

USE OF A BATTERY OF BIOLOGICAL TESTS
TO ASSESS AQUATIC QUALITY IN CANADIAN
PRAIRIE AND NORTHERN WATERSHEDS

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ABSTRACT

In this report are presented results from the examination of water samples, water-extracted sediments and organically extracted sediments from rivers and lakes in western Canada, to which the battery of tests approach was applied. Three basic study areas were chosen to reflect the wide variety of conditions which exist in the Canadian prairie provinces. These study areas included the Saskatchewan River System, the Qu'Appelle River System and the Churchill River diversion route in the Burntwood, Footprint and lower Nelson Rivers. Using a point ranking scheme, the sampling sites (water and sediments) were rated with the top five water and sediment sampling sites of greatest concern (or hot spots) being established by this analytical and ranking scheme.

RÉSUMÉ

Ce rapport présente les résultats de l'analyse d'échantillons d'eau et de sédiments, extraits à l'aide d'eau et de solvants organiques, provenant de rivières et de lacs de l'Ouest canadien. Les analyses ont été réalisées par la méthode de la batterie d'essais. L'étude a couvert trois régions représentatives de la grande diversité des conditions existant dans les provinces des Prairies. Les réseaux hydrographiques des rivières Saskatchewan et Qu'Appelle, de même que la région des rivières Burntwood et Footprint et du fleuve Nelson, vers lesquels les eaux de la rivière Churchill ont été détournées, sont les trois régions retenues par cette étude. Les sites d'échantillon (eau et sédiments) ont été classés à l'aide d'un système de classement numérique. Les cinq sites montrant le plus haut degré de dégradation (zones critiques) ont été déterminés grâce à cette méthode d'analyse et de classement.

MANAGEMENT PERSPECTIVE

The goal of this study is to identify degraded or degrading water bodies so that managers will have a strong data base from which decisions can be made. This information is provided by using the "battery of tests" approach. Another goal of this study is to evaluate a variety of microbiological, biochemical and bioassay tests for their potential of becoming the core group of tests in the "battery of tests" approach. This core group of tests can, and will, be used nationally to prioritize water bodies and sediments on selected areas within water bodies for remedial action, further investigations or, to monitor the effects of remedial actions.

The "battery of tests" approach should make it possible to establish "hot spots" areas of immediate concern which were not previously suspected, due to inappropriate or one-dimensional testing procedures.

In this study, we found that the three sites of greatest concern based on the battery of tests approach and ranking scheme were, in order, Qu'Appelle River at Lumsden, N. Saskatchewan River below Fort Saskatchewan and the Saskatchewan River above Tobin Lake. Data from this study also reaffirmed earlier study data concerning the importance of how sediments are extracted. Each extracting procedure appears to be very specific for the toxicants it extracts.

PERSPECTIVES DE GESTION

Le but de cette étude consiste à identifier les masses d'eau dégradées ou en voie de dégradation de façon à fournir aux gestionnaires une bonne base de données qui leur permettra de prendre des décisions. Ces données sont obtenues par la méthode de la batterie d'essais. Cette étude a aussi pour but d'évaluer une gamme d'essais microbiologiques, biochimiques et biologiques de façon à sélectionner ceux qui pourraient constituer les essais principaux de la méthode de la batterie d'essais. Cet ensemble d'essais principaux peut être utilisé et sera utilisé à l'échelle nationale pour donner la priorité à certaines masses d'eau et à des sédiments localisés dans des endroits précis en ce qui a trait aux mesures de redressement, aux recherches futures et au contrôle continu des effets des mesures de redressement.

La méthode de la batterie d'essais devrait permettre de localiser les zones critiques dont l'état exige une action immédiate et que des essais inadéquats ou unidimensionnels n'avaient pas réussi à détecter.

Cette étude a révélé que les trois sites les plus dégradés, selon la méthode de la batterie d'essais et le système de classement numérique, sont dans l'ordre : la rivière Qu'Appelle à Lumsden, la rivière Saskatchewan Nord au sud de Fort Saskatchewan et la rivière Saskatchewan au nord du lac Tobin. Les données de cette étude confirment les données d'études précédentes concernant l'importance de la méthode d'extraction des sédiments. Chaque méthode d'extraction extrait les substances toxiques de façon très spécifique.

INTRODUCTION

In a series of publications and reports, Dutka et al. (1987, 1986), Dutka and Kwan (1988) and Dutka and Rao (1987), described the results of a series of Canada-wide studies to develop and evaluate a battery of microbiological, biochemical and toxicant screening tests for environmental hazard assessment and priority setting. The goal of these studies was to establish under diverse field conditions a "battery of tests" which could be applied nationally, and perhaps internationally, to designate water bodies or sediments that are degraded or are being degraded. This "battery of tests" approach may also be used to monitor the effectiveness of remedial actions or the effect and extent of specific discharges on ambient riverine or lacustrine ecology.

In this paper, we describe the final study of this series, which entailed the collection and sampling of river and lake waters and sediments from the three Canadian prairie provinces of Alberta, Saskatchewan and Manitoba.

STUDY AREA

Three basic study areas were chosen to reflect the wide variety of conditions which exist in the prairie provinces. These study areas included: the Saskatchewan River system; from upstream of Edmonton on the North Saskatchewan and from Lake Diefenbaker on the South Saskatchewan River to Tobin Lake on the Saskatchewan River; the Qu'Appelle

River system from Buffalo Pound Lake to Katepwa Lake; and along the Churchill River diversion route in the Burntwood, Footprint and lower Nelson Rivers which have been subjected to extensive flooding due to hydroelectricity development. Physical and water quality conditions varied widely between these three study areas.

The North Saskatchewan River originates in the Rocky Mountains south of Jasper, and flows in an eastward direction across Alberta, and into Saskatchewan (Figure 1). In north central Saskatchewan it joins the South Saskatchewan River to become the Saskatchewan River. The South Saskatchewan River also originates in the Rockies as the Bow and Oldman Rivers which flow eastward forming the South Saskatchewan River just west of Medicine Hat and continues eastward through Lake Diefenbaker, created by the construction of the Gardiner Dam in 1966 before passing through Saskatoon and joining the North Saskatchewan River. Tobin Lake, about 120 km downstream of the confluence was created by the construction of the Squaw Rapids dam in 1962, and acts as a sink for much of the sediment transported by the river and the contaminants carried with them. The North Saskatchewan River is situated primarily on forested and agricultural lands and the South Saskatchewan River flows primarily through agricultural land. The cities of Edmonton, Fort Saskatchewan and Prince Albert discharge a variety of industrial and municipal wastes directly to the North Saskatchewan River and the cities of Calgary, Medicine Hat and Saskatoon discharge to the South Saskatchewan River.

The Qu'Appelle River is a mature river situated in southern Saskatchewan which originates near Lake Diefenbaker and flows eastward through agricultural lands to the Manitoba border. The river flows through a number of small lakes along its course. The water is very nutrient rich and the lakes are hypertrophic. The cities of Regina and Moose Jaw are the major users of the Qu'Appelle River. Both cities draw their municipal water from Buffalo Pound Lake, and Regina discharges its sewage to a tributary stream. Flow conditions are typical of prairie streams with high flow during spring runoff and very little flow through the remainder of the year. However, low flow conditions are augmented by diversion waters from Lake Diefenbaker.

The Churchill River Diversion Route in Northern Manitoba includes the Burntwood, Footprint Rivers, Split Lake and the lower Nelson River. In 1976, seventy-five percent of the Churchill River was directed into the Rat and Burntwood River systems. Approximately 214,000 hectares of land in northern Manitoba were flooded. Approximately 41,727 of the flooded hectareage affected Native Reserve lands including some of the selected sites in this study (Nelson House). Hydro generating stations and control dams alter the water flow throughout this watershed. The Burntwood River water quality conditions reflect erosional influences, contributing to suspended solid concentration increases downstream from the diversion.

A total of 34 water and sediment samples were collected (Figure 1) during June-July 1987. Twenty-one sites were within the Saskatchewan River Basin, five in the Qu'Appelle River Basin and eight

were in northern Manitoba in Split and Footprint Lakes. A brief description of each sampling location is presented in Table 1.

METHODS

Sample Collection

Sediments were collected with an Ekman dredge or shovel. Frequently, it was necessary to Ekman many times before sufficient surface (1 to 2 cm layer) sediment was collected. At each site the surface layers were pooled, well mixed, dispensed into aliquots for each testing procedure and refrigerated.

Surface water samples (1 L) were collected at each site for fecal coliform, fecal streptococcus and coliphage tests. These tests were usually processed within eight hours of collection. Also at each site, another 1 L sample of water was collected and preserved at 4°C for toxicant screening tests. Toxicant screening samples were tested after being concentrated 10X by flash evaporation at 45°C.

A one litre surface water sample was also collected at each site, for coprostanol and cholesterol analyses. The sample was preserved with 1 mL concentrated H₂SO₄ and refrigerated at 4°C.

Sediment Extraction and Processing

Prior to performing toxicant screening tests, the sediment samples from each site were homogenized and split into two portions.

One portion of the sediment was extracted with Milli Q water (4 cartridge system, 1 Super C carbon cartridge, 2 Ion-ExTM cartridges, 1 Organet-Q^r cartridge and a Mill-StakTM filter with a glass distilled water feed), by mixing sediment and Milli Q water in a 1:1 ratio (100 g wet weight sediment:100 mL water), shaking vigorously for three minutes, then centrifuging at 5000 rpm in a refrigerated centrifuge for 10 minutes. The supernatant was used in toxicity screening tests.

A 100 gram portion of the above water extracted sediment was freeze-dried, then weighed on pre-fired aluminum foil (550°C overnight). The weighed, freeze-dried sample was added along with 250 mL dichloromethane (DCM) into a 1 L Erlenmyer flask, which was prerinsed twice with DCM, and shaken for approximately 24 hr on a Burrell wrist action shaker at position #2. After settling overnight, the samples were filtered through prewashed Na₂SO₄. To the filtrate, 1.0 mL DMSO was added and the samples were evaporated in a rotary evaporator to 1 mL. The sample was transferred to a test tube with 2 mL DCM rinsings (twice) of the flask. The DCM was evaporated under N₂ in a water bath to 1.0 mL. This 1 mL of 100% DMSO contained sample was used in all tests at the 1% level. A solvent blank was prepared for each testing containing 250 mL DCM plus 1.0 mL DMSO evaporated to 1.0 mL DMSO. A method blank was also prepared as a control containing 250 mL DCM plus 1.0 mL DMSO, shaken, filtered and evaporated as per total sample procedure.

The second portion of sediment was sieved for size distribution, following the procedure outlined by Duncan and LaHaie (1979). Basically, the sample was sieved at 1/2 or 1/4 PH1 scale intervals (Krumbein and Pettijohn, 1938). The size distribution was determined with SIZDIST, a programme used in conjunction with the IBM PC computer (Sandilands and Duncan, 1980).

Microorganism Tests

Fecal coliform five tube MPN tests using A-1 broth and Clostridium perfringens five tube MPN test series using DRCM medium with confirmation in litmus milk were applied to each sediment (Dutka et al., 1987).

Fecal coliform MF, fecal streptococci MF and coliphage tests were performed on all water samples as described by Dutka et al. (1986).

A total of 163 isolates were collected and identified from typical fecal coliform colonies on the MFC agar plates. Identification of these organisms was carried out using the API 20E system.

Biochemical and Toxicity Screening Tests

Coprostanol and cholesterol analyses were performed on water samples and the Microtox toxicant screening test was performed on water and sediment extracts as described by Dutka et al. (1986). SOS genotoxicity tests on water and sediment extracts were performed as

described by Xu et al. (1987) without S-9 addition. ATP-TOX System, a new toxicity screening test, based on toxicant inhibition of bacterial growth and luciferase activity, was applied to water and sediment extracts (Xu and Dutka, 1987). Spirillum volutans, a large aquatic bacterium with a rotating fascicle of flagella at each pole was also used to test samples for toxicity following the procedures described by Dutka and Kwan (1982).

An algal-ATP toxicant screening test was also performed on water and sediment extracts. This test is based on the inhibition of ATP production in cultures of the green alga Selenastrum capricornatum (Blaise et al., 1984). The ATP content of the stressed Selenastrum was measured by the procedure described in the Turner Luminescence Review (1983). The results are reported as a percentage of Relative Light Units (RLU) output by the tested sample, compared to the non-stressed control which is 100%.

A 48-hour Daphnia magna test, using ten organisms per sample and sample dilution was also performed on unconcentrated water and sediment water extracts to assess toxicant activity (APHA, 1985).

Ranking Scheme

The format used to award points for specific data values, in order to rank the sampled water and sediments, from those of most concern to least, is presented in Table 2. The point allocation

scheme is biased but does reflect the authors' evolving experience with data accumulated from the application of a variety of tests to effluents, waters and sediments throughout Canada.

The present point allocation scheme has evolved over a three year period and is an ongoing viable process which may change with increased data accumulations.

Samples with the most points are deemed to contain the greatest potential hazard to man and organisms found in the aquatic ecosystem. High toxicant levels may have reduced microbial levels/activity in sediment samples, however, cause and effect relationships were not investigated.

RESULTS

Sediment Classification

Table 1 presents the composition of each sediment sample based on particle size distribution by sieve analyses and sediment classification by the Shepard (1954) system. As would be expected for river sites, the majority of the sediments from the North Saskatchewan and Saskatchewan Rivers were sand or silty sand.

The sediment samples collected from lakes in this study produced various sediment types, for instance, Lake Diefenbaker sediment samples varied from clay to sand silt clay, Lake Tobin from silty clay to clay while both Pasqua and Katepwa Lakes, part of the Qu'Appelle

system had basically sand sediments. The samples from Split Lake had a variety of sediment types varying from sand to clayey silt and Footprint Lake samples were silty clay. These sediment types were influenced by the erosional influences originating from the diversion route and flooded lands.

MICROBIOLOGICAL AND BIOASSAY TESTS

Water

Table 3 presents the bacteriological, biochemical and toxicological data obtained from the 34 water samples that were collected from the three study areas.

In the Saskatchewan River system only the two sites upstream and downstream of Edmonton showed fecal coliform densities greater than 100/100 mL (142,000/mL and 9600/100 mL, respectively). Both these samples also had fecal streptococci counts greater than 100/10 mL, four other samples also had fecal streptococci count greater than 100/100 mL (Table 3). Samples from eight sites produced coliphage counts with the highest, 660/100 mL, found in the Saskatchewan River below Codette Reservoir (#62). This high coliphage count was unique in that it was greater than the bacteriological counts at the site. A similar situation occurred in a downstream sample at the Highway 55 Bridge (#61) where the coliphage count was nearly equal to the bacterial counts. Four samples had positive coprostanol, the highest

value, 0.2 ppb occurring in the samples from the South Saskatchewan River at Birth Hills (#51). Cholesterol was found in all the samples with the highest, 9.4 ppb, from the Carroll's Cove site on Tobin Lake (#66).

The Daphnia magna 48 hour test was the only bioassay procedure which showed a toxic effect due to exposure to Saskatchewan River basin samples. Thirteen samples caused a toxic response with toxicity ranging from 100% EC₂₀ to 50% EC₅₀. ATP-TOX test did, however, show 66% inhibition in the Battle River, suggesting a toxic effect and low level inhibition, between 30 and 50% was also found in samples from six sites (Table 3).

In the Qu'Appelle River system only the river site near Lumsden (#56) had elevated levels of bacteria and coliphage. This site also had positive coprostanol (0.11 ppb) and higher cholesterol (5.43 ppb) than other sites in the Qu'Appelle.

As in the Saskatchewan River sites, only the Daphnia magna bioassay showed a toxic response to the Qu'Appelle River samples. Samples from four sites display a toxic response ranging from 100% EC₅₀ to 84% EC₅₀. The ATP-TOX test showed inhibition at all five sites ranging from 33% to 80%.

The results for samples from the Churchill River Diversion route showed fecal coliform densities greater than 100/100 mL at three sampling sites. Two were located at Split Lake and one at Footprint Lake. The highest fecal coliform contamination occurred at the Split Lake sewage treatment plant outfall (5800/100 mL). Fecal streptococci

levels were low, the highest density recorded at Footprint Lake, Industrial Causeway #2 (111/1000 mL). Coliphage determinations were less than five, with the exception of the Split Lake sewage treatment plant outfall (10/100 mL). The bioassay results from the water samples from the diversion route were negative. However, the ATP-TOX test did show inhibition in the 30 to 50% range in four samples, which suggests the presence of toxicant activity.

Water samples with the highest point ranking from each study area and thus the greatest potential hazard to man and biota were from the North Saskatchewan River at Devon, Qu'appelle River at Lumsden and Split Lake near the community sewer discharge. This high point score is mainly due to bacteriological pollution occurring at these sites.

Bottom Sediment

Tables 4 and 5 present the results of the analysis of bottom sediments. Table 4 gives the bacteriological data and bioassay data for water extracted sediments and Table 5 the bioassay data for solvent extracted sediments.

Sediment fecal coliform populations were high at the lotic sites in Saskatchewan River system with the highest count (92,000/10 g) occurring at two sites, the North Saskatchewan River at Fort Saskatchewan (#42) and the Saskatchewan River below Codette Reservoir (#61). Populations in samples from reservoir sites in the basin were all very low (26/10 g or less). Clostridium perfringens densities in

the sediments were often at odds with the fecal coliform data. Sites with high fecal coliforms often had low C. perfringens (e.g., #42 North Saskatchewan at Devon) and the reservoir sites had high C. perfringens despite very low fecal coliform densities.

Like the water samples from the Saskatchewan River system, only the Daphnia magna bioassay tests showed a toxic response to water extracted sediment samples. The most toxic sample (42% EC₅₀) was from the Saskatchewan River near Nipawin (#64). The majority of other test procedures showed a stimulatory response. Bioassay testing of the sediment samples following further extraction with dichloromethane (Table 5) showed a toxic response in a number of the test procedures at various sites. The Microtox test showed a toxic response in 9 of the 21 samples collected in the Saskatchewan River system. The most toxic sample was from Site #45 on the North Saskatchewan River near Lloydminster. The Spirillum volutans test was positive in samples from the North Saskatchewan River below Fort Saskatchewan (#42) and the Saskatchewan River near Nipawin (#64) and the SOS Chromotest displayed a genotoxic response to the sample from the North Saskatchewan River at Borden (#48). The ATP-TOX System results indicated low grade toxicity (above 30% inhibition) in samples from the North Saskatchewan River below Fort Saskatchewan (#42) and from Tobin Lake at Carroll's Cove (#67).

In the Qu'Appelle system, the sediment bacteria concentrations were similar to those seen in the Saskatchewan system. The river site at Lumsden had the highest fecal coliforms density (35,000/10 g). The

lake sites had low fecal coliform densities (less than 20/10 g) with C. perfringens densities as high as 1600/10 g. The Daphnia magna bioassay showed a toxic response to all five of the water extracted sediment samples from the Qu'Appelle system. None of the other procedures displayed a toxic response to these extracts and the Microtox and Algal-ATP test showed a stimulatory response. Solvent extraction of the Qu'Appelle sediment samples produced a toxic response by the Microtox test in three samples, a positive response with the Sprillum volutans at one site and very high toxic response (12.0) in the SOS Chromotest from the Qu'Appelle River at Lumsden (#56).

Fecal coliforms in the sediment samples from the sites on the Churchill River diversion route ranged from (2 to 490/10 g), with the highest density occurring in two samples from Split Lake. C. perfringens were present in all the samples from this area ranging from 70 to 600/10 g. As in the other study areas the Daphnia magna bioassay test was the only procedure to display a toxic response to water extracted sediment samples. All sediment samples from this area produced a toxic response, ranging from 20% EC₅₀ to 100% EC₂₀. The most toxic sample was from Footprint Lake, Nelson House School Bay (#75), which receives the Nelson House treated sewage effluent. This sample based on Daphnia magna results was the most toxic of all samples collected during the study.

Point scores based on the sediment analysis show that the site on Qu'Appelle River at Lumsden (#56) had the highest score (18) of all the samples tested from the three areas in both water and solvent

extracted samples. In the Saskatchewan River system the sample from the North Saskatchewan River below Fort Saskatchewan had the highest score (16) in both extracts. However, the sample from the Saskatchewan River below Codette Reservoir (#61) had a score of 17 in the water extracted sample. Two samples from the Churchill diversion route had point scores of 14 in water extracted samples.

Bacterial Isolate Data

A total of 178 typical fecal coliform MF isolates were collected for identification from the 34 sampling sites. The results of the isolate identifications are presented in Appendix A. Six isolates were Klebsiella pneumonia. Two of these K. pneumonia isolates were found in the sample from Tobin Lake at Prudhomme Campground (#65) which also yielded two Escherichia coli, one Salmonella enteritidis and one Enterobacter amigenus. The other four Klebsiella were isolated from samples from the Churchill River diversion route. One from Split Lake at the Split Lake communities sewage discharge (#71) which also had 13 E. coli, one from Footprint Lake at Metis Beach (#73) which only had one typical fecal coliform/100 mL and four from Footprint Lake School Bay (#75) which produced a diverse group of organisms from the typical fecal coliform isolates, one E. coli, one Serratia oderifera, two S. enteritidis, and one S. paratyphi A. Of the 178 typical fecal coliform colonies subjected to identification procedures by the API 20E kit, and using the computer program to

assist in the identification, 123 of the colonies proved to be E. coli of which 27 were atypical E. coli based on computer aided identification.

DISCUSSION

Water sample data shown in Table 3 indicate that the river and lake waters with few exceptions have reasonable to good bacteriological water quality. Isolate data indicate that the main source of fecal coliforms in the Saskatchewan basin is from fecal pollution. The toxicant screening tests for the most part were not able to detect the presence of chemicals with toxicant activity with the exception of the Daphnia magna test. The Daphnia magna test is proving to be the most sensitive toxicant screening test in our battery of tests for assessing toxic activity in environmental samples. Interestingly, water samples positive in the Daphnia magna test were collected from sites #41 to #60, basically the North and South Saskatchewan Rivers, Diefenbaker Lake and the Qu'Appelle sites.

Using the point scheme shown in Table 2, the five water samples of greatest concern are:

1. Site #41, North Saskatchewan River at Devon - ranking due to microbiological load with some toxicant activity.
2. Site #42, North Saskatchewan River at Fort Saskatchewan - ranking due to microbiological load with some toxicant activity.

3. Site #46, Battle River - ranking due to toxicant load with some microbiological contamination.
4. Site #48, North Saskatchewan River at Borden - ranking due to toxicant load with some microbiological contamination.
5. Site #60, Saskatchewan River above Tobin Lake - ranking due to toxicant load with some microbiological contamination.

Fecal coliform and C. perfringens data from sediment samples suggest that the Lake Diefenbaker (sites #49-51) and the Qu'Appelle Lakes (sites #52-55) are the sites least affected by fecal pollution. General impressions from the microbiological data are that all of the Saskatchewan River basin sites appear to have been impacted by fecal pollution, although some sites based on fecal coliform and C. perfringens densities, appear to receive intermittent pollution which may be diminishing. Sites #65 and #66 in Tobin Lake appear to be good examples of intermittent or past fecal pollution contamination. Both water column and sediment fecal coliform counts are extremely low and yet there are large populations of C. perfringens. Whether this finding is due to past or intermittent pollution, only a detailed local study can confirm.

The Native Community sites #68-#75 on the Churchill River diversion route indicate the presence of widespread low grade fecal pollution. The data from Split Lake Community site #70 suggest the low grade fecal pollution is ongoing.

In the water extract sediment samples, only one sample from Lake Diefenbaker (site #50) did not produce a toxic response by the Daphnia magna test. All the other toxicant screening tests showed little or no response to these extracts indicating either a lack of sensitivity to the chemicals present, or a very low level of toxicant.

It was surmised that Tobin Lake sediments would prove to be among the most toxic of the sediments examined within the Saskatchewan River basin, as it was felt that Lake Tobin might act as a sink for all the toxicants and pollutants coming down the Saskatchewan River system. The D. magna test did show a toxic response to sediment extracts from the Tobin Lake sites with EC₅₀ values being obtained from extract concentrations varying from 82% to 100%. These EC₅₀ values were comparable to other sites in the Saskatchewan river system.

Based on the point scheme developed in Table 2, the five sediment samples of greatest concern are:

1. Site #56, Qu'Appelle River at Lumsden - ranking due to microbiological load with some toxicant activity.
2. Site #61, Saskatchewan River below Highway 55 bridge - ranking due to microbiological load with some toxicant activity.
3. Site #66, Tobin Lake of Carroll's Cove - ranking due to microbiological and toxicant activity.
4. Site #42, North Saskatchewan River below Fort Saskatchewan - ranking due to microbiological and toxicant activity.

5. Site #62, Saskatchewan River below Codette Reservoir - ranking due to microbiological and toxicant activity.

The organic extraction of sediment samples produced toxic responses in various screening tests not seen using the water extracted sediments or water samples. The Microtox test showed a toxic response in samples from a number of sites throughout the Saskatchewan and Qu'Appelle River Basins. The highest toxicity occurring in the sample from Buffalo Pound Lake (#52), followed very closely by samples from the North Saskatchewan River below Fort Saskatchewan (#42) and near Lloydminster (#45) and the Qu'Appelle River at Lumsden (#56). Genotoxic activity were also found in these extracts by the SOS Chromotest. The sample from the Qu'Appelle River at Lumsden produced the highest genotoxic effect (12.0) found in these Canada-wide studies (Dutka, 1988). Also two sites were found to contain toxicants which produced a positive test in the Spirillum volutans test. Thus, the use of more stringent extraction procedures has produced a greater incidence of positive responses in the battery of toxicant screening tests. The significance of this increased toxicant response is debatable as there are concerns that these more rigorous extraction procedures may only measure bound toxicants which would not normally return to the environment. Conversely, the other side of the coin is that these toxicants may be biomagnified by biota or biotransformed and become part of the food chain.

A philosophical problem we have with these more rigorously organically extracted toxicants is that the tests are based on a 1% DMSO solution. Some screening tests can be performed with 5 or 7% DMSO without compromising the test, thus if these concentrations of DMSO were used with their potentially greater quantity of dissolved toxicants, we would expect to see an even higher proportion of positive tests.

Using the point scheme shown in Table 2, we can rank the responses of the extracts based on five of the toxicant screening tests used in this study.

Listed below are the top five ranked (most potential hazards) organically extracted sediments.

1. Site #56, Qu'Appelle River at Lumsden
2. Site #42, North Saskatchewan River at Fort Saskatchewan
3. Site #52, Buffalo Pound Lake
4. Site #45, North Saskatchewan River at Lloydminster
5. Site #54, Pasqua Lake near outlet

Site #60, Saskatchewan River above Tobin Lake

Site #64, Saskatchewan River at Nipawin

Site #65, Tobin Lake at Prudhomme Camp

Comparing the top five areas of concern, from each substrate type or extraction method, shows there are three common sites (Table 6), one of which appears in all three columns, e.g., water, water extracted sediment and organically extracted sediment. By implication, those

sites appearing in Table 6, based on the data obtained, are the sites within the study area to which the highest concerns and attention should be given.

The battery of tests evaluated in this study produced basically the expected results. The North Saskatchewan River receives numerous industrial and municipal effluents as it passes through Edmonton and Fort Saskatchewan and as a result the site below Fort Saskatchewan was expected to produce toxic responses and have elevated bacterial populations. Similarly the Qu'Appelle River receives municipal and industrial effluents and agricultural runoff upstream of Lumsden. The Saskatchewan River above Tobin Lake potentially has a summation of all the inputs to the river basin, but is more likely a reflection of the impact of effluent discharges a short distance upstream from the town of Nipawin. Earlier studies (Birkholz et al., 1980) have shown Tobin Lake to be a potential sink of toxic chemicals from the Saskatchewan River basin. As a result, greater toxic response had been anticipated for samples from Tobin Lake. However, the sampling locations cited in this paper may not have included the same deposition areas shown earlier by Birkholz et al. (1980) to have elevated levels of toxic chemicals. This difference may also be a result of the varying sensitivities of the tests used by both studies.

In the isolated part of northern Manitoba where Split Lake and Footprint Lake are located, it was noted that the prime pollution problem in these Native Community sites was related to microbial pollution, yet the positive Daphnia magna tests suggest there are also

some toxicant concentration concerns. Bottom sediments in these areas contain a myriad of heavy metals such as Al, Pb, Zn, Cu, Ni, Fe, Mn, Se, and As. Any combination of these metals may have produced a toxic reaction in the D. magna test.

The results of this study are very illustrative and supportive for the needs of a battery of tests. It is paramount that the composition of the battery of tests be selected very carefully to reflect local conditions. Of the toxicant screening tests evaluated, The Daphnia magna test appears to be the most sensitive, as well as one of the least expensive procedures, for indicating the presence of contaminants with toxic activity.

Use of the "battery of tests" approach reemphasizes that individual toxicant, biochemical and microbiological screening tests do not provide a sufficient data base upon which realistic management decisions can be made.

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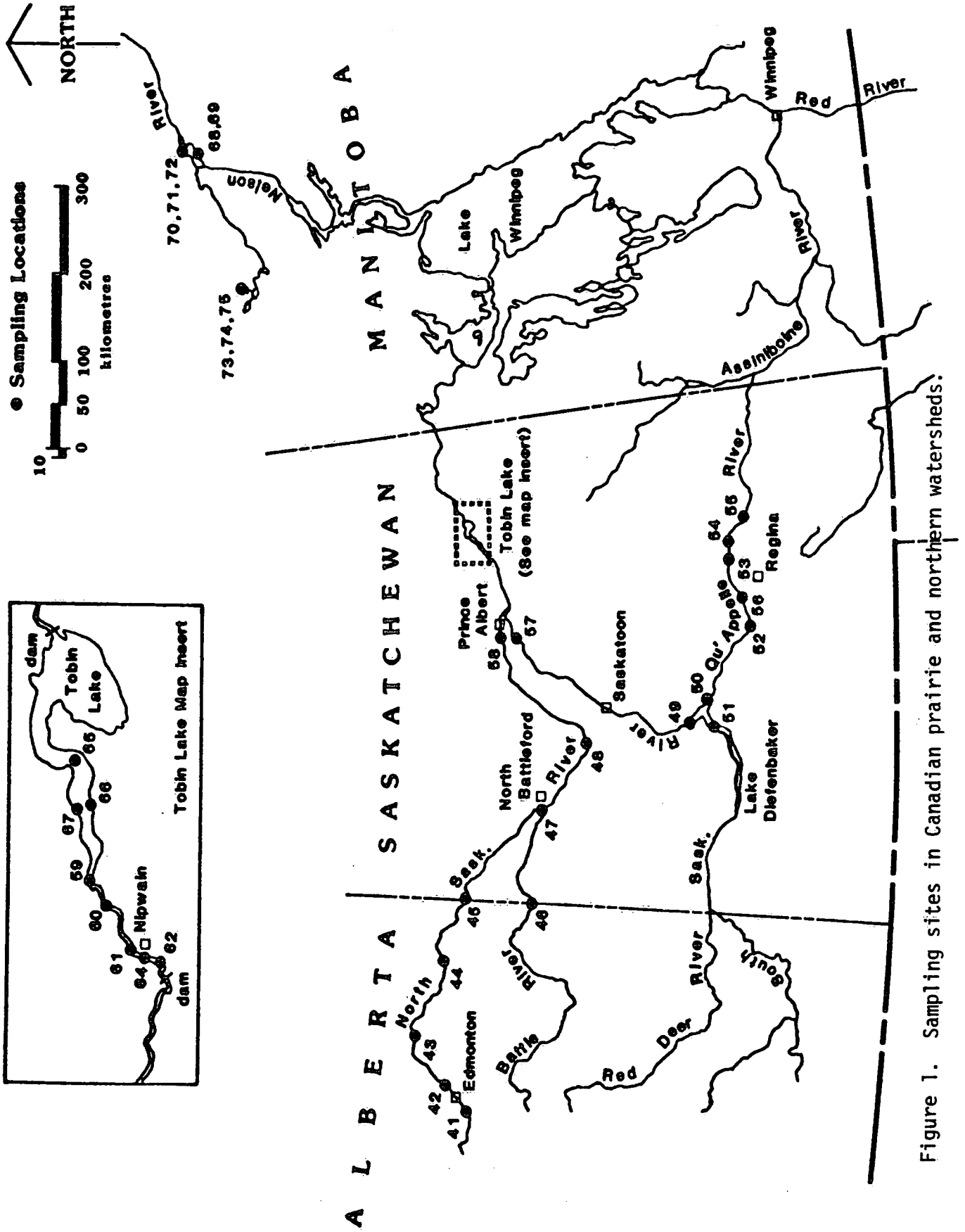


Figure 1. Sampling sites in Canadian prairie and northern watersheds.

Table 1. Sampling Site Locations and Sediment Description by River Basin

Station Name and Number	Latitude	Longitude	Sediment Description and Shepard Classification
SASKATCHEWAN RIVER BASIN			
41. North Saskatchewan R. at Devon, Hwy 60 Bridge	53°22'09"	113°45'06"	sand 85.57%, silt 9.44% and clay 2.98% SAND
42. North Saskatchewan R. at Fort Saskatchewan Hwy 37 Bridge	53°42'22"	113°14'19"	gravel 0.31%, sand 86.00%, silt and clay 13.69% SAND
43. North Saskatchewan R. at Pakan, Hwy 831 Bridge	54°09'00"	112°23'54"	sand 77.72%, silt 13.80%, clay 8.48% SAND
44. North Saskatchewan R. near Myran, Hwy 881 Bridge	54°45'30"	108°19'00"	sand 23.39%, silt 49.25%, clay 27.36% SAND SILT CLAY organic material present
45. North Saskatchewan R. near Lloydminster, Hwy 17 Ferry Crossing	53°38'10"	110°53'09"	sand 22.78%, silt 54.06%, clay 23.17% SAND SILT CLAY
46. Battle River near Urwin	52°56'25"	109°52'25"	sand 91.97%, silt+clay 8.03% SAND
47. North Saskatchewan R. at Battleford, Hwy 16 Bridge	52°45'30"	108°19'00"	sand 90.58%, silt+clay 9.42% SAND
48. North Saskatchewan R. at Borden, Hwy 16 Bridge	52°22'26"	107°09'08"	sand 46.25%, silt 36.01%, clay 17.74% SILTY SAND
58. North Saskatchewan R. upstream of Prince Albert at former Curtwell Ferry Crossing, Hwy 302	53°12'02"	106°06'18"	sand 56.74%, silt 29.36%, clay 13.91% SILTY SAND
49. Lake Diefenbaker off Danielson Provincial Park	51°15'36"	106°49'25"	sand 9.88%, silt 43.92%, clay 46.19% SILTY CLAY
50. Lake Diefenbaker off Douglas Park	51°03'50"	106°31'25"	sand 30.02%, silt 31.81%, clay, 38.17% SAND SILT CLAY

Table 1. (continued)

Station Name and Number	Latitude	Longitude	Sediment Description and Shepard Classification
51. Lake Diefenbaker at Riverhurst Ferry Crossing	50°54'55"	106°55'50"	sand 20.84%, silt 25.86%, clay 53.30% SAND SILT CLAY
57. South Saskatchewan R. at Birch Hills, Hwy 20 Bridge	53°04'44"	105°29'27"	sand 67.80%, silt 24.62%, clay 7.58% SILTY SAND
62. Saskatchewan R. below Codette Reservoir	53°19'28"	104°02'00"	gravel 0.22%, sand 79.65%, silt 14.30%, clay 5.84% SAND
64. Saskatchewan R. at Nipawin, 0.5 km below railway bridge	53°22'18"	104°01'47"	gravel 0.3%, sand 73.45%, silt 23.28%, clay 3.25% SILTY SAND organic material present
61. Saskatchewan R. below Hwy 55 bridge	53°22'58"	104°00'40"	sand 90.74%, silt+clay 9.26% SAND organic material present
60. Saskatchewan R. above Tobin Lake	53°24'19"	103°58'07"	sand 64.53%, silt 22.21%, clay 13.26% SILTY SAND
59. Tobin Lake near inlet of Saskatchewan River	53°28'23"	103°55'27"	sand 56.56%, silt 24.49%, clay 18.95% SILTY SAND
66. Tobin Lake off Carroll's Cove, south side	53°30'49"	103°45'53"	sand 6.42%, silt 29.45%, clay 64.13% SILTY CLAY
67. Tobin Lake off Carroll's Cove, north side	53°31'29"	103°47'00"	sand 3.63%, silt 39.79%, clay 56.58% SILTY CLAY
65. Tobin Lake off Prudhomme Campground	53°33'23"	103°40'00"	sand 0.98%, silt 17.16%, clay 81.87% CLAY
<u>QU'APPELLE RIVER BASIN</u>			
52. Buffalo Pound Lake near Buffalo Pound Provincial Park	50°37'01"	105°25'27"	sand 1.42%, silt 23.28%, clay 75.30% CLAY

Table 1. (continued)

Station Name and Number	Latitude	Longitude	Sediment Description and Shepard Classification
53. Pasqua Lake near mid lake	50°46'27"	104°00'00"	sand 93.10%, silt+clay 6.9% SAND
54. Pasqua Lake near outlet to Qu'Appelle River	50°47'42"	103°54'26"	sand 26.94%, silt+clay 4.06% SAND shells present
55. Katepwa Lake at outlet to Qu'Appelle River	50°39'52"	103°36'33"	sand 93.9%, silt+clay 6.10% SAND shells present
56. Qu'Appelle River at Lumsden	50°39'05"	104°52'15"	sand 15.07%, silt 42.46%, clay 42.46% SILTY CLAY
<u>CHURCHILL RIVER DIVERSION ROUTE</u>			
68. Split Lake, York Landing water treatment plant intake bay	56°04'42"	96°05'20"	sand 1.52%, silt 54.34%, clay 44.15% CLAYEY SILT
69. Split Lake, York Landing, west side of Ferry Dock	56°05'00"	96°06'25"	sand 13.61%, silt 44.96%, clay 41.43% CLAYEY SILT
70. Split Lake, Split Lake Community, beach east of water treatment plant	56°14'20"	96°07'05"	gravel 0.06%, sand 99.22%, silt+clay 0.72% SAND
71. Split Lake, Split Lake Community, Sewage treatment plant outfall	55°14'30"	96°07'05"	sand 72.39%, silt 12.21%, clay 15.40% CLAYEY SAND
72. Split Lake, Split Lake Community, nursing station beach	56°14'30"	96°06'20"	gravel 0.03%, sand 98.07%, silt+clay 1.90% SAND
73. Footprint Lake, Nelson House Metis beach	55°44'30"	98°51'00"	sand 10.36%, silt 22.64%, clay 67.00% SILTY CLAY
74. Footprint Lake, Nelson House Industrial Bay, Causeway #2	55°47'45"	98°52'50"	sand 1.93%, silt 17.61%, clay 70.45% SILTY CLAY
75. Footprint Lake, Nelson House School Bay at sewage treatment plant outfall	55°47'10"	09°53'00"	sand 12.08%, silt 16.54%, clay 71.38% SILTY CLAY

Table 2. Point Awarding Scheme for Sample Ranking, Based on Suspected Contained Hazards.

Fecal Coliform Fecal Streptococci Enterococci per 10 g/100 mL sediment /100 mL water	Coliphage per 100 mL water	Clostridium perfringens per 10 g/100 mL sediment	SOS Chromotest		Algal-ATP		Cholesterol ppb	Points
			Genotoxicity Induction Factor per mL 10x Water Sample 1:1 M111+Q Water Sediment Extract 1% DMSO Sediment Extract	Coprostanol ppb	% Relative Light Units per mL 10x Water Sample 1:1 M111+Q Water Sediment Extract 1% DMSO Sediment Extract	Points		
1 - 100	5 - 24	1 - 25	1.0 - 1.29	<1.0	100 - 50	1	<2.0	1
101 - 500	25 - 100	26 - 100	1.30 - 1.50	1 - 3.0	49 - 20	3	2.1 - 4.0	2
501 - 2500	101 - 250	101 - 500	1.51 - 2.0	3.1 - 5.0	19 - 1.0	5	4.1 - 6.0	3
2501 - 16000	251 - 1000	501 - 2500	2.1 - 3.0	5.1 - 7.0	0.9 - 0.1	7	6.1 - 8.0	4
16001 - 160000	1001 - 5000	2501 - 10000	3.1+	7.1+	0.09+	10	8.1+	5
160000+	5001+	10000+						

ATP-TOX System % Inhibition per mL 10x Water Sample 1:1 M111+Q Water Sediment Extract	Microtox EC50 % per mL 10x Water Sample 1:1 M111+Q Water Sediment Extract	Daphnia magna		Solirillum volutans	
		Points	EC % per mL 1:1 M111+Q Water Sediment Extract	Points	EC % per mL Water Sample 1% DMSO Sediment Extract
1 - 30	40.0+	1	EC20 at 100%	1	negative
31 - 60	40.0 - 25.0	3	EC40 at 100%	2	negative
61 - 90	24.0 - 10.0	5	EC50 at 100%	5	
91 - 99	9.0 - 1.0	7	at 75%	7	
100	0.9+	10	at 50%	8	
			at 25%	10	
			at 10%		

Table 3. Results of Saskatchewan River Basin and Northern Manitoba Native Community Water Analysed by Battery of Tests Approach.

Sample Number	Fecal Coliform MF-mFC /100 mL	Fecal Strepto- cocci MF-KF /100 mL	Collipage /100 mL	Fecal Sterols		Microtox EC ₅₀ % mL	Algal-ATP RLU %	Spirillum volutans 120 min test	SOS Chromotest Induction Factor	ATP-TOX % Inhibition ²	Daphnia ³ Magna EC ₅₀ %	Points	Rank
				Coprostanol	Cholesterol								
				ppb	ppb						Sample		
Saskatchewan River Basin													
41	142,000	340	10		2.86	<0.05	NEG	NEG	1.00	28	92	19	1
42	9,600	290	115		0.65	0.10	NEG	NEG	1.08	6	92	18	2
43	10	34	<5		2.86	0.10	NEG	NEG	1.92	12	50	11	7
44	5	110	<5		3.30	0.05	NEG	NEG	.92	9	NEG	5	13
45	20	13	<5		1.22	<0.05	NEG	NEG	.92	18	72	11	7
46	100	183	25		2.97	<0.05	NEG	NEG	.67	66	86	17	3
47	10	103	<5		1.65	<0.05	NEG	NEG	.92	23	EC ₄₀	7	11
48	60	108	5		1.12	<0.05	NEG	NEG	.73	32	73	14	4
49	3	8	<5		2.48	<0.05	NEG	NEG	.71	15	100	9	9
50	3	7	<5		1.30	<0.05	NEG	NEG	.73	21	EC ₂₀	6	12
51	<1	<1	5		2.59	<0.05	NEG	NEG	.91	30	NEG	4	14
52	<1	<1	<5		1.28	<0.05	NEG	NEG	.36	33	EC ₂₀	5	13
53	<1	9	<5		5.50	0.20	NEG	NEG	.79	17	100	10	8
54	220	18	660		1.82	<0.05	NEG	NEG	.57	33	NEG	10	8
55	1	19	5		1.07	<0.05	NEG	NEG	.50	34	NEG	7	11
56	30	21	20		1.61	<0.09	NEG	NEG	.71	17	NEG	6	12
57	20	16	<5		1.34	<0.05	NEG	NEG	1.71	17	68	13	5
58	2	23	<5		4.14	<0.05	NEG	NEG	.71	10	EC ₄₀	8	10
59	7	40	<5		1.06	<0.05	NEG	NEG	.50	38	NEG	6	14
60	2	46	<5		9.14	<0.05	NEG	NEG	.79	20	NEG	5	12
61	7	47	<5		0.36	<0.05	NEG	NEG	.50	38	NEG	6	12
Qu'Appelle River Basin													
52	<1	<1	<5		2.12	<0.05	NEG	NEG	.79	33	90	10	8
53	<1	4	<5		5.14	<0.05	NEG	NEG	.64	48	84	12	6
54	<1	2	<5		2.85	<0.05	NEG	NEG	.57	78	EC ₃₀	9	9
55	<1	<1	<5		3.89	<0.05	NEG	NEG	.50	80	NEG	3	15
56	88	215	10		5.43	0.11	NEG	NEG	.64	57	EC ₂₀	12	6
Churchill River Diversion Route													
68	<1	11	<5		2.97	<0.05	NEG	NEG	.71	30	NEG	4	14
69	<1	6	<5		0.99	<0.05	NEG	NEG	1.00	37	NEG	6	4
70	237	23	<5		1.85	<0.05	NEG	NEG	1.09	26	NEG	6	12
71	5,800	1	10		.25	<0.05	NEG	NEG	1.20	32	NEG	11	4
72	2	18	<5		.75	<0.05	NEG	NEG	1.00	35	NEG	7	11
73	1	3	<5		.62	<0.05	NEG	NEG	.73	25	NEG	4	5
74	181	111	<5		.85	<0.05	NEG	NEG	.64	31	NEG	8	14
75	7	5	<5		.24	<0.05	NEG	NEG	.73	16	NEG	4	5

Table 4. Results of Saskatchewan River Basin and Native Community Water-extracted Sediment Analyses by Battery of Tests Approach.

Sample Number	Fecal Coliform AI broth 10 g/100 mL MPN	Clostridium perfringens 10 g/100 mL MPN	Microtox EC ₅₀ /mL Water Extract	Algal-ATP %RLU/mL Water Extract	Spirillum volutans 120 min test Water Extract	SOS Chromotest Induction Factor/mL Water Extract	ATP-TOX % Inhibition /mL Water Extract	Daphnia Magna EC ₅₀ Water Extract	Points	Rank
Saskatchewan River Basin										
41	28,000	49	NEG	S	NEG	0.52	NEG	100	14	3
42	92,000	350	S	S	NEG	0.79	NEG	100	16	3
43	14,000	920	S	S	NEG	0.71	NEG	EC ₂₀	9	10
44	2,200	1,600	NEG	S	NEG	0.42	15	EC ₂₀	9	10
45	2,200	1,920	NEG	S	NEG	0.52	20	EC ₃₀	9	10
46	11,000	70	S	S1	NEG	0.71	16	100	13	6
47	2,300	540	NEG	S	NEG	0.91	NEG	92	12	7
48	4,600	1,600	S	S	NEG	0.86	18	EC ₂₀	10	9
58	2,400	2,400	S	S	NEG	1.18	6.9	92	13	6
49	7	180	S	S	NEG	1.00	14	EC ₂₀	7	12
50	2	350	S	S	NEG	0.95	NEG	NEG	4	14
51	5	120	S	S	NEG	1.05	NEG	EC ₃₀	6	13
57	3,300	9,200	S	S	NEG	1.19	2.1	EC ₄₀	15	4
62	1,700	700	S	S	NEG	1.14	NEG	72	15	4
64	8	220	S	S	NEG	0.86	23	45	13	6
61	92,000	280	S	S	NEG	1.14	1.9	84	17	2
60	2,300	1,600	S	S	NEG	1.18	NEG	EC ₃₀	7	12
59	26	700	NEG	S	NEG	1.19	14	82	12	7
66	<2	11,000	S	S	NEG	0.86	2.9	100	16	3
67	22	700	S	S	NEG	1.05	7.9	82	12	7
65	5	2,200	S	S	NEG	0.86	13	92	11	8
Qu'Appelle River Basin										
52	5	130	S	NEG	NEG	1.05	NEG	EC ₃₀	6	13
53	7	1,600	S	NEG	NEG	0.91	NEG	92	12	7
54	11	49	S	S	NEG	1.00	NEG	92	9	10
55	17	110	S	S	NEG	1.00	5.8	78	11	8
56	35,000	2,400	S	S	NEG	1.14	1.5	82	18	1
68	<2	70	S	S	NEG	0.91	NEG	60	9	10
69	7	290	S	S	NEG	0.91	7.6	82	10	9
70	490	600	S	S	NEG	0.86	11	60	14	5
71	490	140	NEG	S	NEG	1.09	NEG	86	6	13
72	49	130	S	S	NEG	1.00	4.9	92	13	6
73	22	540	S	S	NEG	1.00	15	EC ₂₀	8	11
74	230	46	S	S	NEG	1.00	NEG	EC ₂₀	6	13
75	170	70	S	S	NEG	0.89	NEG	20	14	5

Table 5. Results of Saskatchewan River Basin Organically Extracted Sediments Analysed by Battery of Toxicant Screening Tests Approach

Sample number	Microtox EC ₅₀ /mL Organic Extract	Algal-ATP %RLY/mL Organic Extract	<u>Spirillum</u> <u>volutans</u> 120 min test Organic Extract	SOS Chromotest Induction Factor/mL Organic Extract	ATP-TOX % Inhibition /mL Organic Extract	Points	Rank
<u>Saskatchewan River Basin</u>							
41	NEG	S	NEG	.71	NEG	0	-
42	2.1	S	POSITIVE	1.240	45	12	2
43	NEG	S	NEG	.98	NEG	0	-
44	NEG	S	NEG	.86	NEG	0	-
45	1.0	S	NEG	.98	NEG	7	4
46	S	S	NEG	.62	NEG	0	-
47	43.8%	S	NEG	.98	23	2	8
48	NEG	S	NEG	1.49	NEG	3	7
58	37.2%	S	NEG	.91	NEG	3	7
49	NEG	S	NEG	1.69	NEG	0	-
50	NEG	S	NEG	.93	NEG	0	-
51	19.7%	S	NEG	1.81	NEG	5	6
57	NEG	S	NEG	0.94	1	1	9
62	NEG	S	NEG	.76	16	1	9
64	NEG	S	POSITIVE	.94	5	6	5
61	NEG	S	NEG	.75	NEG	0	-
60	19.9%	S	NEG	.96	22	6	5
59	49.6%	S	NEG	.89	NEG	1	9
66	46.4%	S	NEG	.87	24	2	8
67	27.4%	S	NEG	.73	43	6	5
65	NEG	S	NEG	.96	8	1	9
<u>Qu'Appelle River Basin</u>							
52	0.3%	S	NEG	1.19	15	12	3
53	29.2%	S	NEG	1.01	14	5	6
54	NEG	S	POSITIVE	1.26	NEG	6	5
55	NEG	S	NEG	1.11	NEG	1	9
56	1.9%	S	NEG	12.00	11	18	1

Table 6. Sites of Greatest Concern Based on the Battery of Tests Approach and Ranking Scheme

Water Column Rank	Water Extracted Sediment	Organically Extracted Rank	Site Sediment Rank
2	4	2	N. Sask. R. below Fort Saskatchewan
	1	1	Qu'Appelle River at Lumsden
5		5	Sask. R. above Tobin Lake

APPENDIX

IDENTIFICATION OF FECAL COLIFORM ISOLATES BASED ON API 20E SYSTEM

Isolate Number	Site No.	Identification by API Book	Identification Computer Probability
1	41	<u>E. coli</u>	
2		<u>E. coli</u>	
3		<u>E. coli</u>	
4			<u>E. coli</u>
5		<u>E. coli</u>	
6			<u>E. coli</u>
7		<u>E. coli</u>	
8			<u>E. coli</u>
9		<u>E. coli</u>	
10			<u>E. coli</u>
11	42	<u>E. coli</u>	
12		<u>E. coli</u>	
13			<u>E. coli</u>
14		<u>E. coli</u>	
15		<u>E. coli</u>	
16		<u>E. coli</u>	
17		<u>E. coli</u>	
18		<u>E. coli</u>	
19			<u>E. coli</u>
20		<u>E. coli</u>	
21	43	<u>E. coli</u>	
22		<u>E. coli</u>	
23		<u>E. coli</u>	
24	44	<u>E. coli</u>	
25			<u>E. coli</u>
26	45	<u>E. coli</u>	
27		<u>E. coli</u>	
28			<u>E. coli</u>
29			<u>E. coli</u>
30		<u>E. coli</u>	
31		<u>E. coli</u>	
32	46	<u>E. coli</u>	
33		<u>E. coli</u>	
34		<u>E. coli</u>	
35		<u>E. coli</u>	
36		<u>E. coli</u>	
37		<u>E. coli</u>	
38		<u>E. coli</u>	
39		<u>E. coli</u>	
40		<u>E. coli</u>	

APPENDIX
continued
IDENTIFICATION OF FECAL COLIFORM ISOLATES BASED ON API 20E SYSTEM

Isolate Number	Site No.	Identification by API Book	Identification Computer Probability
41			<u>E. coli</u>
42	47	<u>E. coli</u>	
43	48	<u>E. coli</u>	
44		<u>E. coli</u>	
45			<u>E. coli</u>
46			<u>E. coli</u>
47		<u>E. coli</u>	
48		<u>E. coli</u>	
49		<u>E. coli</u>	
50	49	<u>E. coli</u>	
51			<u>E. coli</u>
52		<u>E. coli</u>	
53	56	<u>E. coli</u>	
54		<u>E. coli</u>	
55		<u>E. coli</u>	
56		<u>E. coli</u>	
57			<u>S. paratyphi A</u>
58		<u>E. coli</u>	
59			<u>E. coli</u>
60			<u>E. coli</u>
61		<u>E. coli</u>	
62			
63	58	<u>E. coli</u>	
64		<u>E. coli</u>	
65			<u>S. paratyphi A</u>
81	59		<u>E. cloacae</u>
82			<u>E. cloacae</u>
83	60		<u>E. cloacae</u>
84			<u>E. cloacae</u>
85			<u>E. cloacae</u>
86			<u>E. cloacae</u>
87			<u>E. cloacae</u>
88			<u>E. cloacae</u>
89			<u>E. cloacae</u>
90			<u>E. cloacae</u>
91			<u>E. cloacae</u>
92			<u>E. cloacae</u>
93	61		<u>E. cloacae</u>
94			<u>E. cloacae</u>
95		<u>E. coli</u>	
96			<u>E. cloacae</u>

APPENDIX
continued
IDENTIFICATION OF FECAL COLIFORM ISOLATES BASED ON API 20E SYSTEM

Isolate Number	Site No.	Identification by API Book	Identification Computer Probability
97			<u>E. cloacae</u>
98			<u>E. cloacae</u>
99			<u>E. cloacae</u>
100			<u>E. cloacae</u>
101			<u>E. coli</u>
102			<u>E. coli</u>
103	62		<u>Kluyvera sp.</u>
104		<u>E. coli</u>	
105			
106			<u>E. cloacae</u>
107			<u>E. coli</u>
108			<u>E. coli</u>
109			<u>E. cloacae</u>
110			<u>E. cloacae</u>
111			<u>Shigella</u> <u>dysenteriae</u>
112			<u>E. cloacae</u>
113		<u>E. coli</u>	
114			
115	64		<u>E. cloacae</u>
116	65	<u>E. cloacae</u>	<u>E. cloacae</u>
117		<u>Klebsiella pneumoniae</u>	
118		<u>K. pneumoniae</u>	
119		<u>E. coli</u>	
120			<u>Samonella</u> <u>enteritidis</u> <u>e. amnigenus</u>
121			
122		<u>E. coli</u>	
123	66	<u>E. coli</u>	
124		<u>E. coli</u>	
125		<u>E. coli</u>	
126			<u>E. coli</u>
127		<u>E. coli</u>	
128			<u>E. cloacae</u>
129		<u>E. coli</u>	
130			<u>E. coli</u>
131		<u>E. coli</u>	
133	67		
134	70		<u>E. cloacae</u>
135		<u>E. coli</u>	
136		<u>E. coli</u>	
137		<u>E. coli</u>	
138		<u>E. coli</u>	

APPENDIX
continued
IDENTIFICATION OF FECAL COLIFORM ISOLATES BASED ON API 20E SYSTEM

Isolate Number	Site No.	Identification by API Book	Identification Computer Probability
139		<u>E. coli</u>	
140		<u>E. coli</u>	
141		<u>E. coli</u>	
142		<u>E. coli</u>	
143		<u>E. coli</u>	
144		<u>E. coli</u>	
145		<u>E. coli</u>	
146	71	<u>E. coli</u>	
147		<u>E. coli</u>	
148		<u>E. coli</u>	
149		<u>E. coli</u>	
150		<u>E. coli</u>	
151		<u>E. coli</u>	
152		<u>E. coli</u>	
153		<u>E. coli</u>	
154		<u>E. coli</u>	
155		<u>E. coli</u>	
156		<u>E. coli</u>	
157		<u>E. coli</u>	
158		<u>E. coli</u>	
159			<u>K. pneumoniae</u>
159a	73	<u>K. pneumoniae</u>	
160	74	<u>E. coli</u>	
161			<u>Kluyvera sp.</u>
162		<u>E. coli</u>	
163		<u>E. coli</u>	
164			<u>Tatlockia ptyseos</u>
164a			<u>Proteus mirabilis</u>
165		<u>E. coli</u>	
166			<u>S. paratyphi A</u>
167		<u>E. coli</u>	
168			<u>S. paratyphi A</u>
169		<u>E. coli</u>	
170	75		<u>E. coli</u>
171			<u>Serratia oderifera</u>
172		<u>Salmonella enteritidis</u>	
173			<u>K. pneumoniae</u>
174			<u>S. paratyphi A</u>
175			<u>K. pneumoniae</u>
176			<u>K. pneumoniae</u>
177			<u>K. oxytoca</u>
178			<u>S. enteritidis</u>