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Ground and Surface Water Developmental Toxicity at a Municipal Landfill: Description and Weather-Related Variation

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Contaminated groundwater poses a significant health hazard and may also impact wildlife such as amphibians when it surfaces. Using FETAX (Frog Embryo Teratogenesis Assay-*Xenopus*), the developmental toxicity of ground and surface water samples near a closed municipal landfill at Norman, OK, were evaluated. The groundwater samples were taken from a network of wells in a shallow, unconfined aquifer downgradient from the landfill. Surface water samples were obtained from a pond and small stream adjacent to the landfill. Surface water samples from a reference site in similar habitat were also analyzed. Groundwater samples were highly toxic in the area near the landfill, indicating a plume of toxicants. Surface water samples from the landfill site demonstrated elevated developmental toxicity. This toxicity was temporally variable and was significantly correlated with weather conditions during the 3 days prior to sampling. Mortality was negatively correlated with cumulative rain and relative humidity. Mortality was positively correlated with solar radiation and net radiation. No significant correlations were observed between mortality and weather parameters for days 4–7 preceding sampling. © 1998 Academic Press

INTRODUCTION

There is a growing concern over contamination of groundwater aquifers. One of the greater problems facing scientists and regulatory agencies is that groundwater reservoirs are by their very nature much more difficult to monitor and remediate than surface sites (United States EPA, 1984). Landfills have been identified as one of the major threats to groundwater resources (United States EPA, 1984). It is often not until wells or surface waters become significantly contaminated that a problem is identified. With approximately one-half of United States residents relying on groundwater as their potable water supply (United States EPA, 1984), the need to preserve the integrity of this resource is obvious. Groundwater reservoirs can contaminate surface waters and directly affect amphibians and other wildlife using these surface waters. Contamination with xenobiotics has been found to adversely impact amphibian populations (Fashigbauer, 1957; Hazelwood, 1969; Paulov, 1977; Cooke, 1983) and has been cited as one of many possible causes for the reported worldwide decline in amphibian populations (Wake and Morowitz, 1990; Carey and Bryant, 1995).

Landfills have been the prinicipal disposal method for both industrial and domestic waste. There is a growing list of landfill sites that are known to be leaching contaminants into underlying aquifers (Reinhard *et al*., 1984). Before the widespread use of pollution abatement measures such as waterproof liners, improper waste disposal methods were frequently used with little regard for the potential adverse impacts these practices might have on the environment. Also, too little is known about the health effects of exposure to contaminants, particularly complex mixtures such as those often found in landfill leachate. Toxicological assays such as FETAX (Frog Embryo Teratogenesis Assay-*Xenopus*) (Dumont *et al*., 1983), combined with hydrological studies, can be used to help determine the potential impacts of these contaminants to both humans and other organisms. FETAX is a 96-h whole embryo assay for developmental toxicants that uses the embryos of the South African clawed frog, *Xenopus laevis*, and is thus particularly useful in studies dealing with impacts on amphibians. Additionally, the ability of *Xenopus* to breed year-round allows studies dealing with long-term temporal changes in toxicity.

FETAX has successfully been used to test toxicity of complex mixtures such as industrial effluents (Dumont and Schultz, 1980; Dumont *et al*., 1983; Dawson and Bantle, 1987; Dawson *et al*., 1991a,b), surface water (Dawson *et al*., 1984), groundwater (Bantle *et al*., 1989), and sediment extracts (Dawson *et al*., 1988). This assay can also be modified such that it is applicable for use as an *in situ* biomonitoring technique using plastic enclosures (Linder, *et al*., 1990). Because FETAX is a standardized assay, similar protocols using native species can be applied to test the same material. This is important due to potential differences between species in sensitivity to pollutants (Hall and Swineford, 1980, 1981).

Surface water contamination may play a significant role as a population stressor because amphibians are dependent

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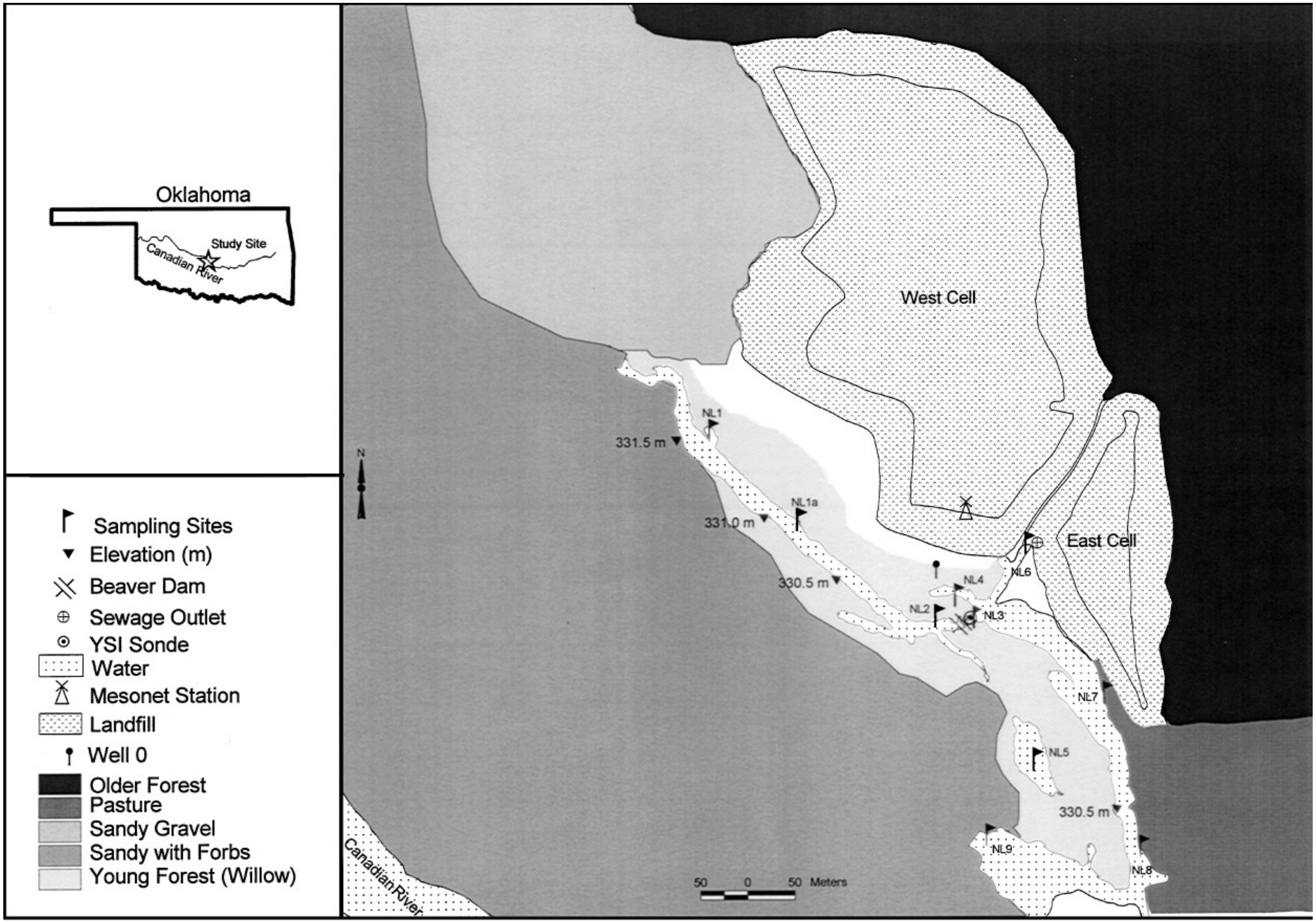

FIG. 1. Map showing the Norman Landfill research site.

on water for reproduction. Rainfall events may alternately dilute toxicity or increase it if rate of transport increases the flow of contaminants to the surface. Therefore, the purpose of this study was to evaluate the developmental toxicity of ground and surface waters near the closed municipal landfill at Norman, Oklahoma. Movement of toxic materials from groundwater to surface water where they could impact amphibians was of particular concern. Additionally, the relationship of temporal changes in surface water toxicity at the landfill to changes in weather parameters was examined.

MATERIALS AND METHODS

Study Site Description

The landfill is located south of the city of Norman in central Oklahoma on alluvium deposited by the Canadian River (Fig. 1). The landfill was operational from 1922 to 1985 with no restrictions on the type of material deposited. It received waste prior to the use of pollution abatement measures such as waterproof liners. In 1985 the landfill was closed, capped with clay, and vegetated (Callender *et al.*, 1993). In 1994 the landfill site was selected to be the focus of the Toxic Substances Hydrology Program of the United States Geological Survey (USGS), Water Resources Division (Lucius and Bisdorf, 1995). Other researchers have identified more than 40 semi-volatile and non-volatile compounds (Dunlop *et al.*, 1976) as well as 35 volatile compounds (Scott Christenson, USGS, personal communication) (Table 1) in the groundwater downgradient from the landfill. Many of these chemicals are known xenobiotics and carcinogens. This groundwater was also found to have low levels of dissolved oxygen and elevated concentrations of hydrogen sulfide and methane (Gibson and Suflita, 1986; Beeman and Suflita, 1987, 1990). Additionally, a toxicity identification evaluation (TIE) was performed on groundwater samples. Preliminary results indicated elevated toxicity may result, in part, from high concentrations of metals. A small stream and associated riparian habitat is adjacent to the landfill. The stream flows into the Canadian River. Treated sewage effluent from the city of Norman is discharged directly into this stream.

TABLE 1

Compounds Identified in Water Samples Collected in September, 1993, by the USGS, Using Gas Chromatography/Mass Spectrometry (EPA Method 8240)

Benzene	1-Methyl-4-propylbenzene
Toluene	1,3-Dimethyl-5-ethylbenzene
Ethylbenzene	1,2-Diethylbenzene
m, *p*-Xylene	1-Methyl-2-propylbenzene
Isopropylbenzene	1,4-Dimethyl-2-ethylbenzene
n-Propylbenzene	1,3-Dimethyl-4-ethylbenzene
1-Ethyl-3-methylbenzene	1,2-Dimethyl-4-ethylbenzene
1-Ethyl-4-methylbenzene	1,3-Dimethyl-2-ethylbenzene
1,3,5-Trimethylbenzene	1,2-Dimethyl-3-ethylbenzene
1-Ethyl-2-methylbenzene	1,2,4,5-Tetramethylbenzene
1,2,4-Trimethylbenzene	1,2,3,5-Tetramethylbenzene
2-Methylpropylbenzene	1,2,3,4-Tetramethylbenzene
1,2,3-Trimethylbenzene	Chloroform
1-Methyl-3-isopropylbenzene	1,1,1-Trichloroethane
1-Methyl-4-isopropylbenzene	Vinyl chloride
1,3-Diethylbenzene	Trichloroethane
1-Methyl-3-propylbenzene	1,4-Dichlorobenzene
o-Xylene	

Surface water samples from a reference location approximately 8 kilometers northwest of the landfill were also analyzed. This site is located in riparian habitat similar to that of the Norman landfill and is also on alluvium deposited by the Canadian River. The reference site also has a small stream that flows through the site and into the Canadian River (Fig. 2).

Sample Collection and Analysis

On 16–17 November, 1995, and 21 January, 1996, groundwater samples were taken from a network of USGS wells adjacent to, and downgradient from, the landfill (Fig. 3). The groundwater is in a shallow, unconfined, alluvial aquifer that extends under the landfill (Callender *et al.*, 1993). Syringes (60 ml) with latex surgical tubing attached were used to draw water from the wells. All water samples were taken from the top 0.5 m of the water column. The water from each well was placed into 250-ml amber bottles with Teflon-lined lids. These bottles were completely filled with water leaving no head space. The syringes were thoroughly rinsed with distilled water between wells and replaced after every 6–8 wells. The latex tubing was replaced after each well. Surface water samples were also taken from various locations along the small stream adjacent to the landfill and from the stream at the reference site. All water samples were cooled to 4°C the day they were taken and stored at this temperature until testing.

Upon obtaining results for this portion of the study, seven permanent surface water sampling locations were established (NL1–NL7) at the landfill along with one groundwater sampling location (Well 0) (Fig. 1). Four permanent surface water sampling locations (AL1–AL4) were established at the reference site (Fig. 2). From June 1996 through May 1997 samples were taken from these locations. The goal was to correlate surface water toxicity at each of the seven landfill surface water sampling locations to changing weather parameters. Both embryo mortality and malformation data were examined, but only mortality data are presented here because malformations did not significantly correlate with any weather parameter.

Weather data were gathered every 5 min by an automated Mesonet weather station installed at the landfill site (Crawford *et al.*, 1992). Weather parameters considered in this study included air temperature, relative humidity, solar

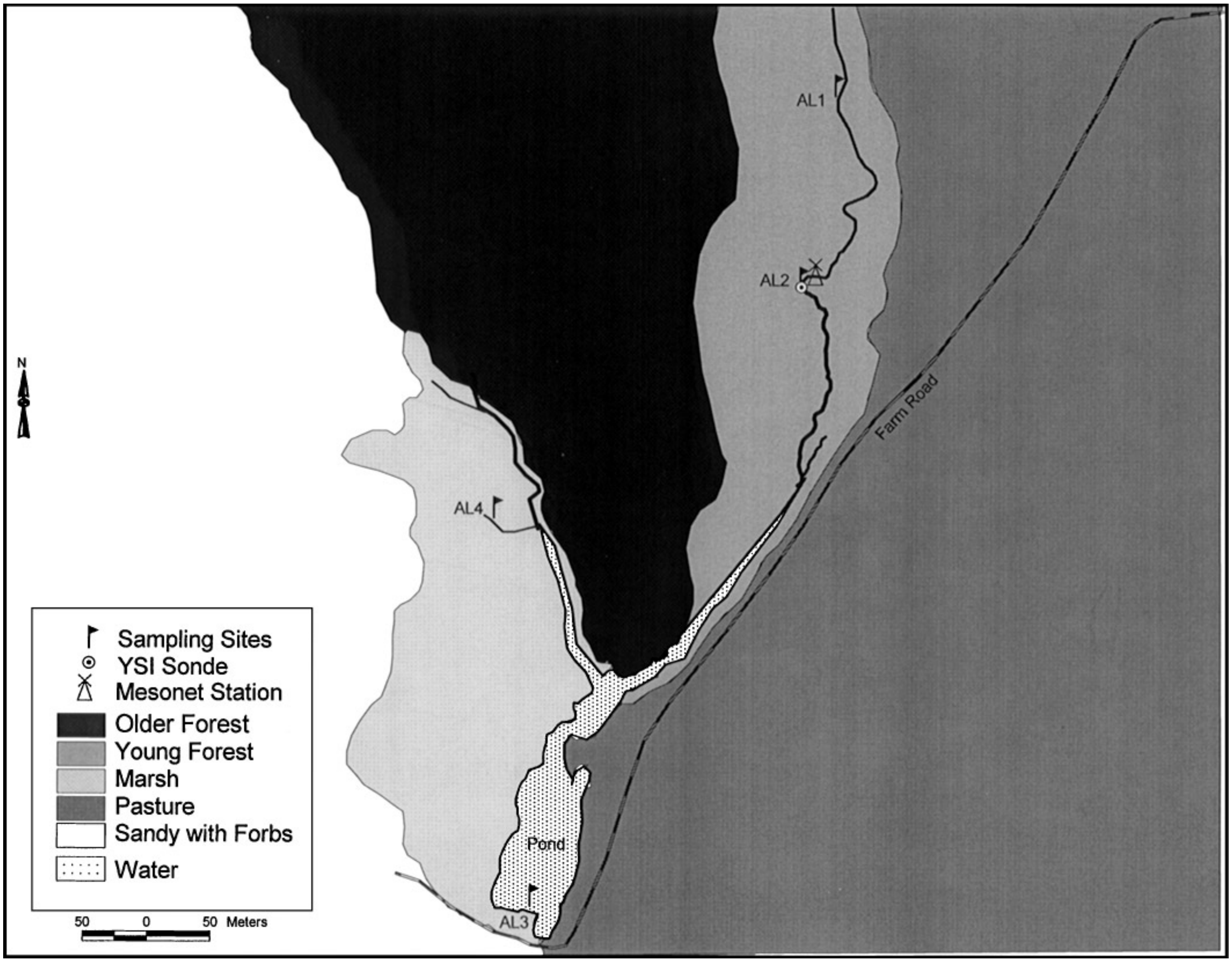

FIG. 2. Map showing the reference site located near Norman, OK.

radiation, net solar radiation, and rainfall. Correlation analyses (Pearson *r*) were used to examine the relationship between variation in toxicity and each of these weather parameters. Since conditions immediately preceding the sampling would presumably have more impact than the earlier conditions at the site, weather data were grouped into two time intervals, days 1–3 preceding sampling and days 4–7 preceding sampling.

All water samples were analyzed at 100% concentration for developmental toxicity using the standardized FETAX assay (Bantle *et al.*, 1990; Bantle, 1995; Bantle and Sabourin, 1991). Experiments where controls exceeded the 10% acceptable limits for mortality or malformation were repeated if possible. In no instance was data used when controls indicated greater than 16% mortality or greater than 14% malformation. Values for pH were always between 6.0 and 9.0. Cosolvents to dissolve hydrophobic contaminants were not used in this study. Water quality parameters including temperature, pH, conductivity, salinity, turbidity, and dissolved oxygen were collected using YSI 6000 (YSI Incorporated, Yellow Springs, OH) multiparameter water quality sondes installed in the streams at both locations.

RESULTS

The data from the YSI water quality meters are summarized to demonstrate that ambient water quality could sustain normal growth and development of amphibians in the absence of xenobiotics (Table 2). Values for pH ranged from 6.45 to 8.79 at the landfill and 6.35 to 9.23 at the reference site. Conductivity at the reference site ranged from 0.48 to 1.91 mS/cm. The conductivity range at the landfill was between 0.00 and 1.87 mS/cm. Salinity values ranged from 0.23 to 0.96 parts per thousand (ppt) at the reference

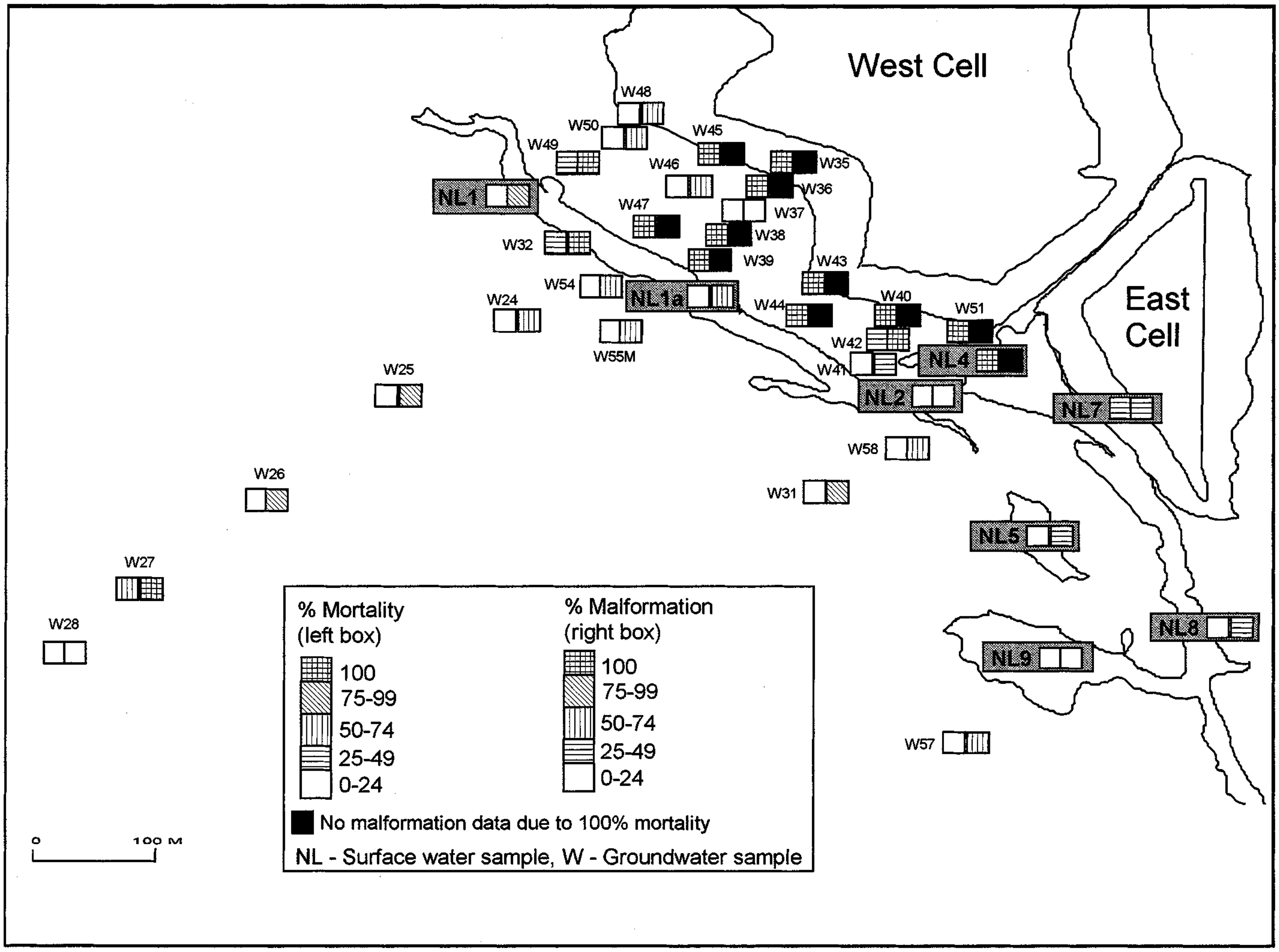

FIG. 3. Results of the FETAX toxicity tests on ground and surface water samples taken from the landfill site (November 16–17, 1995).

site and 0.00 to 1.31 ppt at the landfill. Dumont (unpublished) found that *Xenopus* embryos developed and grew normally in artificial seawater (Instant Ocean) at concentrations up to 1% (10 ppt). Ammonia content for the landfill water was between 0.20 and 3.40 mg/L. Turbidity fluctuated from 0.00 to 867.00 and 0.90 to 785.90 NTU at the landfill and reference sites, respectively. These data suggest that the standard water quality variables (exclusive of toxicants) were within acceptable ranges for FETAX and probably did not affect native populations of amphibians nor the growth and development of test embryos.

Results of the FETAX developmental toxicity tests on the ground and surface water samples taken from the landfill site are presented in Tables 3 and 4. The spatial distribution of the samples taken 16–17 November in relation to the landfill site and the FETAX results are depicted in Fig. 3. Groundwater samples were highly toxic in the area between the landfill and the stream, indicating a plume of toxicants from the landfill. The toxicity diminished with increasing distance from the landfill. Nearly one-half of the groundwater samples taken during these time periods caused 100% mortality. Many surviving embryos of the remainder of the samples were moderately to severely malformed (Table 3). Five of the groundwater samples caused 100% of the surviving embryos to be malformed. Ten of the samples caused malformations in 50 to 100% of the survivors and the remaining samples caused malformations in greater than 12% of the survivors. Finally, all samples except two caused a significant reduction in growth of exposed embryos.

Surface water samples from the landfill site often indicated higher than normal toxicity (Table 4) with mortality values ranging from 0 to 100%. More than one-fourth of the samples tested produced greater than 20% mortality. Toxicity was particularly high at location NL 4, which is a seep

TABLE 2
Monthly Statistics of Water Quality Data for the Landfill (NL) and Reference (AL) Sites

			pH	Conductivity (mS/cm)	Salinity (ppt)	Water temperature (°C)	Ammonia N (mg/L)	Turbidity (NTU)
Jun 1996	AL	Min	7.14	0.66	0.32	21.14	[a]	1.10
		Max	8.66	1.91	0.96	32.72	[a]	89.60
		Mean	7.72	1.33	0.66	27.38	[a]	6.30
	NL	Min	6.78	0.00	0.00	17.49	0.40	0.00
		Max	8.79	1.87	0.94	39.44	1.50	867.00
		Mean	7.78	0.64	0.31	27.19	0.99	60.71
Aug 1996	AL	Min	6.35	0.63	0.30	23.71	[a]	2.50
		Max	8.43	0.97	0.47	31.48	[a]	146.30
		Mean	7.30	0.86	0.42	27.26	[a]	13.17
	NL	Min	6.45	0.04	0.02	19.95	[b]	0.00
		Max	8.15	2.53	1.31	28.65	[b]	820.00
		Mean	6.89	1.53	0.77	22.54	[b]	39.45
Mar 1997	AL	Min	7.72	0.75	0.37	8.75	[b]	5.50
		Max	9.23	1.43	0.72	20.41	[b]	33.20
		Mean	7.97	1.13	0.56	13.72	[b]	9.96
	NL	Min	7.47	0.89	0.44	7.72	0.30	0.00
		Max	8.10	0.97	0.48	10.84	1.20	183.20
		Mean	7.79	0.95	0.47	9.15	0.52	7.32
Apr 1997	AL	Min	7.41	0.66	0.32	12.97	[a]	0.90
		Max	8.86	1.35	0.67	21.98	[a]	785.90
		Mean	7.85	1.04	0.51	16.81	[a]	178.51
	NL	Min	7.02	0.31	0.15	7.61	0.30	5.00
		Max	7.94	1.01	0.50	21.48	3.40	34.00
		Mean	7.39	0.78	0.38	14.91	1.14	12.29
May 1997	AL	Min	7.52	0.48	0.23	16.07	[a]	3.10
		Max	8.36	1.41	0.70	28.51	[a]	362.30
		Mean	7.87	1.05	0.52	22.02	[a]	21.95
	NL	Min	6.90	0.80	0.39	16.24	0.20	0.00
		Max	7.27	1.21	0.60	23.28	1.60	99.40
		Mean	7.10	1.11	0.55	20.42	1.28	3.10

[a] Ammonia not measured at reference site.
[b] Electrode failed during this time period.

where groundwater surfaces (Fig. 1). Samples from this location always caused 100% mortality. Malformation rates ranged from 0 to 95% at the other sampling locations. Nearly one-fourth of these samples had a malformation rate greater than 20% and most samples caused a significant reduction in growth of embryos (Table 4). Control 96-h *Xenopus* embryos had well-formed muscular and nervous systems and organs including eyes, gut, and heart (Fig. 4a). Examples of malformations caused by samples from the landfill site are illustrated in Figs. 4b and 4c. These embryos were exposed for 96 h to a 20 and 30% concentration of water from location NL 4. The malformed embryos were shorter than the controls and, although the eyes appeared normally developed, the gut was improperly coiled. This was more pronounced in the embryo exposed to the 30% concentration of water (Fig. 4c). This embryo also exhibited a reduced development of the head and face. The most distinctive malformation observed in these embryos was the dorsal curvature of the tail, which suggests abnormal development of the notochord. All of these responses increase in severity with increasing concentrations of contaminated water. The dorsal curvature of the tails has been observed in at least one other instance, that of exposure to aqueous extracts of crude shale oil (Dumont, unpublished; Bantle *et al.*, 1990).

Surface water samples taken from the reference site indicated higher than normal toxicity in several cases with mortality values ranging from 0 to 100%. However, the 100% mortality observed with sample AL 4 taken 13 May, 1997, was possibly due to factors other than toxicity because no previous samples from that location were toxic. Occasionally, bacteria or fungi can cause high mortality in samples. Other than this sample, the greatest observed mortality was 37%. Only 15% of all samples from the reference site had mortality greater than 20%. Malformation at the reference site ranged from 0 to 41%, with only one sample

TABLE 3
Results of FETAX Toxicity Tests on Groundwater Samples Taken from the Landfill Site

Site[a]	Sample date	Mortality (%)	Malformation (%)	Growth inhibition (% of control)
W0	18-Aug-96	100	[b]	[b]
	31-Jan-97	100	[b]	[b]
	29-Mar-97	100	[b]	[b]
	28-Apr-97	100	[b]	[b]
W28	16–17-Nov-95	18	12.4	98.9
W31	16–17-Nov-95	4	75	83.1[c]
W32	16–17-Nov-95	28	100	72.9[c]
W35	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W36	16–17-Nov-95	100	[b]	[b]
W37	16–17-Nov-95	10	17.8	93.6[c]
W38	16–17-Nov-95	100	[b]	[b]
W39	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W40	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W41	16–17-Nov-95	16	35.4	91.6[c]
	21-Jan-96	4	52.6	99.3
W42	16–17-Nov-95	40	100	69.4[c]
	21-Jan-96	56	100	83.3[c]
W43	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W44	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W45	16–17-Nov-95	100	[b]	[b]
W46	16–17-Nov-95	14	68	85.0[c]
	21-Jan-96	0	48	91.2[c]
W47	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W48	16–17-Nov-95	16	73.4	92.2[c]
	21-Jan-96	24	92.3	83.6[c]
W49	16–17-Nov-95	38	100	83.5[c]
	21-Jan-96	30	23.8	94.3[c]
W50	16–17-Nov-95	12	56.8	93.0[c]
	21-Jan-96	4	25	93.1[c]
W51	16–17-Nov-95	100	[b]	[b]
	21-Jan-96	100	[b]	[b]
W54	16–17-Nov-95	2	67.3	90.2[c]
	21-Jan-96	22	100	79.8[c]
W55M	16–17-Nov-95	10	67	93.8[c]
	21-Jan-96	10	40.4	90.3[c]
W57	16–17-Nov-95	4	59	86.3[c]
W58	16–17-Nov-95	24	68.9	90.3[c]

[a] For samples taken 16–17 November, 1995, the location and results are shown in Fig. 3.

[b] No malformation/growth inhibition data due to 100% mortality.

[c] Significantly different from controls ($P < 0.05$) using the *t* test for grouped observations.

revealing malformations greater than 20%. Less than one-half of the samples caused a significant reduction in growth of embryos (Table 4).

Surface water samples collected from June 1996 through May 1997 indicated a temporal variation in surface water developmental toxicity at the landfill site. Correlation analyses on this series of water samples indicated relationships between toxicity and weather parameters (Table 5). The most obvious observation was that location NL 4 displayed a complete lack of correlation with any weather parameter. Samples from this location always caused 100% mortality of exposed embryos regardless of weather conditions. For this reason, results for this location are not provided in Table 5. Another notable observation was that location NL 6 did not display the same general trends in relation to the weather variables as the other locations. Water samples from NL 6 were taken directly from the Norman sewage effluent discharge and were thus representative of a completely different source for contaminants.

Several trends were observed across the remaining sample locations. There was a general trend for mortality to be negatively correlated with cumulative rainfall for days 1–3 preceding sampling, and one location, NL 3, demonstrated a statistically significant negative correlation. There was also a trend for mortality to be negatively correlated with average relative humidity for days 1–3 preceding sampling, with two locations, NL 5 and NL 7, indicating significant negative correlations. Mortality in general was positively correlated with average solar radiation for days 1–3 preceding sampling with two locations, NL 1 and NL 3 revealing significant positive correlations. Mortality also tended to be positively correlated with net solar radiation for days 1–3 preceding sampling with one location, NL 3, demonstrating a significant positive correlation. There was no observable trend across sampling locations for mortality to be correlated with average air temperature. However, mortality of samples from NL 6 was negatively correlated with average air temperature of days 1–3 preceding sampling. No significant correlations were observed between mortality and weather parameters for days 4–7 preceding sampling.

Mortality data from all the sampling locations were next averaged and these averages were used in another set of correlation analyses to examine the relationships between toxicity and weather parameters for the landfill site as a whole (Table 5). For reasons previously mentioned, locations NL 4 and NL 6 were not included in this analysis. Mean mortality was negatively correlated with both cumulative rainfall and average relative humidity for days 1–3 preceding sampling. Mean mortality was positively correlated with both average solar radiation and net solar radiation for days 1–3 preceding sampling. Mean mortality was not correlated with average air temperature nor with any weather parameter during the 4–7 days preceding sampling.

DISCUSSION

FETAX results indicated that an area of groundwater downgradient from the landfill was contaminated. Toxicity diminished with increasing distance from the landfill.

TABLE 4
Results of FETAX Toxicity Tests on Surface Water Samples Taken from the Landfill (NL) and Reference sites (AL)

Date	NL1	NL1A	NL2	NL3	NL4	NL5	NL6	NL7	NL8	NL9	AL1	AL2	AL3	AL4
Mortality (%)														
16–17-Nov-95	10	6	18	NT	100	0	NT	44	2	4	20	2	16	NT
21-Jan-96	4	22	18	NT	100	12	NT	NT	18	NT	NT	NT	NT	NT
16-Jun-96[a]	20	NT	15	NT	100	NT	8	12	NT	NT	NT	4	10	6
18-Aug-96[a]	4	NT	NT	13	100	NT	4	6	NT	NT	5	10	17	NT
31-Jan-97[a]	21	NT	20	15	100	25	25	39	NT	NT	22	37	11	6
27-Feb-97	6	NT	6	16	NT	6	8	12	NT	NT	12	8	18	0
29-Mar-97	8	NT	NT	32	100	24	12	34	NT	NT	12	16	10	12
12-Apr-97	12	NT	2	6	NT	6	6	8	NT	NT	8	4	6	8
27-Apr-97	2	NT	6	6	NT	2	14	8	NT	NT	4	26	6	4
13-May-97	32	NT	7	36	100	6	4	8	NT	NT	6	8	8	100
Malformation (%)														
16–17-Nov-95	91.70	68.20	19.40	NT	[b]	26.00	NT	38.80	26.30	12.50	41.00	16.20	4.50	NT
21-Jan-96	41.90	95.70	77.40	NT	[b]	31.80	NT	NT	22.20	NT	NT	NT	NT	NT
16-Jun-96[a]	14.80	NT	11.30	NT	[b]	NT	17.95	33.15	NT	NT	NT	16.10	18.65	18.25
18-Aug-96[a]	3.00	NT	NT	7.05	[b]	NT	5.00	8.10	NT	NT	4.03	4.93	4.37	NT
31-Jan-97[a]	7.73	NT	4.68	33.64	[b]	11.18	4.47	1.04	NT	NT	1.19	2.94	5.58	2.79
27-Feb-97	2.13	NT	0.00	0.00	NT	4.26	2.17	0.00	NT	NT	0.00	0.00	0.00	4.00
29-Mar-97	6.52	NT	NT	8.82	[b]	2.63	2.27	15.15	NT	NT	9.09	0.00	2.22	9.30
12-Apr-97	11.36	NT	6.25	4.27	NT	6.55	8.27	6.52	NT	NT	2.17	4.00	4.27	15.09
27-Apr-97	8.60	NT	4.26	4.26	NT	8.16	11.63	4.35	NT	NT	2.08	10.81	10.64	2.08
13-May-97	8.83	NT	2.00	21.88	[b]	8.00	2.00	10.00	NT	NT	4.26	12.42	17.23	[b]
Growth inhibition (% of control)														
16–17-Nov-95	85.9*	87.7*	90.8*	NT	[b]	92.0*	NT	92.6*	95.8*	96.9*	91.6*	97.3	99.1	NT
21-Jan-96	92.9*	91.9*	90.7*	NT	[b]	95.9*	NT	NT	96.1*	NT	NT	NT	NT	NT
16-Jun-96[a]	102.4*	NT	102.7[c]	NT	[b]	NT	98.2	95.6[c]	NT	NT	NT	100.4[d]	101.9[d]	98.3[d]
18-Aug-96[a]	99.9	NT	NT	97.9	[b]	NT	100.0	96.3[c]	NT	NT	98.4	96.9[c]	99.9	NT
31-Jan-97[a]	95.9*	NT	98.1[c]	89.0	[b]	99.3	95.9[c]	93.3	NT	NT	98.5[b]	94.9	95.3	98.4
27-Feb-97	98.2	NT	98.0	100.9	NT	99.1	91.8*	103.4	NT	NT	92.3*	94.7*	93.1*	94.0*
29-Mar-97	89.1*	NT	NT	95.6*	[b]	100.9	96.7	97.9	NT	NT	96.4	98.3	99.9	96.2
12-Apr-97	98.0	NT	102.0	105.1*	NT	103.2	100.6	95.8	NT	NT	101.6	102.4	104.3	97.6
27-Apr-97	93.9*	NT	95.5*	92.7*	79.6*	94.3*	83.8*	86.6*	NT	NT	93.9*	90.7*	96.1*	93.5*
13-May-97	89.1*	NT	96.3*	92.8*	[b]	95.6*	98.8	89.5*	NT	NT	94.0*	97.1	94.6*	[b]

Note. NT, not tested.

[a] Results shown for these dates are averages of two tests.

[b] No malformation/growth inhibition data due to 100% mortality.

[c] Only one test was significantly different from control ($P < 0.05$).

[d] Result of one test because of error in the other test.

* Significantly different from controls ($P < 0.05$) using the t test for grouped observations.

Therefore, it was concluded that the contamination was due to leachate from the landfill. This conclusion is further supported by an electromagnetic survey conducted by the USGS to determine the extent of the leachate plume. The survey assessed the apparent conductivity of the alluvium, which is determined by several factors including the porosity, pore space saturation, and the conductivity of the water or leachate in these pore spaces. The survey indicated an area of higher conductivity downgradient of the landfill which was interpreted as being caused by leachate flowing from the landfill and mixing with the water in the alluvium (Lucius and Bisdorf, 1995; Fig. 5). Additionally, the surface water at the landfill site is almost certainly receiving contaminants from the leachate plume due to the shallow nature of the aquifer.

It was demonstrated that weather parameters influence toxicity of surface waters at the landfill site. The significant negative correlation between rainfall and mean mortality probably reflects dilution. The negative correlation between relative humidity and mean mortality may be a secondary effect due to the strong correlation between rainfall and relative humidity. Another possibility, however, is that low relative humidity increases evaporation, thereby concentrating toxicants. The positive correlation between average

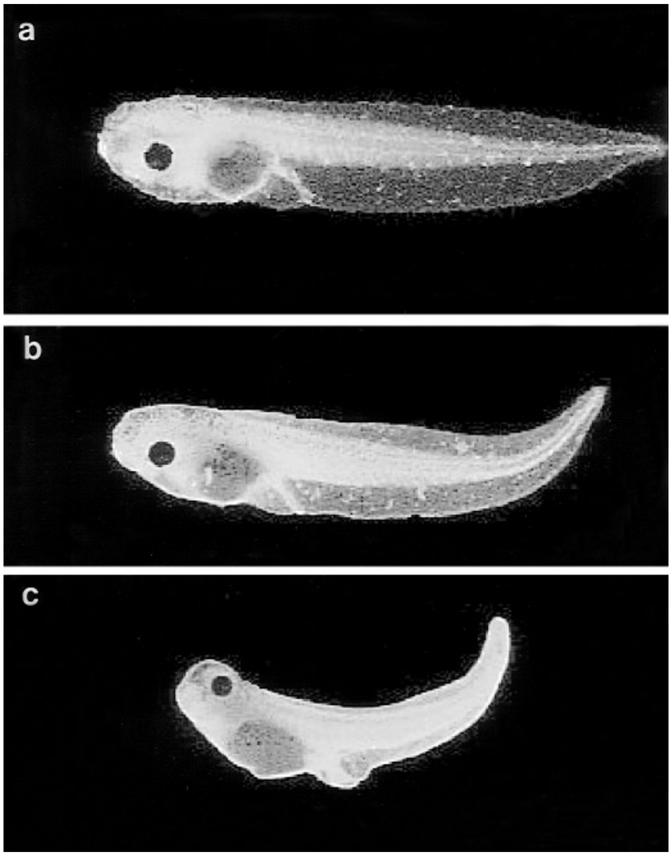

FIG. 4. Effect of water from Norman landfill NL 4 location on *Xenopus* embryo development. (a) Control 96-h *Xenopus* embryos. (b) Embryo exposed to a 20% concentration of water from sample location NL 4. (c) Embryo exposed to a 30% concentration of water from sample location NL 4.

mortality and both average solar radiation and net solar radiation could be explained in several ways. One possibility is that solar radiation is low during rain events when toxicants are being diluted, whereas during periods of high solar radiation evaporation increases, concentrating toxicants. Another possibility is that photochemical reactions caused by solar radiation are increasing toxicity by converting less toxic compounds to more toxic derivatives. Experiments are currently being conducted which are designed to determine if ultraviolet radiation modifies the toxicity of this water.

The weather conditions during the 3 days immediately preceding sampling had a greater effect on toxicity of surface waters than did earlier weather conditions. For days 4–7 preceding the sample date, mean mortality was not correlated with weather parameters, whereas, for days 1–3 all weather parameters except average air temperature were significantly correlated with mortality.

The surface water sampling locations at the landfill site differ in hydrological conditions, which affect the toxicity at these locations (see Fig. 1). Location NL 4 is a seep where the groundwater surfaces and merges with the stream and with water from the sewage discharge. This sampling location appears to be the only surface location where emerging groundwater was not significantly diluted by surface water. Because samples from NL 4 always induced 100% mortality, FETAX results from this location were not included in the correlation analyses for the landfill site as a whole. Future experiments using dilutions of samples from NL 4 will determine the LC_{50}. The LC_{50} data will then be correlated with weather parameters to determine if the toxicity was affected.

TABLE 5

Correlation Coefficients (Pearson *r*) of Mortality vs Environmental Variables for Norman Landfill Surface Water Samples Taken from June 1996 to May 1997

Environmental variable	Period preceding sampling	NL1	NL2	NL3	NL5	NL6	NL7	Mean mortality[a]
Cumulative rain	Days 1–3	– 0.61	– 0.69	– 0.81*	– 0.67	– 0.07	– 0.47	– 0.77*
	Days 4–7	– 0.40	– 0.54	0.08	– 0.28	– 0.29	– 0.12	– 0.18
Average relative humidity	Days 1–3	– 0.22	– 0.45	– 0.68	– 0.91*	– 0.52	– 0.87*	– 0.91*
	Days 4–7	0.36	0.12	0.01	– 0.54	– 0.44	– 0.58	– 0.42
Average solar radiation	Days 1–3	0.70*	0.38	0.87*	0.48	– 0.14	0.28	0.73*
	Days 4–7	– 0.01	– 0.23	0.02	– 0.42	– 0.50	– 0.51	– 0.30
Average net radiation	Days 1–3	0.70	0.31	0.89*	0.49	– 0.19	0.32	0.77*
	Days 4–7	– 0.28	– 0.71	0.05	– 0.41	– 0.36	– 0.37	– 0.34
Average air temperature	Days 1–3	0.26	– 0.08	0.55	– 0.35	– 0.75*	– 0.54	0.22
	Days 4–7	0.23	– 0.21	0.39	– 0.49	– 0.69	– 0.54	0.32

[a] Correlation coefficients calculated based on average mortality of NL1, NL2, NL3, NL5, and NL7.

* Significantly different ($P < 0.05$) using the *t* test.

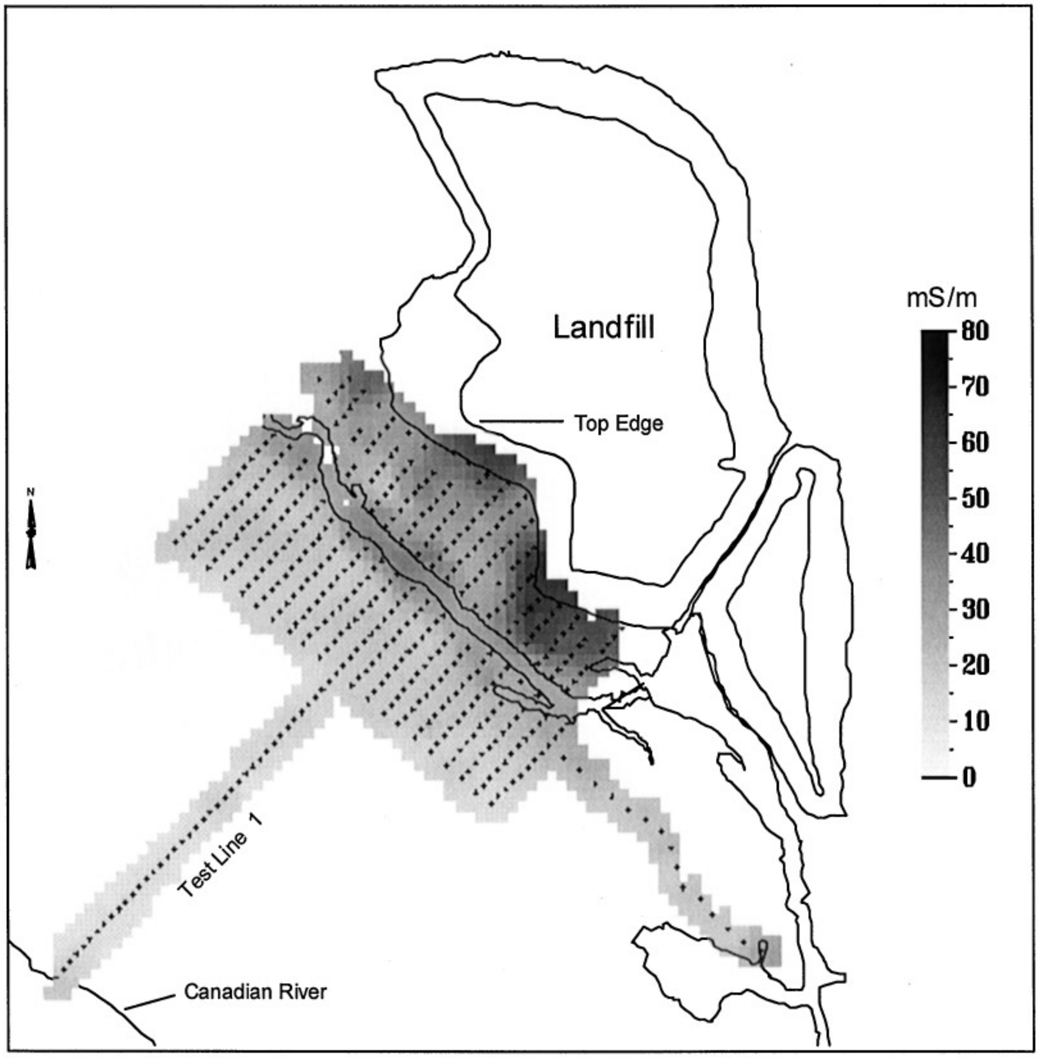

FIG. 5. Image map of EM31-D HMD apparent conductivity for the Norman, OK landfill area (Reproduced, with permission, from Lucius and Bisdorf, 1995).

Samples from NL 6, which were taken directly from the sewage discharge, were not contaminated by the groundwater. Therefore, mortality results from this location were not included in the correlation analyses for the landfill site as a whole.

It is suspected that the negative correlation between mortality and average air temperature at this location is related to the efficiency of the sewage treatment plant. The plant may be more efficient at removing toxicants during warmer weather.

The remaining surface water sampling locations could be receiving contaminants from the shallow groundwater near the landfill (S. Christenson, USGS, personal communication). As the plume of toxicants moving from the landfill contacts the stream, changes in surface water toxicity would be expected. However, the contamination appears to be diluted by surface waters.

FETAX indicated the surface water samples taken from the reference site were generally less toxic than the landfill site. However, there were occasions when the reference samples exhibited elevated toxicity. It should be noted that land use upstream of the reference site is agricultural and agrichemicals may be affecting toxicity.

The evaluation of water toxicity described in this article is part of a larger study of the effects of contamination on amphibians at the landfill. One *in situ* study is using mesh enclosures containing embryos of native anuran species to corroborate the results of the FETAX evaluation. Preliminary results suggest that some locations cannot support growth and development of amphibians. An amphibian monitoring study has also been initiated, and various monitoring techniques for examining amphibian populations at the landfill and the noncontaminated reference site are being evaluated. Amphibians had very low breeding success in the spring of 1996. It was not possible to locate any eggs or larvae. This was attributed to the extremely dry conditions during this period. During the spring of 1997, weather conditions were better and amphibians bred in temporary pools in the sandy areas between the streams and the river at both locations (Figs. 1 and 2). Eggs and larvae of *Bufo woodhousii*, *Rana blairi*, and *Rana catesbeiana* were found in these pools in large numbers. Larvae of *R. blairi* and *R. catesbeiana* were also observed in the streams at both sites. The temporary pools were not present during the spring of 1996. Other researchers have also observed correlations between recruitment failures and drought (Pechman *et al*., 1991). Such nonequilibrium population dynamics typify amphibians and make population declines difficult to distinguish from natural population fluctuations (Berven, 1990; Pechman *et al*., 1991; Blaustein, 1994; Pechman and Wilbur, 1994). Subsequent long-term studies are needed to elucidate the effects of the numerous factors affecting amphibian populations.

CONCLUSIONS

Results of this study indicated a plume of toxicants exuding from the landfill and mixing downgradient with the groundwater. The groundwater between the landfill and the Canadian River is contaminated, with toxicity diminishing as distance from the landfill increases. The small stream which runs adjacent to the landfill also revealed high toxicity, probably as a result of interaction between the stream and the shallow alluvial aquifer. Water samples from the landfill induced mortality, malformations, and growth inhibition in test embryos. Samples from the reference site were generally less toxic.

Surface water developmental toxicity varied through time. Some toxicity at the landfill site was significantly correlated with measurable weather parameters. The variable toxicity of the surface water indicated the need to sample numerous times during the year and suggested that the hazard to amphibians may be dependent on surface water toxicity during the breeding season when eggs and larvae would be exposed.

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