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Analysis of Ottawa River Suspended Sediments and Related Water Fractions for Toxicity, Fecal Sterols and Various Microbiological Parameters

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ANALYSIS OF OTTAWA RIVER SUSPENDED SEDIMENTS AND RELATED WATER FRACTIONS FOR TOXICITY, FECAL STEROLS AND VARIOUS MICROBIOLOGICAL PARAMETERS

by
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EXECUTIVE SUMMARY

The results of an exploratory study to evaluate the importance of suspended sediment as a carrier of toxicants, fecal sterols and bacteria were not as expected. Recent literature reports have stressed the importance of suspended sediment as sources of toxicants and mutagens. The failure of this study to show this relationship may be due to the mode of suspended sediment extraction. In this study a water extraction technique was used in deference to the sensitivity of the screening tests to solvents.

Microbiological data were greatly underestimated, perhaps due to the binding of bacteria and coliphage to particulate matter.

Further studies will be carried out to evaluate nonstressful extraction techniques for toxicant and genotoxic screening tests.

ABSTRACT

A study was initiated to investigate the potential and practicality of using a continuous flow centrifuge to concentrate suspended sediments and testing these suspended sediments for their toxicant and genotoxic activities, their microbiological densities and fecal sterol content. The results of this exploratory study, based on the extraction procedures and testing techniques used, indicate that there was no obvious advantages in using the continuous flow centrifuge to collect suspended sediments for microbiological and toxicant information.

SOMMAIRE ADMINISTRATIF

L'étude préliminaire ayant pour but d'évaluer l'importance du rôle que jouent les sédiments en suspension dans le transport des contaminants toxiques, des stérols fécaux et des bactéries n'a pas donné les résultats escomptés. Nombre d'études publiées dernièrement insistent sur le fait que les sédiments en suspension constituent des sources de contaminants toxiques et d'agents mutagènes. Si cette étude n'a pu démontrer l'existence d'une interaction semblable, c'est peut-être à cause de la technique d'extraction des sédiments en suspension. En effet, vu la susceptibilité des essais de dépistage aux solvants, on a employé une technique d'extraction à l'eau dans la présente étude.

Les données microbiologiques avaient été nettement sous-estimées peut-être en raison de l'agglutination des bactéries et des coliphages aux particules sédimentaires.

D'autres études seront menées dans le but d'évaluer les techniques d'extraction qui n'induisent pas de changement physique violent dans les échantillons pour dépister les contaminants toxiques et les agents mutagènes.

SOMMAIRE

Cette étude visait à déterminer s'il est pratique d'employer un séparateur par centrifugation à flot continu pour concentrer les sédiments en suspension en vue d'analyser leur teneur en contaminants toxiques et en agents mutagènes, en micro-organismes et en stérols fécaux. Compte tenu des techniques d'extraction et d'analyse employées, les résultats de cette étude préliminaire permettent de conclure que le séparateur par centrifugation à flot continu ne présente aucun avantage particulier pour recueillir des données sur les caractéristiques microbiologiques et toxicologiques des sédiments en suspension.

INTRODUCTION

Toxic metals and organic contaminants are often associated with particulates and bottom sediments in aquatic systems. In rivers, particulate matter can play an important role in the fate and transport of these substances (Allan, 1986). With this in mind, a study was initiated to investigate the potential and practicality of using suspended sediments for the estimation of toxicant and genotoxic chemical concentrations as measured by microbial screening tests.

This investigation also provided an opportunity to evaluate fecal sterol concentration estimations as well as the estimation of the traditional health hazard microbial indicators, fecal coliforms, E. coli and coliphage in suspended sediments.

METHODS

Sample Collection

Raw water, centrifuged water and suspended sediments were collected from the Water Quality Branch, Ontario Region (WQB-OR) monitoring station on the Ottawa River on November 4 and 5, November 18 and 19 and December 2 and 3, 1985. Water was drawn from a multi-intake system which was secured to a stainless steel frame, 50 metres north of Lemieux Island and 1 metre above the river bed (McCrea and Fischer, 1986).

The suspended sediment samples were obtained by diverting raw water from one of the intakes through a Westfalia continuous flow centrifuge (model KA-1-06-175) equipped with a four chamber stainless steel bowl rotating at 9300 rpm. This centrifuge model exerts a force of 9500 g and has been shown to be effective in the recovery of suspended sediments (Thomas and McMillan, 1978; Roche, 1985). Approximately 8600 litres of river water were centrifuged during the 24-hour collection period. After collection, the sediments were removed from the bowl and placed into sterile plastic, acid-washed containers and maintained at 4°C until processing, usually within 48 hours.

Raw and centrifuged water samples were collected simultaneously from both the 1st and 24th hour of each sampling period. Samples were collected in 500 mL sterile bottles and kept at melting ice temperatures until processing could be completed.

Genotoxicity Studies

The SOS Chromotest Kit with E. coli K12-PQ37 as supplied by Organics Ltd. (Israel) was used in this study. The technique used was that described by Fish et al. (1985) with a colour development time modification to 90 minutes, with and without S-9 mix (Xu et al., 1986). In preparation for the test, the suspended sediments were extracted with glass distilled water, 10 gm wet wt with 10 mL H_2O.

The mixture was vigorously shaken by hand for three minutes, centrifuged for ten minutes at 4°C at 5000 rpm and the supernatant tested for genotoxicity activity with and without addition of S-9 mix.

Microtox Test

The Microtox toxicant screening test was performed on the raw and centrifuged water samples and on the supernatant prepared for the genotoxicity studies, using the luminescent bacterium Photobacterium phosphoreum. The procedure detailed in the Beckman Microtox Operation Manual (1982) with a 15-minute contact time (Dutka and Kwan, 1981) was followed.

Microbiological Tests

Fecal coliform densities were estimated by the membrane filtration (MF) and most probable number (MPN) five-tube dilution techniques. The membrane filtration procedure used MFC agar and the MPN procedure used A1 broth with incubation at 44.5°C for 22-24 hours (Dutka, 1978).

Fecal streptococci populations were estimated by the membrane filtration technique using KF agar with an incubation period of 48 hours at 35°C.

The procedure used to estimate coliphage concentrations is the one found in section 919c of the 16th edition APHA Standard Methods (1985).

Fecal Sterols

Coprostanol and cholesterol analysis were performed on the suspended sediments using procedures described by Dutka et al. (1974). These procedures provided for 91-97% recoveries of Coprostanol and 74-96% for cholesterol with detection limits of 0.1 ppm.

RESULTS AND DISCUSSION

Table 1 displays the results of various microbiological tests performed on raw and centrifuged Ottawa River water. Included in this table, for general background information, are data obtained from another Ottawa River study, conducted concurrently with this study (Dutka et al., 1986). Also shown in this table are the results of fecal coliform MPN and coliphage tests on the suspended sediments. From this table, it can be seen that the continuous flow centrifugation process usually reduces microbial densities in the water samples. This reduction is believed to reflect natural water conditions where pelagic populations are a variable portion of the total microbial population.

In the three instances where terminal centrifuged water samples contained greater densities than terminal raw water samples, possible explanations for these observations are the extreme variability of pelagic population densities in the original river water, and/or the centrifugation process creates shear forces which release some of the particle attached microorganisms.

An interesting observation can be seen in Table 1, i.e. the wide fluctuations in raw water microbial populations within a 24 hour period: Nov. 4-5, fecal coliform MF 1240→43, fecal coliform MPN 1600→140; as well as the stability of microbial populations within a 24 hr period; Nov. 5-6, fecal coliform MF 43-43, E. coli MF 37-37 and fecal coliform MPN 140-140. With the exception of the Nov. 4 fecal coliform MF and MPN counts the microbial and coliphage populations were remarkably stable over the Nov. 4 to Dec. 3 period, an indication of the basic seasonal stability of rivers (Dutka 1973; Dutka et al. 1986).

Suspended sediment collected as part of the WQB-OR monitoring of the Ottawa River at Lemieux Island, revealed that, under normal conditions, these water contained approximately 2 mg seston per litre of river water.

The total suspended sediments were not accurately measured in this study. However, it was estimated that approximately 5000 L of raw river water would contain on average 10 g of suspended sediment. In view of the fecal coliform MPN counts in the suspended sediment,

the amount of water required to yield 10 gram seston samples and the difference between the raw and centrifuged water fractions, the fecal coliform sediment counts were not representative and greatly underestimate the actual microbial concentrations in the raw water.

In Table 2 toxicity and fecal sterol data are presented. Analysis of the raw and centrifuged water samples indicated that fecal sterols were not present and that these water fractions were not toxic. Similarly, fecal sterols were not detected in the suspended sediment samples, however, positive responses to the Microtox test were obtained from the November 5 and 19 samples.

Kwan et al. (1986), in a concurrent study of the Ottawa River at Lemieux Island, found that by concentrating the raw water samples 10X by flash evaporation, they obtained positive responses in five of the samples tested for toxicant activity by the Microtox test. EC_{50} values for these samples varied from .33 mL to .50 mL of 10X sample.

Microtox EC_{50} values were obtained in this study from .22 g (November 5) and .43 g (November 19) suspended sediments. Based on the typical Ottawa River seston concentration, it was calculated that .22 g of suspended sediment originated from approximately 110 litres of raw water and .43 g of suspended sediment from approximately 215 litres of raw water.

Based on Kwan et al. data (1986) and the results of this study, there appears to be no advantage in collecting suspended sediment samples for toxicant, genotoxicity and fecal sterol analysis under the testing and extraction procedures used in this exploratory study.

In conclusion the results of this exploratory study to evaluate the importance of suspended sediment as a carrier of toxicants, fecal sterols and bacteria were not as expected especially as recent literature reports (Allan 1986) have stressed the importance of suspended sediment as sources of toxicants and mutagens. The failure of this study to show these relationships may be due to the mode of suspended sediment extraction. In this study a water extraction technique was used in deference to the sensitivity of the screening tests to solvents.

Microbiological data were also greatly underestimated, perhaps due to the binding of bacteria and coliphage to particulate matter.

Further studies will be carried out to evaluate nonstressful extraction techniques for toxicants and genotoxic screening tests.

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TABLE 1. Microbiological data collected from Ottawa River raw water, centrifugal water and suspended sediments during three 24-hour period.

Test	Date	First Hour Samples		24th Hour Samples		
		Raw Water Initial	Centrifuged Water	Raw Water	Centrifuged Water	Suspended Sediment
F. coliform MF/100 mL	Nov 4-5	1240	24	43	100	
E. coli MF/100 mL		50	21	37	21	
F. coliform MPN/100 mL		1600	46	140	280	
F. coliform MPN/10 g wet wt						1600
F. strept MF/100 mL		50	20	80	30	
Coliphage /100 mL		5	5	40	15	500
Fecal coliform MF/100 mL	Nov 18-19	110	72	60	44	
E. coli MP/100 mL		51	35	34	22	
F. coliform MPN/100 mL		170	26	23	11	
F. coliform MPN/10 g wet wt						7900
F. strept MP/100 mL		12	28	32	8	
Coliphage /100 mL		30	10	10	50	35
Fecal coliform MF/100 mL	Dec 2-3	21	11	104	36	
E. coli MP/100 mL		16	10	76	24	
F. coliform MPN/100 mL		31	17	140	33	
F. coliform MPN/10 g wet wt						3100
F. strept MF/100 mL		18	3	43	10	
Coliphage /100 mL		15	25	60	50	150
Fecal coliform MN/100 mL	Nov 6	43				
E. coli MF/100 mL		37				
F. coliform MPN/100 mL		140				
Coliphage /100 mL		40				
Fecal coliform MF/100 mL	Nov 29	93				
E. coli MF/100 mL		58				
Fecal coliform MPN/100 mL		140				
Coliphage		45				

TABLE 2. Toxicity screening test and fecal sterol data collected from Ottawa River raw and centrifuged water and suspended sediment samples.

Date	Raw Water		Coprostanol	First hr Centrifuged Water Sample		Suspended Sediment			
	Microtox EC_{50}[1]	SOS Chromotest SOSIP[2]	ppb	Microtox EC_{50}	SOS Chromotest SOSIP	Microtox EC_{50}	SOS Chromotest SOSIP	Coprostanol ppm	Cholesterol ppm
Nov 4	ND[3]	ND	ND	ND	-[4]				
Nov 5	ND	ND	ND	ND	-	.22 g	ND	ND	ND
Nov 18	ND[3]	ND	ND	ND	-				
Nov 19	ND	ND	ND	ND	-	.43 g	ND	ND	ND
Dec 2	ND	ND	ND	ND	-				
Dec 3	ND	ND	ND	ND	-	ND	ND	ND	ND

EC_{50}[1] - water samples and sediments are reported in millilitres and grams required to produce an EC_{50}, i.e. 50% light reduction.
SOSIP[2] - SOS-Inducing Potency is per mg sediment or per mL sample.
ND[3] - not detected.
-[4] - test not performed.