solid phase samples. However, it is often questioned as to what degree the samples have been changed during extraction processes. Therefore, success of detecting the true toxicity in solid phase samples is still very limited.

To circumvent these problems, a qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) was developed by adapting a bacterial bioassay, Toxi-Chromotest (Orgenics Ltd.,1985, 1990), to screen toxicants in sediments, suspended sediments, soil and sludges. This qualitative/semi-quantitative direct sediment toxicity testing procedure is simple, inexpensive, does not require instrumentation and can easily be used under field conditions.

The Toxi-Chromotest which utilizes a 96-well microplate (Fish *et al.*, 1985; Reinhartz *et al.*, 1985; Orgenics 1985, 1990) has been developed and marketed by Orgenics Ltd. (Israel) for the detection of toxic activities in chemicals, pharmaceuticals, food stuffs, food additives and cosmetics. This test was later applied to environmental sediment samples (Xu *et al.*, 1987; Kwan and Dutka, 1990; Kwan and Dutka, 1991). Using the Toxi-Chromotest technique a qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) has been developed.

In this report the procedure will be described and data obtained by this direct sediment toxicity testing procedure will be presented. Data from the DSTTP will be compared to those obtained from various extraction procedures and tested by the 96-well microplate Toxi-Chromotest procedure.

METHODS AND MATERIALS

Sample Collection

Eight sediment samples were collected from Hamilton Harbour (Lake Ontario). This is a heavily industrialized harbour which constantly receives organic and inorganic contaminants from surrounding industries, including Canada's two largest steel companies. Four sediment samples were also collected from the lower Athabasca River in northeastern Alberta. This area contains an extensive oil sands deposit. The sediment samples were collected with an Ekman dredge and were placed into individual sterile plastic bags, iced and returned to the laboratory for toxicity screening tests.

Sediment Extraction Procedures

Sediment extraction procedures described by Kwan and Dutka (1990) were used to extract sediment samples. Sediment sample (wt) and Milli-Q water (v) at 1:1 ratio were shaken by

hand in a sterile centrifuge tube for 2 minutes. The mixture was then centrifuged at 10,000 rpm for 20 minutes. After centrifugation the supernatant was used for toxicity screening tests. This process was also repeated with the organic solvents, 10% methanol and 10% dimethyl sulfoxide (DMSO). Organic solvent extracts were diluted in Milli-Q water to obtain a final testing concentration of 1%.

Direct Sediment Toxicity Testing Procedure (DSTTP)

(1) Set up a series of disposable test tubes (15.5 X 5.6 mm) and label from 1 to 7.

(2) Prepare a bacterial suspension by mixing one vial of lyophilized Escherichia coli (Orgenics Toxi-Chromotest kit) with 10 mL filter sterilized LB medium (Bacto tritone 10g; Bacto yeast extract 1g; sodium chloride 10g; distilled water 1L).

(3) Incubate the bacterial suspension at room temperature for 20 minutes before transferring 0.7 mL into 9.3 mL of filter sterilized Reaction Mixture (sodium chloride 12g; potassium chloride 3.7g; sodium dihydrogen orthophosphate-monobasic 2.8g; Bacto tritone 3g; Bacto yeast extract 1.5g; Isopropyl ß-D-thiogalactopyranoside 0.14g; distilled water 1L) with pH adjusted to 7.5.

(4) Incubate the bacteria reaction mixture at room temperature for 20 minutes before dispensing 1.0 mL of the bacteria reaction mixture into the first tube, and 0.5 mL into the remaining tubes (2-6).

(5) Weigh out 0.5 gm of wet sediment sample and place it into the first tube and mix thoroughly with a vortex mixer for 10 seconds.

(6) Transfer 0.5 mL of the bacteria-sediment mixture into the 2nd tube, 2nd to 3rd, 3rd to 4th till the 6th tube.

(7) After mixing tube 6, 0.5 mL of mixture is discarded.

(8) Before each transfer the bacteria-sediment mixture is vortexed for 5 seconds.

(9) Place 0.5 mL of bacterial suspension into the 7th tube as a negative control.

(10) The tubes are then incubated for 2 hours at 35°C.

(11) While the tubes are incubating, place a Whatman GF/F glass microfibre filter (42.5 mm) into each numbered petri dishes (50 X 9 mm) corresponding to the tube numbers.

(12) Thirty minutes before the two hour incubation time prepare the yellow chromogenic substrate by mixing a bottle of yellow chromogen with a bottle of yellow chromogen diluent (Orgenics Toxi-Chromotest kit).

(13) Pipet 0.75 mL of yellow chromogenic substrate onto each Whatman GF/F glass microfibre filter and replace the petri dish lid to prevent drying and leave at room temperature. Each glass microfibre filter can hold a maximum of four samples or one sample plus three dilutions (Figure 1).

(14) After the 2 hour incubation, vortex each tube for 5 seconds before transferring 20 µL from each tube onto the glass microfibre filter which was previously soaked with yellow chromogenic substrate.

(15) Cover the petri dishes and incubate the glass microfibre filters with samples, sample dilutions and control at 35°C for 30 minutes. (16) After the 30 minute incubation, check each transfer spot for a yellow colour development .

(17) If the sample is toxic, no yellow colour will develop and if the sample is non-toxic, a yellow colour develop around and under the sample (Figure 1).

Interpretations of colour reactions

There are four categories of colour reactions: (a) no yellow colour development, high toxicity level, (-); (b) less than 50% of yellow colour intensity as compared to the control, moderate response, (+); (c) less than 100% but greater than 50% of colour intensity as compared to the control, low response, (++); and (d) yellow colour intensity is equivalent to the control, non toxic, (+++). The more yellow colour developed the less toxic the sample.

Sensitivity of Direct Sediment Toxicity Testing Procedure

To determine the sensitivity of the DSTTP, 20 gm aliquotes of sediment from sample AR-9 were spiked with standard inorganic and organic toxicants (Table 3) and were tested as above.

RESULTS AND DISCUSSION

Table 1 presents data obtained from the direct sediment toxicity testing procedure (DSTTP) and the 96-well microplate Toxi-chromotest procedure using whole sediments and sediment extracts respectively. Data obtained from the extracts are expressed as percentage inhibition of ß-galactosidase production. Data obtained from the DSTTP are expressed as degree of yellow colour intensity ranging from no colour, high

toxicity level (-) to intense yellow colour indicating a completely non-toxic response (+++). From this table it can be seen that the twelve sediment samples tested under the direct sediment toxicity testing procedure produced toxic responses, seven with high toxicity (-) and five with low toxicity (+). However, when the Milli-Q water and the combination of 10% methanol and 10% dimethyl sulfoxide extracts of these samples were tested using the 96 well microplate Toxi-Chromotest procedure they all produced non-toxic responses. These non-toxic responses could have been due to a number of reasons: 1. Milli-Q water extracts solely water soluble toxicant(s) and the toxicant(s) may not have been water soluble; 2. the concentration of water soluble toxicant(s) (if present) could have been in very low concentrations which were below the detection limit of the test; 3. due to the toxic nature of the organic solvents themselves, the solvents must be diluted to their Maximum Allowable Concentration (MAC) (Kwan and Dutka, 1990) which could have diluted the toxicant(s) below the detection level of the test; and 4. the solvent strength or mixture was inappropriate to the toxicant(s) in the sediments. No ß-galactosidase inhibition was observed in the negative control (tube 7), to which the positive and negative responses were compared.

Table 2 presents the minimum detectable concentrations of several toxicants, Hg^{++}, Cd^{++}, Pb^{++}, Al^{+++}, Zn^{++}, Cu^{++}, Ni^{++}, Phenol, 2,4 Dichlorophenol, Aldrin, Dieldrin and N,N,N,'N'-tetraethyl-p-phenylenediamine dihydrochloride, using the DSTTP. Concentrations are expressed in mg per litre. It is surprising to see that the DSTTP was sufficiently sensitive to detect a Hg^{++} level as low as 8.5 ppt. Reinhartz *et al.*, 1987 using the 96-well microplate Toxi-Chromotest procedure obtained a minimum inhibitory concentration for Hg^{++} of 0.12 ppm which is 70,000X less sensitive than the DSTTP. Table 3 presents the minimum detectable concentrations and minimum

inhibitory concentrations of some of toxicants tested using the DSTTP and the 96-well microplate Toxi-Chromotest procedure respectively. From Table 3 it can be seen that the DSTTP is 80 to 70,000 times more sensitive in detecting toxic presence than the 96-well microplate Toxi-Chromotest procedure with all toxicants tested. Although the extreme sensitivity of the DSTTP may have been due to or partially due to the synergistic and/or additive effects between the spiked toxicant and the toxicant(s) present in the sample, the results demonstrates that the DSTTP is a more sensitive procedure. From Table 2 and 3 it appears that some metals are detected at lower toxic concentrations than some organics in the DSTTP.

The DSTTP is cost effective since solvent extraction procedures are not required. The cost of specific or detailed organic solvent extraction can sometimes run into thousands of dollars and thus can cost 10 to 100 times more than the bioassay test itself. Based on the reagents (lyophilized bacteria and chromogenic substrate) used in this procedure, which can be purchased from the Orgenics Ltd. (Isreal), the total cost for analyzing one sample with four dilutions is approximately $10 (Canadian). The DSTTP is sensitive and representative as well.

In summary, the qualitative/semi-quantitative direct sediment toxicity testing procedure (DSTTP) has definite advantages over many other toxicity procedures. It is simple, quick, and inexpensive, and the results can be obtained in 2-3 hours. The sample size required for the test is small (0.5 - 1.0 gm) as compare to the larger quantity used during extraction procedures. The test does not require the use of any instruments and can be easily used in the field. The DSTTP can detect the toxic responses of soluble and insoluble, organic and inorganic, volatile and non-volatile contaminants in tested samples.

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TABLE 1: Toxicity data obtained from sediments using the 96-well microplate Toxi-Chromotest procedure and DSTTP. Data are expressed as percentage of inhibition of ß-galactosidase production.

	EXTRACTS[1]		
SAMPLE #	**Milli-Q H2O**	**10% MeOH+10%DMSO**	**DSTTP**
Negative Control	0%	0%	NON-TOXIC(+++)
HH-1	NEG	NEG	MODERATE (+)
HH-2	NEG	NEG	HIGH (-)
HH-3	NEG	NEG	HIGH (-)
HH-4	NEG	NEG	HIGH (-)
HH-5	NEG	NEG	HIGH (-)
HH-6	NEG	NEG	HIGH (-)
HH-7	NEG	NEG	HIGH (-)
HH-8	NEG	NEG	MODERATE (+)
AR-9	NEG	NEG	MODERATE (+)
AR-10	NEG	NEG	HIGH (-)
AR-11	NEG	NEG	MODERATE (+)
AR-12	NEG	NEG	MODERATE (+)

[1] All results are based on 3 replicates

HH Hamilton Harbour

AR Athabasca River

TABLE 2: Concentrations of toxicants giving 100% inhibition of β-galactosidase production in DSTTP

Toxicant	Concentration
Al^{+++}	0.05 ppm
Cd^{++}	0.02 ppm
Pb^{++}	0.18 ppm
Hg^{++}	8.50 ppt
Zn^{++}	0.02 ppm
Cu^{++}	0.09 ppm
Ni^{++}	0.18 ppm
Phenol	6.06 ppm
2,4-D	>6.06 ppm
TPDD	6.06 ppm
Aldrin	1.51 ppm
Dieldrin	0.18 ppm

Table 3: Minimum detectable concentrations of toxicants in DSTTP and 96-well microplate Toxi-Chromotest procedure.

Toxicant	DSTTP	96-well*
Hg^{++}	8.50ppt	0.12ppm
Cd^{++}	0.02ppm	15.00ppm
Zn^{++}	0.02ppm	25.00ppm
Aldrin	1.52ppm	300.00ppm
Dieldrin	0.18ppm	15.00ppm

*data obtained from Reinhartz *et al.* 1987

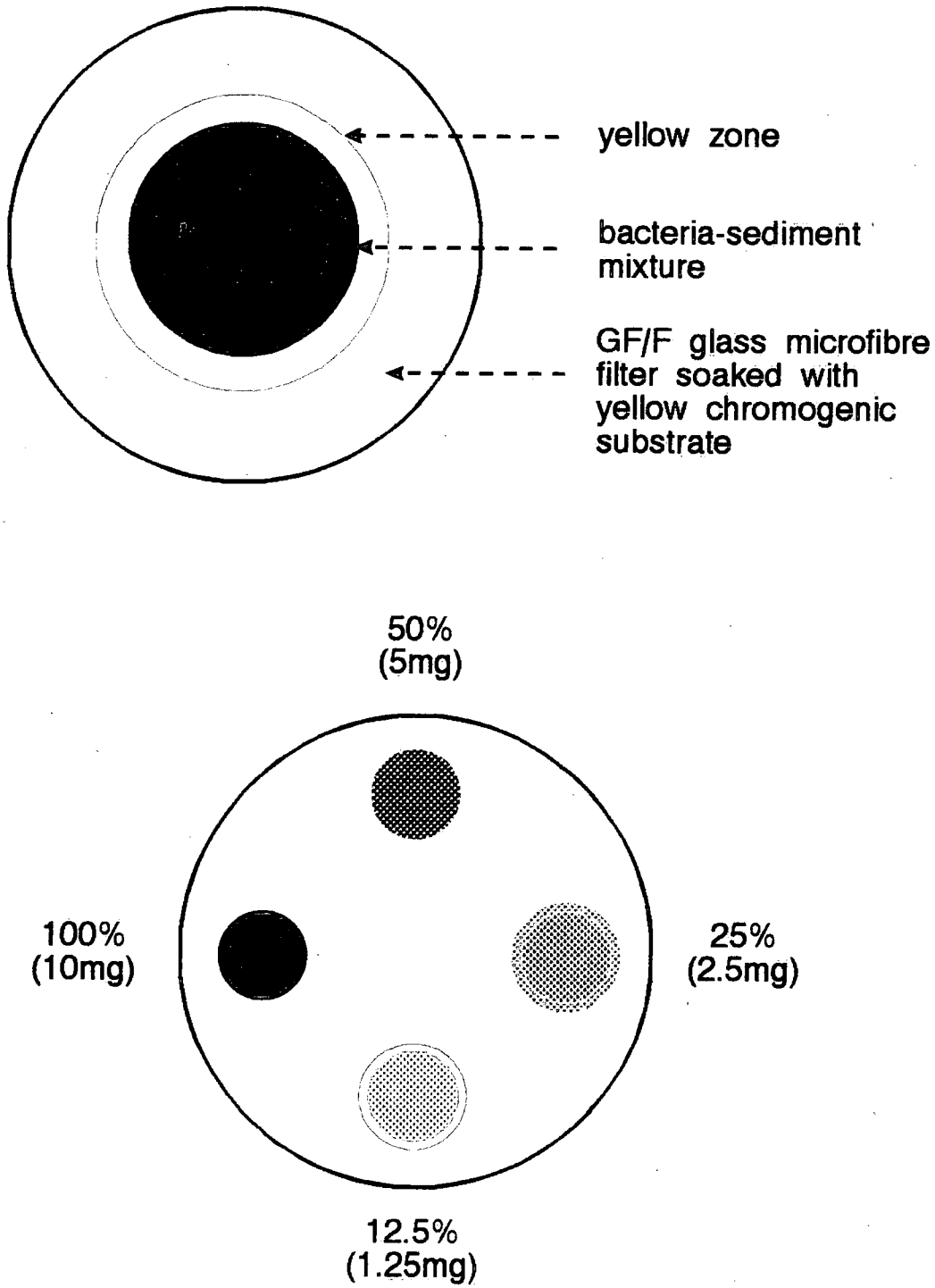
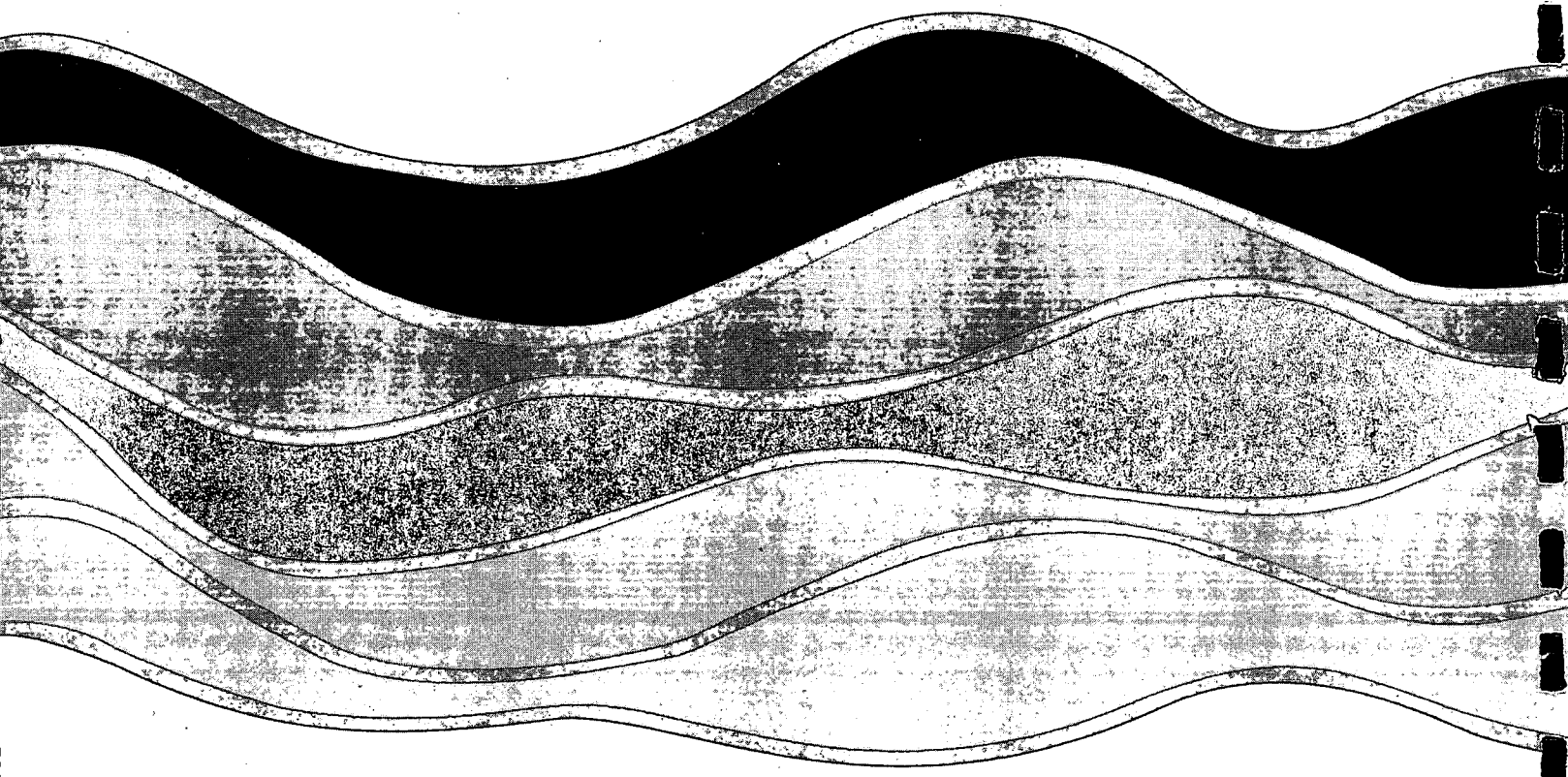


Figure 1. Colour development of sample concentrations (100% to 12.5%) and sample arrangement in DSTTP



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