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Toxicity of Municipal Wastewater to Two Species of Fish, the Cladoceran *Daphnia magna*, and the Mollusc *Anodonta grandis*

Alberta
ENVIRONMENTAL CENTRE

TOXICITY OF MUNICIPAL WASTEWATER TO TWO SPECIES OF FISH, THE CLADOCERAN *Daphnia magna*, AND THE MOLLUSC *Anodonta grandis*

by

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SUMMARY

The purpose of this study was to determine the toxicity of influent and effluent from ten large municipal wastewater treatment plants, representing three process types, in Alberta. This permitted an evaluation to be made of the efficiency of the treatment system and of the toxicity of wastewater discharged to the environment. Both acute and chronic toxicity were determined using rainbow trout (*Oncorhynchus mykiss*), and Cladocera (*Daphnia magna*). Molluscs (*Anodonta grandis*) and fathead minnow (*Pimephales promelas*) were also used in acute toxicity tests. Histopathologic evaluation of tissues was also conducted following exposure of fish to wastewater.

The following plants were evaluated: Calgary (Bonnybrook), Camrose, Edmonton (Capital Region), Edmonton (Goldbar), Fort McMurray, Grande Prairie, Lethbridge, Medicine Hat, Red Deer, Wetaskiwin. To assess the effect of season (and hence, temperature) on the plants' abilities to reduce toxicity, samples were collected on two occasions, generally during the winter and summer. Heavy metal and ammonia-N analyses were conducted on each sample. Prior to implementation of the toxicologic procedure, each sample was amended to remove ammonia-N; in addition, all samples were aerated to eliminate the possibility of suffocation of test species.

Based on the samples collected, it was observed that:

1. influent and effluent from all plants were generally not acutely toxic (LC_{50} ≥100%) to the four test species;
2. the primary lesions found in fish following exposure to influent and effluent were minor disorders associated with the skin and, to a lesser degree, the gills;
3. there were no seasonal differences in potential toxic effects;
4. the basic plant design (aerated lagoon versus mechanical-biological versus lagoon treatment system) did not produce differences in potential toxic effects;
5. exposure of *Daphnia magna* for 21 d to effluent from Edmonton (Goldbar) and Edmonton (Capital Region) resulted in a statistically significant increase in the production of neonates, implying no long-term toxicity of the effluent to this species;

6. there was no change in body weight or fork length when rainbow trout were exposed to effluent from Edmonton (Goldbar) for 29 d and to effluent from Edmonton (Capital Region) for 28 d.

It was concluded that, under the conditions of this study, influent and effluent wastewater from the ten plants was nontoxic to the test species. It is recommended that follow-up studies be conducted to investigate the effects of unamended samples on test species.

1 INTRODUCTION

1.1 Background

Municipal wastewater and sludges may contain a substantial amount of potentially toxic agents. Hydrocarbons from vehicular traffic, heavy metals from metal working industries, plus solvents, degreasers and asbestos may all be discharged to the municipal treatment system. In one study on sewage sludges from 40 plants in England, chromium, copper, lead, manganese and zinc were all above 600 mg/kg dry weight (Table 1). The decomposition of organic material may also lead to the production of toxic gases, such as H_2S, other breakdown products, such as ammonia, and high suspended solid levels.

Table 1. Concentration of heavy metals in 40 sewage sludges from England.

Metal	Concentration (mg/kg dry weight)	
	Mean	Range
Cadmium	25	2 - 110
Chromium	707	57 - 5190
Cobalt	105	11 - 2490
Copper	721	170 - 2080
Lead	1550	28 - 45,400
Manganese	667	131 - 6120
Molybdenum	16	0.1 - 214
Nickel	290	16 - 2020
Tin	58	3 - 329
Zinc	1930	94 - 9210

Source: Sterritt and Lester (1981).

Municipal wastewater treatment plants are designed to reduce the toxicity of many agents prior to discharge to surface waters. Plant performance varies with the type of waste, season, plant design, and maintenance of the treatment facilities. Hannah *et al.* (1986), for example, showed that the effectiveness in removal of heavy metals was approximately similar among activated sludge, aerated lagoons, and facultative lagoons (Table 2). Primary clarification, on the other hand, was least effective, followed by trickling filter. Organic compounds were most efficiently removed by facultative lagoon and activated sludge processes (Table 3).

Table 2. Percentage removal of heavy metals from municipal wastewater using different processes.

Metal	Process				
	A	B	C	D	E
Cadmium	12	28	24	ND	32
Chromium	7	52	82	71	79
Copper	19	60	82	74	79
Lead	30	48	65	58	50
Nickel	4	30	43	35	43
Average	14	44	59	50	57

Source: Hannah *et al.* (1986). ND=no data; A=primary clarification; B=trickling filter; C=activated sludge; D=aerated lagoon; E=facultative lagoon.

If the type of treatment system is not designed to remove specific compounds, toxic wastewater may be produced. For example, Wylie *et al.* (1990) showed that fathead minnow (*Pimephales promelas*) and Cladocera (*Ceriodaphnia dubia*) did not survive for more than a few hours in effluents from two treatment plants in Joplin, Missouri. This toxicity was due to relatively high concentrations (0.13-0.97 mg/L) of pentachlorophenol from a wood-preservative plant. Neiheisel *et al.* (1988), working on six treatment plants in Ohio, noted that all plant influent was toxic to fathead minnow and *Ceriodaphnia dubia*. All plant effluents showed some residual toxicity, and the plant receiving the most toxic influent showed the largest reduction in toxicity. A study of plants in Ontario showed that toxicity was generally due to high concentrations of ammonia-N, poor biological oxygen demand (BOD) removal, and possibly chlorination (Ontario Ministry of Environment, 1990).

Table 3. Perçentage removal of volatile organic compounds from municipal wastewater using different processes.

Compound	Process						
	A	B	C	D	E	F	G
Bromoform	18	2	6	57	65	80	84
Carbon tetrachloride	19	22	13	59	74	70	77
1,1-Dichloroethane	2	32	21	34	94	68	87
1,1-Dichloroethylene	5	22	25	58	92	60	85
Chloroform	7	18	20	25	86	51	80
1,2-Dichloroethane	7	34	22	33	84	70	90
Ethylbenzene	9	35	31	71	93	70	96

Source: Hannah *et al.* (1986). A=primary clarification; B=primary plus filtration; C=chemical clarification; D=trickling filter; E=activated sludge; F=aerated lagoon; G=facultative lagoon.

1.2 Purpose of Study

The purpose of this study was to determine the toxicity of influent and effluent from large municipal treatment plants, representing three types, in Alberta. Both acute and chronic toxicity were determined using rainbow trout (*Oncorhynchus mykiss*), and Cladocera (*Daphnia magna*). Molluscs (*Anodonta grandis*) and fathead minnow (*Pimephales promelas*) were also used in acute toxicity tests. Such information is useful in determining (i) the efficiency of the treatment system of different systems, and (ii) toxicity of effluent discharged to surface waters.

2 MUNICIPAL WASTEWATER TREATMENT PLANTS

Ten municipal wastewater treatment plants were located in the Edmonton region, Calgary, Grande Prairie, Fort McMurray, Camrose, Wetaskiwin, Lethbridge, Medicine Hat, and Red Deer (Table 4). During this study, Camrose used a lagoon treatment system, whereas Fort McMurray and Wetaskiwin used aerated lagoons. The Medicine Hat plant also used an aerated lagoon system prior to the spring (1990), but then switched to mechanical-biological treatment. All other plants also used a mechanical-biological system.

Collections of influent and effluent were made from the ten plants during 1989 and 1990. The dates for collection of samples used in acute toxicity are in Table 5 and those from chronic toxicity tests are in Table 6.

Table 4. Municipal waste treatment methods used at the ten plants.

Calgary (Bonnybrook)
Screening > grit removal > primary clarification > aeration (activated sludge) > secondary clarification and phosphorus removal (alum precipitation) > continuous discharge (Bow River)
Camrose
Wastewater stabilization ponds (four anaerobic cells, one facultative cell, and four storage cells - seven months storage) > discharge twice per year (spring and fall) to Battle River via Camrose Creek
Edmonton (Capital Region)
Screening > grit removal > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (North Saskatchewan River)
Edmonton (Goldbar)
Grit removal > bar screens > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (North Saskatchewan River)
Fort McMurray
Aerated lagoons > continuous discharge (Athabasca River)
Grande Prairie
Screening > grit removal > primary clarification > biological treatment (rotating biological contact process) > secondary clarification > storage lagoon (30 days) > continuous discharge (Wapiti River)

Lethbridge
Mechanical screening > grit removal > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (Oldman River) Note: Plant consists of two parallel plants, one for domestic wastewater treatment and one for industrial wastewater treatment. Final discharge is a mixture of effluents from the two plants.
Medicine Hat
Prior to March (1990): Grit removal > primary clarification > aeration (four aerated lagoons) > facultative lagoon > continuous discharge (South Saskatchewan River) (NOTE: some wastewater irrigation) After March (1990): Grit removal > primary clarification > biological treatment (trickling filter-solids contact process) > secondary clarification with phosphorus removal > disinfection using Cl_2 > polishing and dechlorination discharge (South Saskatchewan River)
Red Deer
Screening > grit removal > primary clarification > aeration (activated sludge) > secondary clarification > continuous discharge (Red Deer River)
Wetaskiwin
Screening > aerated lagoons > polishing ponds > storage lagoon (seven months) > discharge, twice per year (Battle River via a tributary)

Table 5. Collection dates for samples used in acute toxicity tests.

Plant	Sampling Dates
Calgary (Bonnybrook)	18 January 1990; 6 June 1990
Camrose	16 November 1989; 3 May 1990
Edmonton (Capital Region)	30 November 1989; 21 June 1990
Edmonton (Goldbar)	23 November 1989; 14 June 1990
Fort McMurray	6 October 1989; 1 March 1990
Grande Prairie	12 October 1989; 4 January 1990
Lethbridge	8 February 1990; 31 May 1990
Medicine Hat	12 January 1990; 24 May 1990
Red Deer	7 December 1989; 21 June 1990
Wetaskiwin	2 November 1989; 24 April 1990

Table 6. Collection dates for samples used in chronic toxicity tests.

Plant	Sampling Dates
Edmonton (Capital Region)	16 November; 23 November; 30 November; 7 December, 1990
Edmonton (Goldbar)	12 October; 19 October; 26 October; 2 November, 1990

3 COLLECTION PROCEDURES AND SAMPLE MANIPULATION

All influent samples were collected immediately after screening whereas all effluent samples were taken immediately prior to discharge to the receiving waters. Unlike all other systems, the Lethbridge plant had two parallel processes, one for treatment of domestic wastewater and the other for treatment of industrial wastewater (Table 4). The influent samples used for toxicity evaluation were obtained by manually combining equal amounts of wastewater from the two processes. On the other hand, the effluent from the two processes was mixed within the plant as part of the treatment process prior to discharge. Hence there was no need to manually combine wastewater from the two processes.

Grab samples were collected in a 10-L stainless steel pail and placed in 20-L polypropylene containers. A total of 120 L of both influent and effluent was taken on each sampling date. All samples were collected over a relatively short time period (1-2 min), so potential daily (or longer term) changes in hydraulic flow and wastewater quality may have gone undetected by the sampling regime. However, since the toxicological procedures used in this study yielded consistent data (see Results), the extent of diurnal changes in the toxicological properties of the wastewater was probably minimal.

During transport, the samples were held at ambient temperature. On receipt at the laboratory, the influent and effluent samples were poured into individual 300-L polypropylene tanks. The sample temperature, pH, conductivity, and dissolved oxygen were then determined using a Hydrolab® meter. A 500-mL aliquot was taken for analysis of heavy metals, preserved by acidification, and analyzed by Chemistry Division using standard methods (Alberta Environmental Centre, 1987).

Preliminary analysis indicated that the concentration of ammonia-N in several samples was high and might result in rapid death of experimental organisms. Because the toxicity of ammonia-N had already been well established by other investigators, the samples were manipulated using the following procedure to remove ammonia-N. All samples were aerated at 20°C for 48 h to raise pH to approximately 8.5. At this pH, ammonia-N dominates the ammonia/ammonium complex, and results in the volatilization of free ammonia. pH was then adjusted to 6.0 using 1-10 N HCl to reduce the proportion of residual ammonia-N to <0.01%.

A 125-mL aliquot was taken and cooled to 4°C for approximately 24 h prior to analysis of ammonia-N. The analysis was conducted by a commercial laboratory (Norwest Labs, Edmonton, AB) using an automated colorimetric method. The samples were then poured for implementation of toxicologic procedures (Section 4). It is recognized that these manipulative procedures may have amended the samples in ways other than the removal of ammonia-N. However, the concentration of ammonia-N in samples that were not manipulated was so high that all test species would have died in a relatively short time period. Manipulation of samples makes possible the evaluation of toxicity not related to ammonia-N.

4 TOXICOLOGIC PROCEDURES

A number of toxicologic procedures were used to evaluate the toxicity of influent and effluent (Table 7). It was not possible to implement all tests on all samples because of manpower restraints. All aquatic animals were maintained according to Standard Operating Procedures (Aquatic Biology Branch, 1991). A summary of water quality during maintenance of the four species is given in Table 8.

Table 7. Toxicologic procedures used on samples from ten municipal waste treatment plants.

Plant	Procedure					
	A	B	C	D	E	F
Calgary (Bonnybrook)						
influent	x	x	x			
effluent	x	x	x	x		
Camrose						
influent	x	x	x			
effluent	x	x	x	x		
Edmonton (Capital Region)						
influent	x	x	x			
effluent	x	x	x	x	x	x
Edmonton (Goldbar)						
influent	x	x	x			
effluent	x	x	x	x	x	x
Fort McMurray						
influent	x	x	x			
effluent	x	x	x	x		
Grande Prairie						
influent	x	x	x			
effluent	x	x	x	x		

Plant	Procedure					
	A	B	C	D	E	F
Lethbridge						
influent	x	x	x			
effluent	x	x	x	x		
Medicine Hat						
influent	x	x	x			
effluent	x	x	x	x		
Red Deer						
influent	x	x	x			
effluent	x	x	x	x		
Wetaskiwin						
influent	x	x	x			
effluent	x	x	x	x		

A = 96-h LC_{50} rainbow trout
B = 96-h LC_{50} fathead minnow
C = 96-h LC_{50} *Anodonta grandis*
D = 48-h LC_{50} *Daphnia magna*
E = 21-d multigeneration study *Daphnia magna*
F = 28-d growth study rainbow trout

All acute toxicologic procedures involving rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*), Cladocera (*Daphnia magna*), and Molluscs (*Anodonta grandis*) were conducted using Standard Operating Procedures (Aquatic Biology Branch, 1991). Histopathologic evaluation was conducted on tissues of a representative number of fish exposed to wastewater from each plant. Because of resource restraints, it was not possible to conduct histopathologic evaluation of all exposed fish. The 21-d chronic procedure with *Daphnia magna* was also conducted following Standard Operating Procedures (Aquatic Biology Branch, 1991). Collection and replacement dates of samples for the chronic *Daphnia magna* test were:

Capital Region (1990): 16 November, 23 November, 30 November, 7 December

Goldbar (1990): 12 October, 19 October, 26 October, 2 November

Table 8. Summary (average, range) of water quality conditions used during the maintenance of the four test species.

Species	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Conductivity (µS/cm)
Rainbow trout	12.9 (10.1 - 15.0) *N=425	10.6 (9 - 13) N=386	7.7 (7.0 - 8.3) N=386	222 (200 - 280) N=386
Fathead minnow	22.1 (13.6 - 28.0) N=590	7.1 (3.5 - 8.4) N=590	8.0 (7.1-9.7) N=590	289 (251 - 646) N=590
Daphnia magna	20.0 (18.4 - 21.3) N=130	7.1 (4.8 - 8.3) N=128	8.3 (7.4 - 9.5) N=128	596 (324 - 727) N=128
Anodonta grandis	21.0 (17.6 - 22.9) N=231	7.3 (6.3 - 9.6) N=58	8.3 (7.8 - 8.6) N=58	570 (298 - 877) N=58

*N = number of observations

A 28/29-d growth study involving rainbow trout was used to evaluate potential effects of the effluent on growth. The Edmonton (Capital Region) procedure was conducted for 28 d whereas the Edmonton (Goldbar) procedure was extended an additional day because of work schedule restraints. Collection and replacement dates are as outlined for *D. magna*. The method included a number of procedures.

i. **Acquisition and Maintenance of Rainbow Trout**: Conducted by Standard Operating Procedures 2350-AJ4/AN/AQ/11/91 and 2350-AJ4/AN/AQ/10/91 (Aquatic Biology Branch, 1991).

ii. **Feeding**: Conducted by Standard Operating Procedure 2350-AJ4/AN/AQ/10/91 (Aquatic Biology Branch, 1991).

iii. **Monitoring and Water Quality**: Conducted by Standard Operating Procedure 2350-AJ4/FAC/AQ/2/91 (Aquatic Biology Branch, 1991).

iv. **Number of Fish**: Three groups of ten rainbow trout were tested.

v. **Test Concentrations**: 100% effluent, 50% effluent, control.

vi. **Test Duration**: 28/29 days.

vii. **Exposure Chambers**: Polypropylene pails containing 40 L of medium; temperature 15 ± 1°C.

viii. **Growth Measurements**: Wet weight and fork length of all fish measured prior to the start of the experiment and at 28/29 days. Size of fish at the start of the experiment was relatively homogeneous (see Results).

ix. **Termination and Disposal**: Conducted by Standard Operating Procedures 2350-AJ4/PR/EUTH/1/91 and 2350-AJ4/PR/NEC/6/91 (Aquatic Biology Branch, 1991).

Examples of water quality conditions during implementation of the above-noted toxicologic procedures are listed in Tables 9a and 9b.

Table 9a. Summary (average, range) of water quality conditions in control chambers during implementation of toxicologic procedures.

Procedure	**Temperature (°C)**	**Dissolved Oxygen (mg/L)**	**Conductivity (µS/cm)**	**pH**
96-h LC_{50} rainbow trout	14.8 (12.7 - 20.6) *N=99	8.7 (7.4 - 9.7) N=99	292 (274 - 316) N=99	7.5 (6.1 - 8.1) N=99
96-h LC_{50} fathead minnow	20.4 (18.7 - 22.5) N=100	7.8 (6.6 - 9.0) N=100	296 (274 - 322) N=100	7.6 (6.5 - 8.5) N=100
96-h LC_{50} *Anodonta grandis*	18.5 (13.0 - 22.1) N=44	8.2 (7.2 - 10.5) N=44	309 (208 - 361) N=44	7.6 (5.8 - 8.3) N=44
48-h LC_{50} *Daphnia magna*	20.5 (18.5 - 22.3) N=40	7.9 (7.0 - 9.1) N=40	506 (254 - 670) N=40	8.1 (7.0 - 8.7 N=40
21-d *Daphnia magna*	20.4 (19.7 - 20.9) N=91	7.4 (5.8 - 8.6) N=144	475 (460 - 484) N=72	8.2 (7.8 - 8.7) N=144
Chronic rainbow trout	14.9 (14.4 - 15.9) N=64	8.4 (6.6 - 9.4) N=64	285 (267 - 310) N=64	7.9 (7.4 - 8.3) N=64

*N = Number of observations

Table 9b. Summary (average, range) of water quality conditions in chambers containing undiluted (100%) wastewater (influent and effluent) during implementation of toxicologic procedures.

Procedure	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity (µS/cm)	pH
96-h LC_{50} rainbow trout	15.1 (12.7 - 20.5) *N=99	8.6 (7.0 - 9.8) N=99	1386 (827 - 2330) N=99	7.5 (5.9 - 8.1) N=99
96-h LC_{50} fathead minnow	20.2 (19.2 - 22.5) N=100	7.5 (4.0 - 9.3) N=100	1563 (954 - 4020) N=100	7.4 (5.9 - 8.5) N=100
96-h LC_{50} *Anodonta grandis*	18.5 (16.6 - 23.7) N=44	7.9 (3.4 - 9.9) N=44	1523 (840 - 4300) N=44	7.5 (5.1 - 8.3) N=44
48-h LC_{50} *Daphnia magna*	20.6 (18.7 - 22.0) N=40	7.5 (4.6 - 8.6) N=40	1272 (403 - 2200) N=40	7.1 (5.8 - 8.2) N=40
21-d *Daphnia magna*	20.6 (19.5 - 21.1) N=44	7.0 (5.4 - 8.0) N=44	1100 (887 - 1264) N=44	6.5 (5.7 - 7.8) N=44
Chronic rainbow trout	14.6 (14.2 - 16.1) N=64	7.2 (2.8 - 9.1) N=64	1054 (856 - 1274) N=64	6.5 (4.6 - 8.0) N=64

*N = Number of observations

pH of the samples used during the 21-d *Daphnia magna* survival and reproduction test of Goldbar wastewater was 8.3 (8.0 - 8.7) for the control tank and 6.8 (5.7 - 7.8) for the 100% effluent tank. The corresponding values for the Capital Region plant were 8.1 (7.9 - 8.4) and 6.3 (5.8 - 7.4), respectively. pH of samples used during the 28-d rainbow trout study of Goldbar wastewater was 7.9 (7.6 - 8.3) for the control tank and 6.6 (4.6 - 8.0) for the 100% effluent tank. The corresponding values for the Capital Region plant were 7.9 (7.4 - 8.2) and 6.4 (5.0 - 7.0), respectively.

5 RESULTS

5.1 Calgary (Bonnybrook)

5.1.1 Chemical Analysis

Of the 15 heavy metals listed in Table 10, the majority underwent a reduction in concentration during the treatment process. Aluminum did, however, increase substantially between the influent and effluent, a result of the use of alum for coagulation during the treatment process. An anomalous iron level was found in the effluent collected on 6 June 1990, possibly the result of the addition of unidentified treatment chemicals. Ammonia-N was reduced by a factor of approximately ten during the treatment process.

5.1.2 Acute Toxicity

Rainbow trout, fathead minnow and *Anodonta grandis* were exposed to influent and effluent collected from the Calgary (Bonnybrook) plant on 18 January and 6 June 1990. In addition, *Daphnia magna* was exposed to effluent collected on 18 January and 6 June. In all cases, the influent and effluent were not acutely toxic; the LC_{50}'s were ≥100%.

5.1.3 Histopathologic Evaluation

Rainbow trout and fathead minnow exposed to influent (collected on 6 June 1990) at dilutions of 60-100% developed multifocal to diffuse, mild lamellar epithelial cell hypertrophy in the gills (Table 11). This effect was not observed at 20 and 40% concentrations or in the control fish. No abnormalities were noted in any other tissue, regardless of concentration.

Table 10. Chemical analysis (mg/L) of municipal wastewater collected from Calgary (Bonnybrook).

PARAMETER	DATE			
	18 JANUARY 1990		6 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.121	0.741	0.098	0.860
Ammonia-N*	21.0	2.7	17.7	1.09
Arsenic	0.0003	0.0002	0.0004	0.0005

PARAMETER	DATE			
	18 JANUARY 1990		6 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Barium	0.067	0.027	0.084	0.004
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.002	0.002	0.004	0.003
Chromium	0.007	0.004	0.008	0.008
Cobalt	<0.001	<0.001	0.001	0.002
Copper	0.031	0.005	0.024	0.009
Iron	0.240	0.082	0.282	0.720
Lead	0.003	0.006	<0.002	<0.002
Manganese	0.016	0.012	0.020	0.018
Molybdenum	0.003	0.004	0.003	0.007
Nickel	0.002	0.010	0.007	0.020
Selenium	0.0004	0.0001	0.0011	0.0001
Zinc	0.062	0.076	0.053	0.100

*Concentration after reduction (p. 7).

When rainbow trout were exposed to effluent (collected on 6 June 1990) at concentrations of 60-100%, multifocal to diffuse, mild lamellar epithelial cell hypertrophy of the gills was again observed (Table 11). The same condition occurred in fathead minnow, but only at a concentration of 40%. No abnormalities were noted in any other tissue of either species.

Table 11. Summary of histopathologic findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected on 6 June 1990 from Calgary (Bonnybrook).

INFLUENT	
Rainbow trout	Gills: multifocal to diffuse, mild lamellar epithelial cell hypertrophy
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: multifocal to diffuse, mild lamellar epithelial cell hypertrophy

EFFLUENT	
Rainbow trout	Gills: multifocal to diffuse, mild epithelial cell hypertrophy
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: multifocal, minimal lamellar epithelial hypertrophy (at 40% dilution only)
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%. No abnormalities observed at concentrations of 40%, 20% or control fish, unless otherwise noted.

When rainbow trout were exposed to influent collected on 15 January 1990, there was mild hyperplasia and hypertrophy of mucous cells in the skin (Table 12). No other tissues were affected by the influent. The same condition was noted following exposure of trout to effluent; in addition, mild blunting of the gill lamellae was observed. Because of resource limitations, it was not possible to evaluate the effects of wastewater collected on 15 January 1990 on fathead minnow.

Table 12. Summary of histopathologic findings of rainbow trout exposed to municipal wastewater* collected on 15 January 1990 from Calgary (Bonnybrook).

INFLUENT	
Rainbow trout	Skin: diffuse to multifocal, minimal to mild mucous cell hyperplasia and hypertrophy (also noted at 40% and 20% concentration)
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: multifocal, mild blunting of lamellae
	Skin: multifocal, minimal to mild mucous cell hypertrophy and hyperplasia (also noted at 40% concentration)
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%. No abnormalities noted at 40%, 20% or control fish, unless otherwise noted. No evaluation of fathead minnow conducted.

5.2 Camrose

5.2.1 Chemical Analysis

The majority of heavy metals, including aluminum, underwent a reduction in concentration during the treatment process (Table 13). Ammonia-N in the 16 November collections was similarly reduced from 29.0 to 0.3 mg/L, but for the 3 May samples, the reduction was relatively small (27.1 to 21.7 mg/L).

Table 13. Chemical analysis (mg/L) of municipal wastewater collected from Camrose.

PARAMETER	DATE			
	16 NOVEMBER 1989		3 MAY 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.149	0.033	4.715	0.157
Ammonia-N*	29.0	0.3	27.1	21.7
Arsenic	0.0021	0.0064	0.0004	0.0031
Barium	0.134	0.020	0.129	0.026
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.004	0.003	0.003	0.003
Chromium	0.008	0.003	0.011	0.005
Cobalt	0.002	0.002	0.003	0.002
Copper	0.155	0.003	0.164	0.007
Iron	4.089	0.103	2.794	0.339
Lead	0.069	<0.002	0.364	<0.002
Manganese	0.139	0.013	0.191	0.294
Molybdenum	0.005	0.002	0.009	0.002
Nickel	0.012	0.006	0.013	0.009
Selenium	0.0001	0.0001	0.0009	0.0003
Zinc	0.240	0.006	0.215	0.016

*Concentration after reduction (p. 7).

5.2.2 Acute Toxicity

Rainbow trout, fathead minnow and *Anodonta grandis* were exposed to influent and effluent collected from the Camrose lagoon on 16 November 1989 and 3 May 1990. In addition, *Daphnia magna* was exposed to effluent collected on 16 November 1989 and 3 May 1990. In all cases but one, the influent was not acutely toxic, with LC_{50}'s exceeding 100%. In the one exception, the 96-h LC_{50} for rainbow trout exposed to influent collected on 3 May was 92.8%. The effluent was not acutely toxic to any species, with LC_{50}'s exceeding 100%.

5.2.3 Histopathologic Evaluation

When rainbow trout were exposed to influent collected from the Camrose lagoon on 16 November 1989, no anomalies were noted in the gills, even for fish exposed to 100% influent (Table 14). There was a mild subacute hepatitis of the liver at concentrations of 20-100%, but all other tissues were unaffected at 20-100% concentration. Effluent from Camrose apparently had no effect on rainbow trout (Table 14). Fathead minnow showed some extensive bronchitis, but only at 60% concentration.

Table 14. Summary of histopathologic findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected on 16 November 1989 from Camrose.

INFLUENT	
Rainbow trout	Gills: no abnormalities noted
	Liver: multifocal, mild subacute hepatitis
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: multifocal to focally extensive, subacute bronchitis (60% concentration only)
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%, 40%, 20%. No abnormalities noted in control fish.

5.3 Edmonton (Capital Region)

5.3.1 Chemical Analysis

The majority of the metals underwent a reduction in concentration during the treatment process (Table 15). Ammonia-N was relatively high during both collection periods, but also fell sharply in the effluent. No chemical analysis was conducted on samples collected for evaluation of chronic toxicity.

Table 15. Chemical analysis (mg/L) of municipal wastewater collected from Edmonton (Capital Region).

PARAMETER	DATE			
	30 NOVEMBER 1989		21 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.488	0.074	0.393	0.218
Ammonia-N*	35.9	0.050	32.0	6.4
Arsenic	0.0015	0.0011	0.0014	0.0014
Barium	0.069	0.014	0.082	0.040
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.002	0.001	0.003	0.030
Chromium	0.037	0.027	0.008	0.012
Cobalt	0.002	<0.001	0.002	0.002
Copper	0.023	0.002	0.026	0.011
Iron	1.321	0.064	0.573	0.411
Lead	0.022	0.0032	0.006	0.010
Manganese	0.154	0.017	0.108	0.078
Molybdenum	0.004	0.004	0.004	0.005
Nickel	0.010	0.005	0.020	0.010
Selenium	0.0009	0.0002	0.0007	0.0005
Zinc	0.093	0.066	0.062	0.047

*Concentration after reduction (p. 7).

5.3.2 Acute Toxicity

Rainbow trout, fathead minnow and *Anodonta grandis* were acutely exposed to influent and effluent from the Edmonton (Capital Region) plant on 30 November 1989 and 21 June 1990. In addition, *Daphnia magna* was exposed to effluent collected on 30 November 1989 and 21 June 1990. In all cases the influent and effluent were not acutely toxic; the LC_{50}'s were ≥100%.

5.3.3 Histopathologic Evaluation

When rainbow trout were exposed to influent collected on 30 November 1989, blunting and edema of gills were observed at 100% concentration (the only concentration used) (Table 16). Although the skin of this species showed epithelia hyperplasia, no anomalies were noted in other tissues. Because of resource restraints, it was not possible to determine the effect of effluent collected on 30 November 1989 on the tissues of fathead minnow, or of effluent on rainbow trout and fathead minnow.

Table 16. Summary of histopathologic findings of rainbow trout exposed to municipal wastewater* collected on 30 November 1989 from Edmonton (Capital Region).

INFLUENT	
Rainbow trout	<u>Gills</u>: diffuse mild atrophy, blunting and edema of gill lamellae (also noted in control fish)
	<u>Skin</u>: multifocal to focally extensive epithelial hyperplasia
	<u>Other Tissues</u>: no abnormalities noted

*Abnormalities noted at concentration: 100% (no other concentration used). No abnormalities noted in control fish. No evaluation of fathead minnow conducted.

Histopathologic evaluation was completed on rainbow trout and fathead minnow exposed to influent and effluent collected on 21 June 1990. No abnormalities were noted in any tissue, regardless of exposure concentration.

5.3.4 Chronic Toxicity

There was no dose-related or statistically significant ($p>0.05$) pattern of mortality when rainbow trout were exposed to effluent concentrations of 100% and 50% for 28 d (Table 17). Similarly fish body weight, fish length, and body weight gains were not different ($p>0.05$) from control fish (Table 17).

There was a statistically significant ($p<0.05$) increase in the production of neonates of *Daphnia magna* at all effluent concentrations compared to controls (Table 18). Hence, it was not possible to calculate the No-Observed-Effect Concentration or the Lowest-Observed-Effect Concentration. Likewise, mortality was significantly less ($p<0.05$) at all effluent concentrations except 100% compared to controls (Table 18).

Table 17. Mortality, body weight and fork length of rainbow trout exposed for 28 d to different concentrations of effluent from Edmonton (Capital Region) plant.

PARAMETER	CONTROL (±SD)	50% EFFLUENT	100% EFFLUENT
Mortality (Number)	0	1	1
Body weight (g)	1.80 ± 0.19	1.87 ± 0.35	1.79 ± 0.54
Fork length (cm)	5.6 ± 0.3	5.6 ± 0.3	0.58 ± 0.54
Weight gain (g)	0.59 ± 0.19	0.66 ± 0.35	0.58 ± 0.54
Sample size at 28 d	10	9	9

NOTES: Initial mean body weight, 1.21 ± 0.09 g, based on 35 fish, was used to calculate weight gain data for each group;
Each group contained 10 rainbow trout at the start of the experiment;
Mortality data are not different ($p>0.05$);
Weight data are not different ($p>0.05$).

Table 18. Mortality and production of neonates by *Daphnia magna* exposed for 21 d to different concentrations of effluent from the Edmonton (Capital Region) plant.

PARAMETER	EFFLUENT CONCENTRATION					
	CONTROL	6%	12%	25%	50%	100%
Sample size	10	10	10	10	10	10
Mortality (number)	7	2*	1*	0*	0*	3
Neonates/adult	5.1	32.9	42.4	48.0	68.7	45.5

NOTES: *Different from control at $p<0.05$;
Number of neonates/adult different than control at all effluent concentrations ($p<0.05$).

5.4 Edmonton (Goldbar)

5.4.1 Chemical Analysis

The majority of metals underwent a reduction in concentration during the treatment process (Table 19). The 14 June 1990 collection did, however, yield one anomaly - chromium increased from 0.135 to 0.186 mg/L. It is not known if this increase represents a sampling artifact or an actual increase in concentration. Ammonia-N was relatively high in the influent and showed only a modest decline in the effluent. No chemical analysis was conducted on samples collected for evaluation of chronic toxicity.

Table 19. Chemical analysis (mg/L) of municipal wastewater collected from Edmonton (Goldbar).

PARAMETER	DATE			
	23 NOVEMBER 1989		14 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.117	0.014	0.930	0.019
Ammonia-N*	45.0	19.0	28.9	20.6
Arsenic	0.0009	0.0006	0.0027	0.0008
Barium	0.052	0.013	0.127	0.022

PARAMETER	DATE			
	23 NOVEMBER 1989		14 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.005	0.002	0.006	0.003
Chromium	0.119	0.008	0.135	0.186
Cobalt	0.003	<0.001	0.003	0.002
Copper	0.028	0.002	0.043	0.004
Iron	0.640	0.053	2.995	0.002
Lead	0.016	0.0112	0.052	<0.002
Manganese	0.07	0.018	0.370	0.110
Molybdenum	0.008	0.006	0.011	0.016
Nickel	2.907	0.015	0.029	0.025
Selenium	0.0006	0.0002	0.0010	0.0001
Zinc	0.096	0.078	0.143	0.039

*Concentration after reduction (p. 7).

5.4.2 Acute Toxicity

Rainbow trout, fathead minnow and *Anodonta grandis* were acutely exposed to influent and effluent collected from the Edmonton (Goldbar) plant on 23 November 1989 and 14 June 1990. In addition, *Daphnia magna* was exposed to effluent collected on 23 November 1989 and 14 June 1990. In all cases the effluent was not acutely toxic; the LC_{50}'s were ≥100%. The influent was also not acutely toxic (LC_{50}>100%) with one exception. That exception was observed when fathead minnow was exposed to samples collected on 23 November 1989; an LC_{50} of 95.6% was obtained.

5.4.3 Histopathologic Evaluation

The rainbow trout used to evaluate the potential histopathologic effects of influent collected from the Goldbar plant during November 1989 exhibited diffuse mild blunting, atrophy and edema of the gill lamella (Table 20). This lesion was found in the controls as well as principals. No other lesion, indicative of wastewater toxicity, was found in the gills of the trout.

The skin of the rainbow trout exposed to 100% influent developed hyperplasia of the epithelium. This condition was not observed in controls.

Table 20. Summary of histopathological findings of rainbow trout exposed to municipal wastewater* collected 23 November 1989 from the Edmonton (Goldbar) plant.

INFLUENT	
Rainbow trout	Gills: diffuse mild blunting, atrophy and edema of gill lamellae (also noted in control fish)
	Skin: multifocal to focally extensive hyperplasia of epithelium
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentration: 100% and control (no other concentrations used). No evaluation of fathead minnow conducted.

Minor gill and skin lesions were noted when rainbow trout were exposed to influent collected from the plant on 14 June 1990 (Table 21). However, no abnormalities were noted when fathead minnow were exposed to the same wastewater. No lesions developed when rainbow trout and fathead minnow were exposed to effluent collected on 14 June 1990.

Table 21. Summary of histopathologic findings of rainbow trout exposed to municipal wastewater* collected 14 June 1990 from Edmonton (Goldbar) Plant.

INFLUENT	
Rainbow trout	Gills: diffuse mild lamellar blunting (80% concentration only)

*Abnormalities noted a concentrations: 100%, 80% and 60%. No abnormalities noted at 40% and 20% or in control fish.

5.4.4 Chronic Toxicity

There was no dose-related or statistically significant ($p>0.05$) pattern of mortality when rainbow trout were exposed to effluent concentrations of 100% and 50% for 28 d (Table 22). Similarly, fish body weight, fish length, and body weight gains were not different ($p>0.05$) from control fish (Table 22).

Rainbow trout exposed to Goldbar effluent (100%) developed slight lamellar blunting of the gills. No lesions were seen in other tissues or when fish were exposed to 50% effluent for 28 d.

There was a statistically significant increase ($p<0.05$) in the production of neonates of *Daphnia magna* at all effluent concentrations compared to controls (Table 23). Mortalities of *D. magna* in 25% and 100% effluent concentrations were significantly less ($p<0.05$) than controls (Table 23). At other concentrations there was no significant difference ($p>0.05$) in mortality (Table 23).

Table 22. Mortality, body weight and fork length of rainbow trout exposed for 28 d to different concentrations of effluent from the Edmonton (Goldbar) plant.

PARAMETER	CONTROL (±SD)	50% EFFLUENT	100% EFFLUENT
Mortality (number)	1	1	2
Wet weight (g)	5.15 ± 0.74	5.73 ± 0.86	4.77 ± 0.47
Fork length (cm)	7.5 ± 0.3	7.9 ± 0.4	7.3 ± 0.7
Weight gain (g)	1.87 ± 0.73	2.45 ± 0.85	1.49 ± 1.47
Sample size at 28 d	9	9	7

NOTES: Initial body weight (g), 1.21 ± 0.09 g, was based on 35 fish and was used to calculate weight gain data for each group;
Each group contained ten rainbow trout at the start of the experiment;
Mortality data are not different ($p>0.05$);
Weight data are not different ($p>0.05$).

Table 23. Mortality and production of neonates by *Daphnia magna* exposed for 21 d to different concentrations of effluent from the Edmonton (Goldbar) plant.

PARAMETER	EFFLUENT CONCENTRATION					
	CONTROL	6%	12%	25%	50%	100%
Sample size	10	10	10	10	8	10
Mortality	4	1	1	0*	1	0*
Neonates/adult (average)	26.7	56.6	77.7	69.8	75.9	110.9

NOTES: *Different from controls at $p<0.05$.
Number of neonates/adult significantly greater than control at all effluent concentrations (($p<0.05$).

5.5 Fort McMurray

5.5.1 Chemical Analysis

The majority of metals underwent a moderate to substantial reduction in concentration during the treatment process (Table 24). Ammonia-N was substantially reduced by the treatment process on the 6 October 1989 collection, but the reduction in the 1 March 1990 sample was far more modest (Table 24). This is probably a reflection of reduced microbial metabolism of nitrogenous compounds during the cooler months.

5.5.2 Acute Toxicity

Rainbow trout, fathead minnow and *Anodonta grandis* were acutely exposed to influent and effluent collected from the Fort McMurray plant on 6 October 1989 and 1 March 1990. In addition, *Daphnia magna* was exposed to effluent collected on 6 October 1989 and 1 March 1990. In all cases but one, the effluent was not acutely toxic (the LC_{50}'s were ≥100%). The exception was *Anodonta grandis* which had an LC_{50} of 56.1% when exposed to effluent collected on 1 March 1990.

The influent was acutely toxic in a number of cases as specified below:

	6 October 1989	1 March 1990
Rainbow trout (96-h LC_{50})	>100%	80.0%
Fathead minnow (96-h LC_{50})	69.3%	76.3%
Anodonta grandis (96-h LC_{50})	>100%	<50%
Daphnia magna (48-h LC_{50})	test not conducted	test not conducted

Table 24. Chemical analysis (mg/L) of municipal wastewater collected from Fort McMurray.

PARAMETER	DATE			
	6 OCTOBER 1989		1 MARCH 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	5.951	0.138	1.462	0.127
Ammonia-N*	21.0	0.300	45.0	26.0
Arsenic	0.0048	0.0018	0.0012	0.0007
Barium	0.099	0.011	0.101	0.022
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.002	0.001	0.002	0.001
Chromium	0.005	0.002	0.005	0.003
Cobalt	0.002	0.002	<0.001	<0.001
Copper	0.043	0.004	0.052	0.004
Iron	2.098	0.102	1.159	0.287
Lead	<0.002	<0.002	0.017	<0.002
Manganese	0.123	0.026	0.069	0.061
Molybdenum	0.011	0.006	0.005	0.003
Nickel	0.007	0.005	0.004	0.004
Selenium	<0.0001	<0.0001	0.0006	0.0002
Zinc	0.103	0.019	0.181	0.066

*Concentration after reduction (p. 7).

5.5.3 Histopathologic Evaluation

When rainbow trout were exposed to influent from the Fort McMurray system, hyperplasia of skin mucous cells developed (Table 25). No abnormalities were noted in any other tissue, or in any tissue of fathead minnow exposed to influent.

Exposure of rainbow trout to effluent caused mild blunting of the lamellae (Table 25). There was also some hyperplasia of the mucous cell of the skin, but no other abnormalities were noted. Histopathologic evaluation of fathead minnow exposed to effluent from the system was not conducted.

Table 25. Summary of histopathologic findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected on 6 October 1989 from Fort McMurray.

INFLUENT	
Rainbow trout	Gills: multifocal to diffuse, minimal to mild mucosa cell hyperplasia
	Other Tissues: no abnormalities noted
Fathead minnow	All Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: diffuse, mild blunting of lamellae
	Skin: diffuse, minimal to mild mucous cell hyperplasia (also noted at 40% concentration)
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80% and 60%. No abnormalities noted at 40%, 20% and control fish, unless otherwise noted. No evaluation of fathead minnow exposed to effluent.

5.6 Grande Prairie

5.6.1 Chemical Analysis

The concentrations of heavy metals and ammonia-N in effluent samples collected on 12 October 1990 were generally lower than in influent samples collected on the same date (Table 26). In the 4 January 1990 collections, however, percentage reductions were often small (especially for ammonia-N, barium and iron) and, in two cases (aluminum and manganese), concentrations actually increased (Table 26). The reason(s) for this increase are unknown at the present time.

Table 26. Chemical analysis (mg/L) of municipal wastewater collected from Grande Prairie.

PARAMETER	DATE			
	12 OCTOBER 1989		4 JANUARY 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.770	0.062	0.0468	0.339
Ammonia-N*	72.8	0.069	26.9	13.1
Arsenic	0.0008	0.0010	0.0015	0.0001
Barium	0.128	0.03	0.093	0.047
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.003	0.002	0.002	0.002
Chromium	0.004	0.002	0.003	0.002
Cobalt	0.001	<0.001	0.002	0.002
Copper	0.034	0.006	0.041	0.019
Iron	0.471	0.099	0.309	0.240
Lead	0.007	0.002	0.006	<0.002
Manganese	0.059	0.035	0.058	0.070
Molybdenum	0.006	0.004	0.008	0.009
Nickel	0.006	0.006	0.005	0.005
Selenium	0.0051	0.0061	0.0002	0.0001
Zinc	0.077	0.043	0.060	0.065

*Concentration after reduction (p. 7).

5.6.2 Acute Toxicity

There was no mortality of either rainbow trout or fathead minnow following exposure to both influent and effluent collected on 12 October 1989 and 4 January 1990. The LC_{50}'s in all cases were >100%. *Daphnia magna* and *Anodonta grandis* were also relatively insensitive; the 48-h LC_{50} for *D. magna* and the 96-h LC_{50} for *A. grandis* were ≥100% for both effluent collections. *A. grandis* was sensitive to influent collection on 12 October 1989 (96-h LC_{50} 84.1%) and 4 January 1990 (96-h LC_{50} 77.1%). No assay was conducted on the toxic effects of influent to *D. magna*.

5.6.3 Histopathologic Evaluation

Exposure of rainbow trout to 100% influent led to some mild blunting of the gill lamellae, but no lesions in any other tissue (Table 27). Effluent-exposed rainbow trout showed atrophy and blunting of the gill lamellae.

Table 27. Summary of histopathological findings of rainbow trout exposed to municipal wastewater* collected 12 October 1989 from Grande Prairie.

INFLUENT	
Rainbow trout	Gills: multifocal, mild blunting of gill lamellae
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: (i) diffuse, mild lamellar atrophy and blunting; (ii) focal epidermal hyperplasia and mild erosion with attached metazoan parasite (also noted in control fish)
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentration: 100% (no other concentrations used). No abnormalities noted in control fish. No evaluation of fathead minnow conducted.

5.7 Lethbridge

5.7.1 Chemical Analysis

The majority of metals underwent an appreciable reduction in concentration during the treatment process (Table 28). Ammonia-N was substantially reduced by the treatment process on the 31 May 1990 collection, but the reduction in the 8 February 1990 sample was far more modest (Table 28). This is probably a reflection of reduced biological metabolism of nitrogenous compounds during the winter.

Table 28. Chemical analysis (mg/L) of municipal wastewater collected from Lethbridge.

PARAMETER	DATE			
	8 FEBRUARY 1990		31 MAY 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.53	0.057	0.495	0.069
Ammonia-N*	28.9	10.1	28.7	2.1
Arsenic	0.0009	0.0006	0.0018	0.0005
Barium	0.128	0.055	0.154	0.071
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.003	0.003	0.009	0.003
Chromium	0.005	0.003	0.019	0.002
Cobalt	0.002	0.002	0.006	<0.001
Copper	0.026	0.005	0.028	0.003
Iron	1.006	0.166	3.236	0.230
Lead	0.008	0.002	0.006	0.003
Manganese	0.084	0.037	0.157	0.031
Molybdenum	0.005	0.003	0.009	<0.001
Nickel	0.008	0.007	0.019	0.002
Selenium	0.0021	0.0006	0.0023	0.0026
Zinc	0.113	0.068	0.103	0.040

*Concentration after reduction (p. 7).

5.7.2 Acute Toxicity

There were no mortalities (LC_{50}>100%) when rainbow trout were exposed to wastewater influent and effluent collected from the Lethbridge plant on 8 February 1990 and 31 May 1990.

The LC_{50}'s for fathead minnow were also >100% with the exception that influent collected on 8 February 1990 produced an LC_{50} of 94.5%. That same sample was also acutely toxic to *Anodonta grandis* (LC_{50} <50%), but, in all other cases, the wastewaters caused no mortality. The LC_{50}'s for *Daphnia magna* were all >100% (no mortality).

5.7.3 Histopathologic Evaluation

Rainbow trout and fathead minnow exposed to influent and effluent from Lethbridge (31 May 1990) developed no lesions at any concentration (Table 29). When rainbow trout were exposed to Lethbridge influent collected on 8 February 1990, a number of lesions were noted including atrophy and blunting of gill lamellae, skin mucous cell hyperplasia, and skin epithelial swelling (Table 30). Effluent-exposed fish exhibited atrophy of the gill lamellae and skin mucous cell hyperplasia.

Table 29. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected 31 May 1990 from Lethbridge.

INFLUENT	
Rainbow trout	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted

*Concentrations: 100%, 80%, 60%, 40%, 20% and control.

Table 30. Summary of histopathological findings of rainbow trout exposed to municipal wastewater* collected 8 February 1990 from Lethbridge.

INFLUENT	
Rainbow trout	Gills: diffuse, mild atrophy and blunting of lamellae
	Skin: diffuse to multifocal, mild mucous cell hyperplasia and epithelial cell swelling
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: diffuse to multifocal, minimal to mild atrophy of lamellae (also noted in control fish)
	Skin: diffuse to multifocal, minimal to mild mucous cell hyperplasia
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%, 40%. No abnormalities noted at 20% and control fish unless otherwise noted. No evaluation of fathead minnow conducted.

5.8 Medicine Hat

5.8.1 Chemical Analysis

The majority of metals underwent an appreciable reduction in concentration during the treatment process (Table 31). Ammonia-N was substantially reduced by the treatment process on the 24 May 1990 collection, but the reduction in the 12 January 1990 sample was relatively modest (Table 31). This is probably a reflection of reduced biological metabolism of nitrogenous compounds during the winter. Aluminum levels were always higher in the effluent than influent, reflecting the input of alum, a coagulant for phosphorus removal.

Table 31. Chemical analysis (mg/L) of municipal wastewater collected from Medicine Hat.

PARAMETER	DATE			
	12 JANUARY 1990		24 MAY 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.254	0.378	0.200	0.663
Ammonia-N*	32.0	24.0	32.0	11.4
Arsenic	<0.0001	<0.0001	0.0016	0.0006
Barium	0.145	0.036	0.157	0.044
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.002	0.002	0.004	0.003
Chromium	0.015	0.003	0.009	0.005
Cobalt	0.003	0.002	0.007	0.018
Copper	0.051	0.006	0.061	0.005
Iron	1.967	0.103	0.552	0.085
Lead	0.025	0.047	<0.002	<0.002
Manganese	0.078	0.050	0.071	0.037
Molybdenum	0.120	0.011	0.081	0.142
Nickel	0.059	0.012	0.033	0.040
Selenium	0.0001	0.0001	0.00010	0.00006
Zinc	0.073	0.012	0.072	0.023

*Concentration after reduction (p. 7).

5.8.2 Acute Toxicity

Effluent samples collected from the Medicine Hat plant on 12 January 1990 and 24 May 1990 were not acutely toxic (LC_{50} >100%) to all four test species. Influent was also not acutely toxic with two exceptions (rainbow trout, 24 May 1990, LC_{50} 84.1%; *Anodonta grandis*, 12 January 1990, LC_{50} 70.7%).

5.8.3 Histopathologic Evaluation

Rainbow trout exposed to influent collected on 24 May 1990 from Medicine Hat developed hypertrophy and hyperplasia of gill epithelial cells, and mild branchial edema (Table 32). There was also some necrosis and degeneration of the gill arch and pharynx, but no other tissue was affected. Although fathead minnow showed mild to moderate effects when exposed to influent from the plant (Table 32), these effects were also present in control fish.

Rainbow trout exposed to effluent collected on 24 May 1990 from Medicine Hat developed blunting of gill lamellae and lamellar epithelial hypertrophy and hyperplasia (Table 32). No abnormalities were seen in other tissues. When fathead minnow was exposed to effluent, no abnormalities developed in any tissue.

Table 32. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected 24 May 1990 from Medicine Hat.

INFLUENT	
Rainbow trout	Gills: (i) diffuse, mild epithelial cell hypertrophy and hyperplasia, (ii) multifocal, minimal to mild branchial edema
	Gill Arch and Pharynx: multifocal to diffuse, mild epithelial degeneration and necrosis
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: (i) multifocal, mild fusion of lamellae, (ii) diffuse, mild to moderate congestion (also present in control fish)
	Gill Arch and Pharynx: multifocal, mild to moderate epithelial cell degeneration and necrosis (also present in control fish)
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: (i) multifocal, mild blunting of lamellae, (ii) multifocal, mild lamellar epithelial hypertrophy and hyperplasia
	Gill Arch and Pharynx: multifocal, minimal epithelial degeneration and necrosis of gill arch
	Other Tissues: no abnormalities noted
Fathead minnow	All Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%. No abnormalities noted at 40%, 20% and control fish, unless otherwise noted.

When rainbow trout were exposed to influent collected on 12 January 1990, swelling of lamellar epithelial cells and blunting of lamellae were noted (Table 33). Mucous cells of the skin underwent hyperplasia, and there was epithelial cell swelling (also noted on ventral body wall). No evaluation was conducted using effluent.

Table 33. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected 12 January 1990 from Medicine Hat.

INFLUENT	
Rainbow trout	Gills: multifocal swelling of lamellar epithelial cells; multifocal minimal blunting of lamellae (100% concentration only)
	Skin: multifocal to focally extensive to coalescing mild to moderate epithelial cell swelling and cell hyperplasia (also noted at 40% concentration)
	Skin (ventral body wall): focally extensive moderate epithelial cell swelling (20% concentration only)
Fathead minnow	All Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%. No abnormalities noted at 40%, 20% and control fish, unless otherwise noted.

5.9 Red Deer

5.9.1 Chemical Analysis

The majority of metals underwent a moderate to substantial reduction in concentration during the treatment process (Table 34). Ammonia-N was substantially reduced by the treatment process on both collection dates (Table 34).

Table 34. Chemical analysis (mg/L) of municipal wastewater collected from Red Deer.

PARAMETER	DATE			
	7 DECEMBER 1989		21 JUNE 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.261	0.054	0.394	0.025
Ammonia-N*	43.0	11.3	40.0	0.77
Arsenic	0.0012	0.0008	0.0014	0.0008
Barium	0.091	0.024	0.206	0.109
Beryllium	<0.001	<0.001	<0.001	<0.001
Cadmium	0.002	0.001	0.004	0.003
Chromium	0.008	0.009	0.141	0.008
Cobalt	0.001	0.001	0.002	0.001
Copper	0.037	0.004	0.062	0.006
Iron	1.066	0.112	1.499	0.138
Lead	0.033	0.005	0.028	<0.002
Manganese	0.054	0.037	0.072	0.009
Molybdenum	0.004	0.003	0.004	0.004
Nickel	0.006	0.003	0.009	0.006
Selenium	0.0005	0.00001	0.0011	0.0002
Zinc	0.136	0.037	0.134	0.042

*Concentration after reduction (p. 7).

5.9.2 Acute Toxicity

Influent and effluent samples collected on 7 December 1989 and 21 June 1990 were not acutely toxic (LC_{50} >100%) to any of the four test species.

5.9.3 Histopathologic Evaluation

When rainbow trout were exposed to influent collected on 7 December 1989, some effects to the skin were noted (Table 35). The gills of principal fish also developed some mild blunting and atrophy of gill lamellae, a condition also noted in control fish. When rainbow trout were exposed to influent collected on 21 June 1990, some mild skin lesions developed (Table 36). No abnormalities were noted when rainbow trout were exposed to effluent from either collection period. There were also no abnormalities noted when fathead minnows were exposed to influent and effluent on both collection dates (Table 36).

Table 35. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected on 7 December 1989 from Red Deer.

INFLUENT	
Rainbow trout	<u>Gills</u>: diffuse, mild blunting and atrophy of gill lamellae (also noted in control fish)
	<u>Skin</u>: (i) multifocal to focally extensive epithelial hyperplasia, (ii) focally extensive serocellular crust and focal vesicle of skin
Fathead minnow	<u>All Tissues</u>: no abnormalities noted
EFFLUENT	
Rainbow trout	<u>All Tissues</u>: no abnormalities noted
Fathead minnow	<u>All Tissues</u>: no abnormalities noted

*Abnormalities noted at concentration: 100% (no other concentrations used). No abnormalities noted in control fish, unless otherwise noted.

Table 36. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected on 21 June 1990 from Red Deer.

INFLUENT	
Rainbow trout	<u>Skin</u>: multifocal, minimal epithelial cell hypertrophy and mucous cell hyperplasia
	<u>Other Tissues</u>: no abnormalities noted

Fathead minnow	All Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	All Tissues: no abnormalities noted
Fathead minnow	All Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 80%, 60%. No abnormalities noted at 40%, 20%, or control fish.

5.10 Wetaskiwin

5.10.1 Chemical Analysis

The majority of metals underwent an appreciable reduction in concentration during the treatment process (Table 37). The concentration of ammonia-N was not determined during either sampling period.

5.10.2 Acute Toxicity

Effluent collected on 2 November 1989 and 24 April 1990 was not acutely toxic (LC_{50} >100%) to any of the four test species. Influent was also not acutely toxic with two exceptions (rainbow trout, 24 April 1990, LC_{50} 56.3%; fathead minnow, 24 April 1990, LC_{50} 42.2%).

Table 37. Chemical analysis (mg/L) of municipal wastewater collected from Wetaskiwin.

PARAMETER	DATE			
	2 NOVEMBER 1989		24 APRIL 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Aluminum	0.459	0.136	0.669	0.121
Ammonia-N	no data	no data	no data	no data
Arsenic	0.0010	0.0041	0.0013	0.0006
Barium	0.122	0.022	0.104	0.027
Beryllium	<0.001	<0.001	<0.001	<0.001

PARAMETER	DATE			
	2 NOVEMBER 1989		24 APRIL 1990	
	INFLUENT	EFFLUENT	INFLUENT	EFFLUENT
Cadmium	0.002	0.002	0.003	0.002
Chromium	0.004	0.003	0.008	0.005
Cobalt	0.001	0.003	0.003	0.003
Copper	0.122	0.005	0.082	0.009
Iron	0.809	0.729	1.593	0.884
Lead	0.075	<0.002	0.062	0.008
Manganese	0.159	0.133	0.160	0.165
Molybdenum	0.003	0.002	0.004	0.003
Nickel	0.007	0.008	0.010	0.010
Selenium	0.00002	0.00002	0.00001	0.00001
Zinc	0.136	0.016	0.135	0.034

5.10.3 Histopathologic Evaluation

Rainbow trout exposed to both influent and effluent collected from Wetaskiwin on 2 November 1989 showed minimal lamellar epithelial hypertrophy (Table 38). No abnormalities were noted in any other tissue. When fathead minnow was also exposed to influent and effluent collected on 2 November 1989, no lesions developed (Table 38).

Table 38. Summary of histopathological findings of rainbow trout and fathead minnow exposed to municipal wastewater* collected 2 November 1989 from Wetaskiwin.

INFLUENT	
Rainbow trout	Gills: multifocal, minimal lamellar epithelial hypertrophy (100% influent concentration only)
	Other Tissues: no abnormalities noted

Fathead minnow	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted
EFFLUENT	
Rainbow trout	Gills: multifocal, minimal lamellar epithelial hypertrophy (60% effluent concentration only)
	Other Tissues: no abnormalities noted
Fathead minnow	Gills: no abnormalities noted
	Other Tissues: no abnormalities noted

*Abnormalities noted at concentrations: 100%, 60%. No abnormalities noted at 80%, 40%, 20% or control fish.

6 DISCUSSION

The wastewater samples used in this study were generally not acutely toxic to the four test species. There appeared to be little difference in the toxicity of influent and effluent samples, and the type of treatment system (for example, aerated lagoon versus mechanical-biological) did not influence toxicity. Although rainbow trout and, to a lesser degree, fathead minnow developed some skin and gill lesions following exposure to certain samples, these disorders were relatively minor and did not affect the short-time survival of fish.

When *Daphnia magna* was exposed to effluent from the Capital Region and Goldbar plants, there was an increase in neonate production over the 21-d exposure period, likely due to an improvement in the quantity and/or quality of food in the samples. This increase in neonate production meant that it was not possible to determine if there were any chemically induced toxicologic effects of the wastewater. The enhanced food conditions apparently masked any potential toxic effects of the wastewater sample.

The chronic rainbow trout growth test also indicated low toxicity of the Capital Region and Goldbar effluent samples. Unlike the 21-d *Daphnia magna* procedure, the results of this test were unaffected by the organically enriched wastewater.

All of these data indicate that the wastewater samples used in this study posed little threat to the four species. Total ammonia-N in the amended samples was relatively low due to the stripping process, with maximum concentrations falling in the 20-73 mg/L range. At 15°C and a pH of 7, the percentage of unionized ammonia (the highly toxic form) is only 0.27 of total ammonia (Piper *et al.*, 1982). This places the maximum concentration of unionized ammonia of our samples at 0.20 mg/L. The corresponding percentages of unionized ammonia at pH 6 are 0.027 and 2.7 (Piper *et al*, 1982), yielding maximum concentrations of unionized ammonia of 0.02 and 2.0 mg/L, respectively, in our samples. Although this latter value (recorded for the Grande Prairie sample from 12 October 1989) might be expected to induce toxic effects over the long term, the period of exposure to pH 8 was relatively short. The samples were also aerated, thereby reducing the problem of high BOD. In addition, none of the samples had been chlorinated with the exception of the May 1990 sample from Medicine Hat. However, since Medicine Hat has dechlorination ponds prior to effluent discharge, no chlorine related toxicity was expected and none was detected. All remaining potentially toxic agents such as heavy metals and industrially derived chemicals (such as hydrocarbons) had little or not effect on the test species.

At present, no information is available on the toxicity of unamended municipal wastewater samples from Alberta treatment plants. It is therefore recommended that appropriate toxicologic studies be initiated on wastewater from several plants using the same techniques as those employed in this study.

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