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Toxicity of Landfill Leachates to Underyearling Rainbow Trout (*Salmo gairdneri*)

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Sanitary landfilling is the method used for the disposal of domestic and commercial refuse in the Greater Vancouver Regional District, British Columbia. Within this District in excess of 900,000 tonne of general urban refuse are generated and deposited in 6 major landfills. Five of these landfills discharge leachates into the Fraser River estuary.

The Fraser River Estuary is an important segment of the migration route utilized by five species of Pacific salmon, steelhead trout and coastal cutthroat trout (NORTHCOTE 1974). In addition, the estuary is utilized as a nursery by several species of salmonids and is of special significance to all anadromous salmonids making the transition from fresh to sea water.

The technology of landfilling, the generation of leachate and its characterization are well documented (ROVERS et al. 1974, BRUNNER and KELLER 1972, JOHANSEN and CARBON 1976, CHIAN 1977). However,.with the exception of an acute toxicity study (VIGERS 1977) no information is available in the literature on the deleterious effects of sanitary landfill leachates on fishes. The objective of this study was to document the sublethal response of a salmonid (Salmo gairdneri) to leachate from a Greater Vancouver Regional District landfill site.

MATERIALS AND METHODS

Study area

The Burns Bog sanitary landfill is situated on a 405 hectare site of which approximately 65 hectares have been utilized. The landfill is situated on a peat bog averaging 2.7 m in thickness, underlain by approximately 1.2 m of relatively impervious clay (CORBETT 1975).

The area receives approximately 112 cm of precipitation per annum, of which about 75 cm enters the landfill following evaporation losses (CORBETT 1975). Following the consolidation of the underlying peat and improved ditching nearly 100% of the leachate generated flows into Crescent Slough and subsequently into the Fraser River.

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Leachates

Two samples of the leachate were collected in plastic lined drums from a upwelling at the periphery of the landfill. The first was used to establish the acute toxicity and the second, collected 4 weeks later, was utilized in the sublethal assessments. Leachates held in storage were maintained at 5° ± 1°C and to ensure homogeneity the samples were impellor stirred with mixing timed to an alternating 15 minute on-off cycle. To determine the composition of the leachate and assess for stability during storage a partial chemical and physical profile was programmed for Leachate Sample No. 2. The analysis was as outlined in the Fisheries Service and Environmental Protection Service Laboratory Manual (ANON 1979).

Fish

All bioassays were conducted with underyearling rainbow trout, obtained from the Provincial Fish Hatchery, Abbotsford, British Columbia. Fish were not fed for 48 hours prior to nor during the tests.

Acute toxicity

After 48 hours acclimation to the holding conditions static 96-h LC_{50} bioassays (SPRAGUE 1969) were carried out on both leachate samples, using first fresh and then material that had been stored for 2 weeks. The average weight of the fish used in the bioassays was 3.4 g. All aquaria were maintained at a constant 15° ± 1°C, aerated at a rate of 200 mL/min using oil free compressed air and retained on a 14 L:10 D photoperiod (no twilight).

Sublethal toxicity

This phase of the study was divided into 2 components; histopathology and plasma corticosteroid concentrations. In each case static bioassays were employed with the maximum concentration of leachate (Sample No. 2) set at a point slightly below that of the 96-h LC_{50} value obtained with the first leachate sample. Additional 10 fold serial dilutions of the leachate were included. Test solutions were prepared from stock leachate that had been held in storage for 6 days.

Histopathology

Paired tanks each with 7 fish, average weight 30 g, were employed for assessing the different concentrations of leachate plus the control. Aquaria conditions were as noted in the acute toxicity section. None of the test solutions or water for the controls were renewed during the 7 day period of the study. Fish loading density at the start of the study was approximately 1 g/L. Preparation of the tissues for histological examination was as previously outlined (McBRIDE and VAN OVERBEEKE 1971, VAN OVERBEEKE and McBRIDE 1971).

Plasma cortisol

To ensure a basal corticosteroid level at the start of the study and to minimize the possibility of external changes influencing the test results the following conditions were rigidly enforced. Light was excluded from all aquaria by the application of black plastic masking tape to the outside of the glass. To facilitate rapid capture 1 fish was assigned per tank. Noise, tank movement or other physical activity in the immediate area was kept to a minimum. Fish were acclimated to these conditions for 3 days prior to the addition of the leachate. Five fish were assigned to each time/concentration combination. The time of initiation of exposure was regulated so that the sampling period always occurred between 1:00 and 2:30 p.m. This precluded any possible effects of circadian changes in plasma cortisol concentration. At the end of the treatment period, fish were rapidly netted and stunned by a blow on the dorsal surface of the head. A blood sample was removed from a blood vessel in the caudal peduncle using a heparinized 1 mL syringe fitted with a 21 G 1 1/4" hypodermic needle. Plasma was separated by centrifugation at 4000 rpm for 10 min in a Sorvall RC-5 refrigerated centrifuge and stored at -50°C prior to analysis. Cortisol determinations were performed in duplicate on 10 µL aliquots of plasma using the Clinical Assay GammaCoat ^{125}I cortisol radioimmunoassay kit.

RESULTS

Leachate analysis

The results of the leachate analysis are given in Table 1.

Acute toxicity

The 96-h LC_{50} bioassays for leachate Samples No. 1 and 2 are shown in Table 2.

TABLE 2

Bioassay 96-h LC_{50} values of leachates.

Leachate	Storage (days)	96-h LC_{50} value
Sample 1	0	6.5%
Sample 1	14	5.9%
Sample 2	0	7.5%
Sample 2	14	5.8%

Histopathology

Leachates did not visibly affect the structure of liver, kidney, gill, skin or alimentary tract. In contrast, the interrenal, i.e., adrenocortical tissue homologue, of those

TABLE 1

Effect of storage on the stability of landfill leachate (Sample No. 2).

Parameter (mg/L)	Storage period (days) 0	3	7	10	14	Mean	S.D.
COD	710	730	690	700	640	694	± 33.6
Ph	8.0	8.1	8.1	8.2	8.2	8.1	± .08
Chloride	735	740	750	755	768	749	± 12.9
Suspended solids (NFR)	32	31	23	23	20	25.8	± 5.3
Dissolved solids (FR)	3200	3200	3400	3300	3300	3280	± 83.7
Total alkalinity	2490	2670	2600	2640	2640	2608	± 70.5
Total hardness	480	---	421	---	---	450	± 42
Nitrite-N	.124	.067	.066	.074	.078	.082	± .024
Nitrate-N	.311	.433	.464	.404	.403	.403	± .057
Ammonia-N	370	395	363	425	389	388	± 24.3
Un-ionized NH_3							
at 15°C	9.9	13.2	12.1	17.7	16.2	13.8	± 3.1
at 5°C	4.6	6.1	5.6	8.2	7.5	7.8	± 3.9

fish exposed to the maximum concentration of the pollutant exhibited profound changes.

In the controls, the interrenal tissue consisted of small clusters of closely packed cells with spherical nuclei containing mostly inconspicuous nucleoli and showing a fine evenly dispersed granular cytoplasm. Mitotic activity was not noted. The interrenal structure of the fish exposed to the maximum concentration of leachate displayed the typical histological characteristics of heightened activity: hypertrophy of the nuclei (Table 3) as well as the nucleoli, numerous small vaculoles in the cytoplasm, abundant mitotic figures and prominent sinusoids. Haemorrhages or pyknotic nuclei, however, were not observed. The above features were more clearly defined after 7 days of treatment. A similar, albeit less marked response was noted in the interrenal tissue of those animals exposed to a 200 fold dilution of the leachate at day 7. No apparent alteration was indicated in these latter fish during the shorter exposure periods.

TABLE 3

Interrenal nuclear diameters (mean $\pm$ SD) in rainbow trout exposed to landfill leachate. Each value based on the results obtained with 2 fish. Significant differences from control fish denoted by *, $P < 0.05$.

Leachate (%)	Exposure (days)	Interrenal nuclear diameter (μ)
0	2	5.92 $\pm$ 0.26
5.0	2	6.64 $\pm$ 0.45*
0.5	2	5.74 $\pm$ 0.47
0.05	2	5.76 $\pm$ 0.37
0.005	2	5.69 $\pm$ 0.37
0	4	5.59 $\pm$ 0.37
5.0	4	6.72 $\pm$ 0.36*
0.5	4	5.71 $\pm$ 0.39
0.05	4	5.49 $\pm$ 0.33
0.005	4	5.65 $\pm$ 0.43
0	7	5.66 $\pm$ 0.36
5.0	7	7.12 $\pm$ 0.48*
0.5	7	6.10 $\pm$ 0.51*
0.05	7	5.66 $\pm$ 0.45
0.005	7	5.70 $\pm$ 0.42

Plasma cortisol

The plasma cortisol concentrations in fish exposed to leachates are presented in Table 4. In fish exposed to 5%

leachate, cortisol concentrations were significantly elevated at all times from 1 to 48 hours. The maximum concentration, 17.8 ng/mL, was observed after 8 hours of exposure. The lowest concentrations in fish exposed to 5% leachate were 8.0 ng/mL at 1 hour and 8.2 ng/mL at 48 hours. The only other fish showing significantly elevated cortisol concentration were those exposed to 0.5% leachate for 1 hour. The mean cortisol concentrations in the control groups of trout ranged from 0.04 to 3.2 ng/mL.

TABLE 4

Plasma cortisol concentrations (mean $\pm$ SD) in rainbow trout exposed to landfill leachate. There were initially 5 fish in each group. Mortality occurred in some of the 5% leachate groups prior to sampling. Significant differences from control fish denoted by *, $P < 0.05$.

Leachate (%)	Exposure (hours)	No. of fish	Cortisol concentration ng/mL
0	1	5	3.23 $\pm$ 3.12
5.0	1	5	8.02 $\pm$ 2.99*
0.5	1	5	8.45 $\pm$ 1.99*
0.05	1	5	2.25 $\pm$ 2.15
0	4	5	0.52 $\pm$ 0.87
5.0	4	4	15.99 $\pm$ 3.25*
0.5	4	5	2.70 $\pm$ 3.10
0.05	4	5	0.62 $\pm$ 1.16
0	8	5	1.71 $\pm$ 1.67
5.0	8	4	17.79 $\pm$ 9.01*
0.5	8	5	0.90 $\pm$ 0.69
0.05	8	5	2.35 $\pm$ 1.10
0	24	5	2.62 $\pm$ 3.33
5.0	24	2	14.13 $\pm$ 2.98*
0.5	24	5	3.33 $\pm$ 4.53
0.05	24	5	1.44 $\pm$ 1.30
0	48	5	0.04 $\pm$ 0.07
5.0	48	5	8.18 $\pm$ 4.02*
0.5	48	5	0.68 $\pm$ 1.07
0.05	48	5	1.46 $\pm$ 3.09

DISCUSSION

Exposure of trout to leachates clearly induced a stress response. The return of the serum cortisol to the resting range in those fish exposed to a 200 fold dilution of the leachate indicated a complete compensation, i.e., adaptation. Such was not the case, however, for the trout introduced to the 20 fold dilution where the cortisol remained at an elevated level for the full term of the study. The occurrence of

mortalities among the latter group suggests that these fish had entered the exhaustive stage in Selye's General Adaptation Syndrome (SELYE 1950). Similar results were observed when juvenile sockeye salmon were exposed to 10^{-6} and 10^{-5}M Cu respectively (DONALDSON and DYE 1975).

The histological alterations corroborated the stressed state of those groups identified in the serum cortisol portion of the study. Interestingly, structural changes in the interrenal consistent with stress were not noted in the 200 fold dilutions until day 7. The latter may indicate that at this dilution the fish is capable of adaptation by increasing the level of interrenal activity. On the other hand, in the fish exposed to a 20 fold dilution interrenal nuclear diameters were considerably greater than any of the other groups even after only 2 days and continued to increase with further exposure suggesting that the fish were under a severe stress that they were unable to adapt to.

The concentration of un-ionized NH_3 was the only component in the chemical-physical profile of the leachate that met and possibly exceeded the published LC50 values for short term toxicity tests in rainbow trout. In the 20 fold leachate dilution of this study the un-ionized NH_3 concentration of 0.7 mg/L fell in the upper range of recorded LC50 toxicity values for salmonids (rainbow trout, Salmo gairdneri; cutthroat trout, S. clarki; Atlantic salmon, S. salar) of 0.2 to 0.8 mg/L NH_3 (LLOYD 1961, BALL 1967, THURSTON et al. 1978). However, in this study, no degenerative alterations were noted in the structure of the gill or kidney, a pathology commonly noted in fishes exposed to toxic concentrations of NH_3 (SMART 1976, THURSTON et al. 1978). An analysis for individual heavy metals was carried out (R. Swingle, personal communication); the individual concentrations, however, all registered below the recorded threshold levels for toxins in rainbow trout.

With the exception of the decrease in nitrites and increase in un-ionized NH_3, little change in the chemical-physical profile was noted during storage. Generally, a decrease in toxicity would be anticipated with a reduction in nitrites (RUSSO et al. 1974, SMITH and WILLIAMS 1974). This was not the case as less of the stored leachate was required to produce a 96-h LC_{50}. The increase in un-ionized NH_3 would, however, be in agreement with the increased toxicity.

Any assessment of possible deleterious effects to fishes exposed to leachates must be predicated on the knowledge that: (1) the chemical-physical profile of leachates reflects not only the waste composition of the landfill, but also the landfill conditions (JOHANSEN and CARLSON 1976, CHIAN 1976); (2) the resident or migrant fish may experience a series of exposures to the leachate; (3) any induced stress may influence a host of physiological activities (SPRAGUE 1971, MAZEAUD et

al. 1977); (4) the stressed state may be transitory in nature with a return to homeostasis, or it may lead to a state of exhaustion followed by death; (5) one stress at a moderate level of intensity may not in itself be deleterious, but the effects of a simultaneous exposure to two or more stressors, e.g., leachate exposure combined with esturine osmotic stress could be very serious indeed.

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