Pergamon

Chemosphere, Vol. 33, No. 11, pp. 2303–2320, 1996

PII: S0045-6535(96)00322-0
0045-6535/96 $15.00+0.00

ESTIMATION OF THE HAZARD OF LANDFILLS THROUGH TOXICITY TESTING OF LEACHATES

I. Determination of leachate toxicity with a battery of acute tests.

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(Received in USA 9 February 1996; accepted 31 July 1996)

ABSTRACT

Twenty-seven landfill leachates were tested on a battery of conventional toxicity tests (microalgae, daphnids, duckweeds) and new microbiotests (rotifers, crustaceans, protozoans, luminescent bacteria).

The toxicity varied substantially from one test species to the other, from one site to the other, as well as from one type of landfill to the other. Leachates of domestic wastes were significantly more toxic than those of pure industrial wastes; the most toxic leachates were found for landfills receiving hazardous industrial wastes mixed with domestic wastes.

The highest sensitivity was found for the protozoan assay, followed by the crustacean microbiotests. All other types of bioassays appeared to be substantially less sensitive to the toxicants present in the landfill leachates.

The results of a Principal Component Analysis suggest that in approximately 90% of the cases the toxicity of landfill leachates can be assessed by applying a test battery composed of a bacterial assay, a protozoan test and an assay with micro-algae, jointly with one of the following bioassays: higher plants, rotifers or crustaceans.

The application of a factor 100 to to the highest toxicity figure for each landfill leachate to extrapolate a Predicted No-Effect Concentration (PNEC) revealed that in quite a number of cases, the leachates need to be diluted by more than 10.000 times to make them innocuous for environmental biota.

Keywords: landfill leachate, acute toxicity, microbiotests, multivariate analysis.

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INTRODUCTION

The assessment of the hazard to the environment resulting from the disposal of solid wastes in landfills is, in many countries, still performed exclusively by chemical analysis of the solid wastes and/or their percolates. During recent years, however, it has become generally accepted that chemical data by themselves do not allow to evaluate the global toxic effect which may result from the leaching out of the chemicals from the landfills. As a result, increasing attention is to date focussed on the incorporation of toxicity tests in hazard evaluations of waste dumps, whereby acute and chronic impacts are assessed on "terrestrial" species in "contact tests" and bioassays are performed on leachates with aquatic organisms. Although a variety of hazard studies on landfill leachates has already been undertaken in various countries, very few of these investigations were *in situ* evaluations of the ecological impacts of leachate discharge on surface waters (Nuttall, 1973; SRAE, 1988; Keck and Jean, 1990, 1991). Furthermore, in most studies the toxicity of percolates has been determined in the laboratory on only one type of organism: fish, crustacean or microalgae (Vigers and Ellis, 1977; Walker and Adrian, 1977; McBride *et al.*, 1979; Cameron and Koch, 1980; Wong, 1989; Cheung *et al.*, 1993), or in the best cases on a limited set of test species, e.g. fish/microalgae/daphnid/bacteria (Atwater *et al.*, 1983; Plotkin and Ram, 1984; Deneuvy, 1987; Lambolez *et al.*, 1993; Ernst, 1994; Devare and Bahadir, 1994; Jean and Fruget, 1994).

For complex effluents such as landfill leachates, the use of a battery of tests seems particularly appropriate due to the number of potential toxicants, the effects of which may be species-dependent and chemical-dependent. In the majority of the studies on solid wastes referred to above, little attention has been paid to aspects such as the selection of representative test species, the sensitivity of the tests and the simplicity and the costs of the assays. Neither have serious endeavors been made either to find out which minimum battery of tests is in fact needed to make an ecologically realistic evaluation of the hazard of landfills.

The present study attempts to address some of these questions by determination of the toxicity of 25 landfill leachates collected from various sites in France, and of 2 additional "artificial" (lab-made) leachates, with a battery of conventional toxicity tests and new microbiotests. The investigations were made on various categories of landfills receiving domestic wastes, either or not mixed with non hazardous or hazardous industrial wastes and on (artificial) landfill wastes reconstituted in the laboratory with household refuse.

In order to take the (often neglected) ecological realism in toxicity testing in consideration, the battery of bioassays selected for this study was composed of test species belonging to the three trophic levels of aquatic food chains: producers, consumers and decomposers.

MATERIALS AND METHODS

Effluent sampling

Twenty three leachates were collected over a period of 36 months, at 14 landfills filled with various kinds of wastes:

a) domestic wastes exclusively (L1a, L1b, L1c, L2, L5, L6a, L6b, L11a, L11b, L14), or domestic wastes mixed with non hazardous industrial wastes such as e.g. wood, paper and cardboard refuse, and/or sludge from wastewater treatment plants (L3, L4)

b) non hazardous industrial solid wastes (L8a, L8b)

c) hazardous industrial solid wastes such as e.g. paint residues, waste water treatment sludges and fly ashes from incineration plants (L9a, L9b, L17a, L17b), or hazardous industrial wastes mixed with domestic wastes (L10a, L10b, L15, L16a, L16b).

Two additional samples were collected from $70m^3$ lysimeters filled with domestic solid wastes, either or not mixed with lime (L7a and L7b respectively).

Samples marked with the same number originate from the same landfill; the small letters refer to different samples taken at a particular site.

The leachates were collected at various points of the dump sites :

a) the inflow of a lagoon (L1a and L1c, collected at an interval of 4 months, and L6a)

b) the outflow of a lagoon (L1b, collected the same day as L1a; L8a, and L6b, which was taken the same day as L6a)

c) storage ponds (L4, L15 and L16a)

d) the outflow of a pipe (L2, L3, L5, L10a, L11a)

e) the bottom of a waste cell (L7a, L7b, L9a, L9b, L17a, L17b).

Sample L8b was collected subsequently to treatment of L8a, which consisted of filtration through scoria and stabilization in a lagoon.

Samples L9a and L9b were taken from a cell operated 7 years ago, and from a cell in operation respectively.

Sample L10b resulted from oxidation and lime coagulation treatment of L10a.

Sample L11b was obtained following lime treatment and decantation of L11a.

Sample L16b resulted from oxidation of L16a, and L14 was obtained after a nitrification/ultrafiltration treatment.

Samples L12 and L13 were "artificial" leachates generated in the laboratory by filling lysimetric columns with biodegradable household refuse (meat, vegetables, fish, wrapping paper); for leachate L13 lime had been added to the lysimetric column.

Sampling of leachates was performed by collecting 20 litres with a bucket and transferring into a plastic container. Time of transportation to the laboratory did not exceed 6 hours. All leachates were immediately stored frozen in 1 litre-polyethylene flasks, at -18°C. Prior to testing the leachates were thawed at room temperature during a period of 15 hours, followed by 2 hours of sedimentation of the suspended material. The supernatant was subsequently collected for the toxicity tests. Preliminary investigations (not published) revealed that the former treatment of the samples had no significant effects on the physico-chemical characteristics of the leachates, nor on their toxicity.

Toxicity tests

Acute toxicity tests were performed on all leachates with 8 different test species:
- for the producers: *Scenedesmus subspicatus* (micro-algae) and *Lemna minor* (duckweed)
- for the consumers: *Brachionus calyciflorus* (rotifers) and *Daphnia magna*, *Ceriodaphnia dubia* and *Thamnocephalus platyurus* (crustaceans)
- for the decomposers: *Vibrio fisheri* (bacteria) and *Spirostomum ambiguum* (ciliate protozoan).

Table 1 lists the test species used, the type of test, the test duration and the endpoints measured and shows that these bioassays comprised acute as well as chronic tests, with exposures ranging from 30 minutes to 5 days.

The micro-algae test was performed according to the (experimental) AFNOR standard NT90-304 (AFNOR, 1980), modified in 1990 (AFNOR, 1990a). The initial algal density was 6.10^5 to 1.10^6 cells.ml^{-1} and the volume of test solution in the Erlenmeyers 10 ml. The sensitivity of the microalgae was controlled in parallel experiments with potassium dichromate as reference toxicant.
The protocol used for the duckweed test is described in Clément and Bouvet (1993). The growth inhibition is based on measurement of frond increase after 5 days of exposure.
Daphnia magna assays were performed in both the French and the Belgian laboratory. The protocol followed in France was the AFNOR NF T 90-301 standard (AFNOR, 1990b), whereas in Belgium the tests were performed according to OECD Guideline 202 (OECD, 1993).
The rotifer, ciliate and crustacean microbiotests with *Brachionus calyciflorus*, *Spirostomum ambiguum*, *Ceriodaphnia dubia*, and *Thamnocephalus platyurus* were performed according to the Toxkit Standard Operational Procedures (Snell and Persoone, 1989; Van Steertegem and Persoone, 1993; Centeno *et al*, 1995).
The bacterial luminescence inhibition test was performed according to the French standard AFNOR NF T90-320 (AFNOR, 1991), using the Lumistox equipment (Dr Lange, Düsseldorf, Germany), with measurement of the luminescence after 30 minutes exposure.

Table 1. Characteristics of the battery of test-organisms used for toxicity assessment of landfill leachates

Trophic level	Organisms	Type of test	Endpoint	Test duration
Producers	Micro-algae *Scenedesmus subspicatus*	conventional	growth inhibition	5 days
	Duckweed (*Lemna minor*)	conventional	growth inhibition	5 days
Consumers	Rotifers *Brachionus calyciflorus*	microbiotest	mortality	24 hours
	Crustaceans *Daphnia magna*	conventional	mortality	24 hours
	Ceriodaphnia dubia	microbiotest	mortality	24 hours
	Thamnocephalus platyurus	microbiotest	mortality	24 hours
Decomposers	Bacteria *Vibrio fisheri*	microbiotest	luminescence inhibition	30 mn
	Protozoans *Spirostomum ambiguum*	microbiotest	mortality	24 hours

All leachates used for the bacterial test were prefiltered on 0.45 µm filters to preclude light interference by particles in suspension.

All bioassays performed in the French laboratory were carried out within less than 2 months conservation of the leachates. In turn, the replicated *Daphnia magna* assay and the ciliate and crustacean microbiotests were carried out in the Belgian laboratory on samples which had been stored for periods ranging from one and a half year up to 4 years and which had been sent on ice from France to Belgium shortly prior to performance of the tests.

Data treatment

For all the tests LC50 or EC50's were calculated using the Spearman-Karber method (Hamilton *et al.*, 1977), except for the *Daphnia magna* assays performed in France, for which a probit-derived method (Litchfield and Wilcoxon, 1949) was used.

For a more convenient graphical expression and interpretation of the toxicity data, all median toxicity values were converted in Toxic Units (TU), i.e. the inverse of the LC/EC50 expressed in %, according to the formula of Sprague and Ramsay (1965): TU = [1/L(E)C50] x 100. This expression is the dilution factor which must be applied to the effluent so as to obtain a 50% effect, and is directly proportional to toxicity.

In an attempt to define the minimum number of toxicity tests necessary to assess the hazard of landfills through the toxicity of their leachates, Principal Components Analysis (PCA) has been performed on the whole set of toxicity data.

RESULTS

Toxicity of the leachates

The results of the toxicity tests with the 8 different bioassays, on all the samples, are given in Table 2. The number of Toxic Units (TU) are represented graphically in Figure 1 for domestic wastes, Figure 2 for domestic wastes mixed with non-hazardous or hazardous industrial wastes, Figure 3 for hazardous and non-hazardous industrial wastes and Figure 4 for the 2 artificial wastes. From these graphs it clearly appears that the toxic effects found vary very substantially from one test species to the other, from one site to the other, as well as from one type of landfill to the other. The number of TU indeed ranges from 0 up to 500, exceeding 100 in a substantial number of cases.

Figures 1 to 4 furthermore show that the leachates of domestic wastes, alone or mixed with hazardous or non-hazardous industrial wastes are substantially more toxic than those of pure industrial wastes. For the laboratory generated leachates of household refuse, the lime treated sample (L13) was substantially less toxic than the non-treated one (L12); in turn oxidation or lime treatment did not seem to have much influence on the toxicity of the leachates of domestic wastes either or not combined with hazardous industrial wastes (samples L10b, L11b, L16b).

Table 2. Results of toxicity tests on 23 landfill leachates and on 2 artificial leachates, expressed in toxic units (with 95% confidence limits).

S.s.: *Scenedesmus subspicatus*; *L.m.*: *Lemna minor*; *V.f.*: *Vibrio fisheri*; *S.a.*: *Spirostomum ambiguum*; *B.c.*: *Brachionus calyciflorus*; *T.p.*: *Thamnocephalus platyurus*; *D.m.*: *Daphnia magna*; *C.d.*: *Ceriodaphnia dubia*.

Sample	S.s.	L.m.	V.f.	S.a.	B.c.	T.p.	D.m. (Ghent)	D.m. (Lyon)	C.d.
Leachates from domestic wastes									
L1a	8.3	20.8 NC	6.9 NC	37 (33-42)	10.1 (9-12)	200 (167-250)	4.3 (NC)	13.5 (NC)	17.5 (15-21)
L1b	2.5 NC	5.4 NC	8.1 (NC)	270.3 (233-385)	2.6 (2.2-2.9)	19.2 (18-21)	29.8 (26-35)	2.2 (NC)	83.3 (67-100)
L1c	5.0 NC	12 NC	14.5 (NC)	370.4 (323-435)	2 (1.8-2.2)	100 (NC)	30.6 (26-36)	9.4 (9.3-9.4)	111.1 (100-125)
L2	1.2 (NC)	3.4 (NC)	1.1 (NC)	3.9 (2.8-5.3)	2.7 (2.5-2.9)	2.1 (1.7-2.6)	NT	1.9 (NC)	3 (2.6-3.4)
L5	4.3 (NC)	26.3 (NC)	5.4 (NC)	117.6 (10-137)	13.2 (13-14)	52.6 (46-59)	21.6 (19-24)	15.6 (14-24)	33.3 (29-37)
L6a	3.3 (NC)	6.3 (NC)	43.5 (NC)	41.7 (37-48)	3.4 (3.2-3.5)	25 (NC)	7.8 (7-8)	7.9 (6-9)	15.2 (13-17)
L6b	2.6 (NC)	10 (NC)	3 (NC)	83.3 (71-91)	7.2 (6.8-7.6)	43.5 (NC)	14.5 (12-17)	4.6 (NC)	25.6 (21-29)
L11a	18.2 (NC)	33.3 (NC)	9.9 (NC)	285.7 (250-333)	11.6 (NC)	71.4 (63-83)	31.8 (27-39)	22.2 (20-24)	111.1 (100-111)
L11b	16.7 (NC)	16.1 (NC)	2.4 (NC)	135.1 (111-159)	7.4 (6.5-8.3)	66.7 (50-91)	18.9 (16-22)	14.5 (14-15)	83.3 (71-100)
L14	3.7 (NC)	10.4 (NC)	11.1 (NC)	NP	100 (NC)	NP	NP	NP	NP
L3	1 (NC)	4.3 (NC)	2.5 (NC)	29.4 (25-50)	1.7 (1.4-1.9)	7.3 (6-9)	3.6 (2.9-5.8)	2.8 (NC)	10 (9-12)
L4	7.7 (NC)	13.5 (NC)	18.2 (NC)	83.3 (71-100)	16.7 (15-18)	111.1 (91-125)	16.3 (14-19)	9.3 (NC)	43.5 (36-50)

Table 2 (continued). Results of toxicity tests on 23 landfill leachates and on 2 artificial leachates, expressed in toxic units (with 95% confidence limits).

Sample	S.s.	L.m.	V.f.	S.a.	B.c.	T.p.	D.m. (Ghent)	D.m. (Lyon)	C.d.
Leachates from lysimeters (domestic wastes)									
L7a	6.7 (NC)	25 (NC)	4.8 (NC)	243.9 (222-270)	24.4 (23-26)	500 (500-1000)	21.5 (19-25)	22.7 (NC)	71.4 (63-77)
L7b	5 (NC)	14.5 (NC)	2.5 (NC)	90.9 (77-125)	5.6 (5.5-5.8)	30.3 (23-42)	5.3 (4.6-6.2)	13 (12-14)	18.5 (15-23)
Leachates from non-hazardous industrial solid wastes									
L8a	1.6 (NC)	6.8 (NC)	8.8 (NC)	100 (83-111)	4.4 (3.9-4.9)	9.3 (8.6-10.1)	4.2 (NC)	3.1 (1-3.9)	13.7 (12-16)
L8b	1.3 (NC)	2.6 (NC)	5.3 (NC)	13.3 (12-15)	1.3 (1.3-1.4)	4.4 (NC)	2.4 (2.1-2.9)	1.1 (NC)	3.2 (2.4-3.7)
Leachates from hazardous industrial solid wastes									
L9a	3.7 (NC)	2.2 (NC)	14.7 (NC)	NT	1 (NC)	NT	NT	1.3 (NC)	1.7 (1.5-1.9)
L9b	8.5 (NC)	4.5 (NC)	40 (NC)	2.6 (2.2-3.1)	1 (NC)	3.4 (2.9-4)	1.6 (1.4-1.9)	1.8 (1.7-1.9)	3 (2.6-3.4)
L17a	NP	>32.3 (NC)	NP	11.9 (11-13)	NP	12.5 (NC)	8.8 (7.7-11)	NP	17.9 (16-21)
L17b	NP	NT	NP	4.7 (NC)	NP	<3.1 (NC)	NT	NP	1.9 (1.7-2.2)

NP: not performed

NT: not toxic

NC: not calculable

Sensitivity of the test organisms

In order to compare the relative sensitivity of the test species of the different assays for the individual leachates, the toxicity data have been ordered according to the classification proposed by Bulich (1982). This classification considers 6 categories of toxicity ranking from class 1 (>100 TU) to class 6 (<1 TU).

The procentual distribution for each test organism over the 6 toxicity classes, calculated for the total number of leachates, is represented graphically in Figure 5.

Table 2 (continued). Results of toxicity tests on 23 landfill leachates and on 2 artificial leachates, expressed in toxic units (with 95% confidence limits).

Sample	S.s.	L.m.	V.f.	S.a.	B.c.	T.p.	D.m. (Ghent)	D.m. (Lyon)	C.d.
Leachates from hazardous industrial solid wastes + domestic wastes									
L10a	2.4 (NC)	6.2 (NC)	13.3 (NC)	400 (345-500)	7.2 (6.7-7.7)	22.7 (16.1-32.3)	5.3 (4.8-5.9)	8.3 (NC)	21.7 (18-26)
L10b	33.3 (NC)	2.8 (NC)	10.6 (NC)	222.2 (179-278)	5.5 (5-6)	12.5 (NC)	5 (NC)	4.6 (NC)	29.4 (26-32)
L15	16.7 (NC)	40 (NC)	37 (NC)	400 (333-476)	19.6 (18-22)	200 (NC)	41.7 (35-48)	NP	125 (111-143)
L16a	9.1 (NC)	14.3 (NC)	43.5 (NC)	263.2 (NC)	19.6 (18-22)	90.9 (77-100)	15.6 (14-17)	NP	55.6 (48-63)
L16b	6.8 (NC)	15.6 (NC)	9.7 (NC)	208.3 (175-244)	16.4 (14-19)	35.7 (NC)	28.6 (24-35)	NP	76.9 (NC)
Artificial leachates from household refuse									
L12	71.4 (NC)	217.4 (NC)	142.9 (NC)	NP	55.6 (NC)	NP	NP	50 (46-56)	NP
L13	7.7 (NC)	40 (NC)	76.9 (NC)	NP	5.6 (NC)	NP	NP	26.3 (24-29)	NP

NP: not performed
NT: not toxic
NC: not calculable

This graph clearly reveals that some of the assays gave quantitatively substantially "more sensitive" effect-signals than others. For example, for more than 50% of the samples, the ciliate bioassay gave a toxic signal at the >100 TU level (class 1) and for another 20% of the leachates at the 30-100 TU level (class 2). The crustacean microbiotests with *Thamnocephalus platyurus* and *Ceriodaphnia dubia* were also quite sensitive to the toxicants in many leachates (10-20% score in class 1 and 25-30% in class 2 respectively). For most other test species in turn the highest percentages were situated in classes 3 and 4.

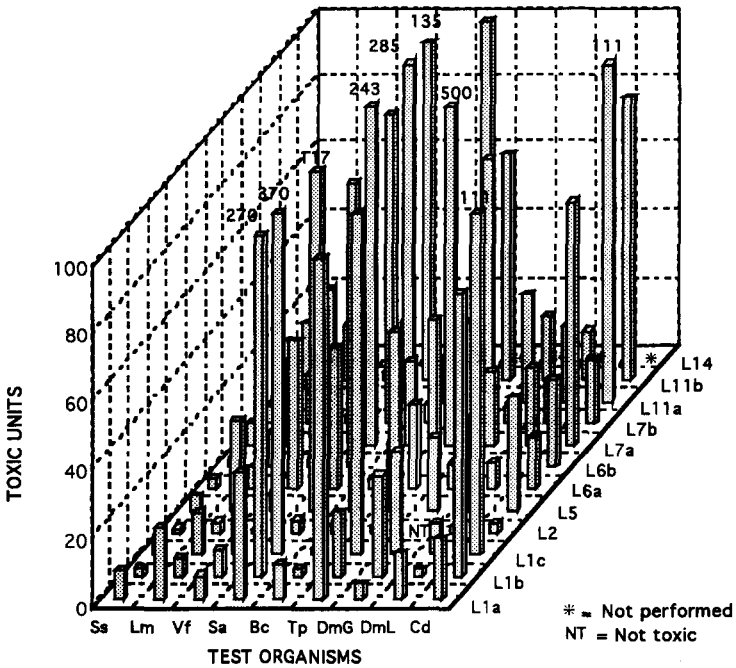

Figure 1. Toxicity of leachates of domestic wastes (results expressed in Toxic Units)

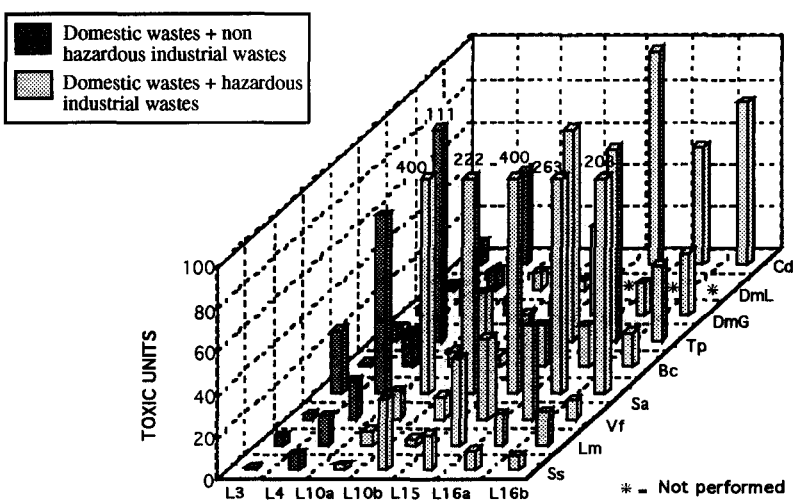

Figure 2. Toxicity of leachates of domestic wastes mixed with non-hazardous or hazardous industrial wastes (results expressed in Toxic Units)

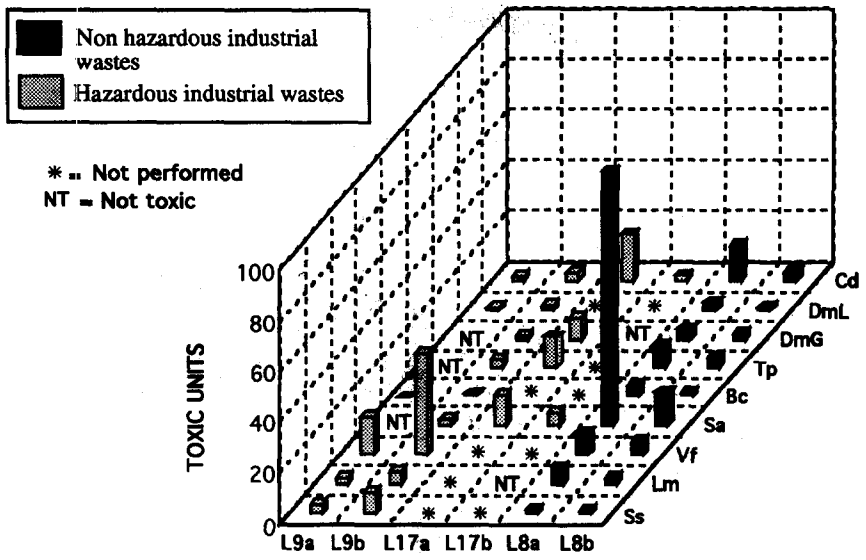

Figure 3. Toxicity of leachates of hazardous and non-hazardous industrial wastes (results expressed in Toxic Units)

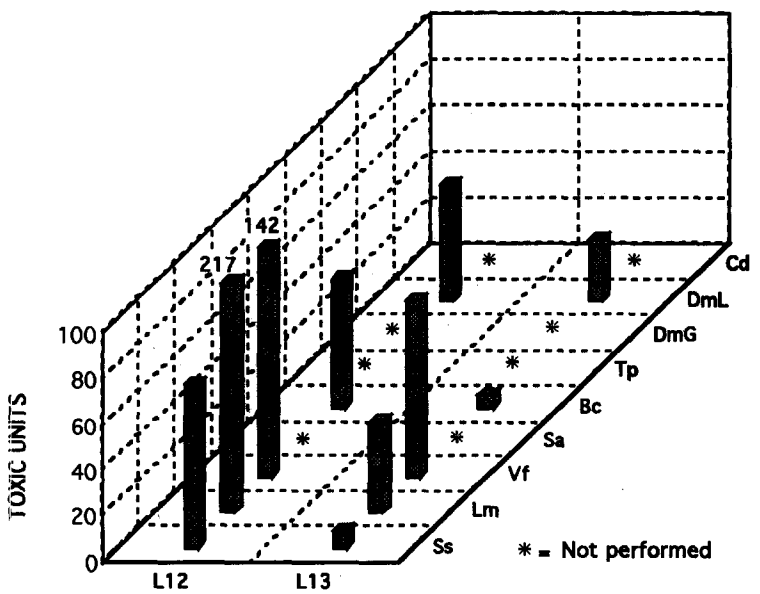

Figure 4. Toxicity of leachates of artificial solid wastes (results expressed in Toxic Units)

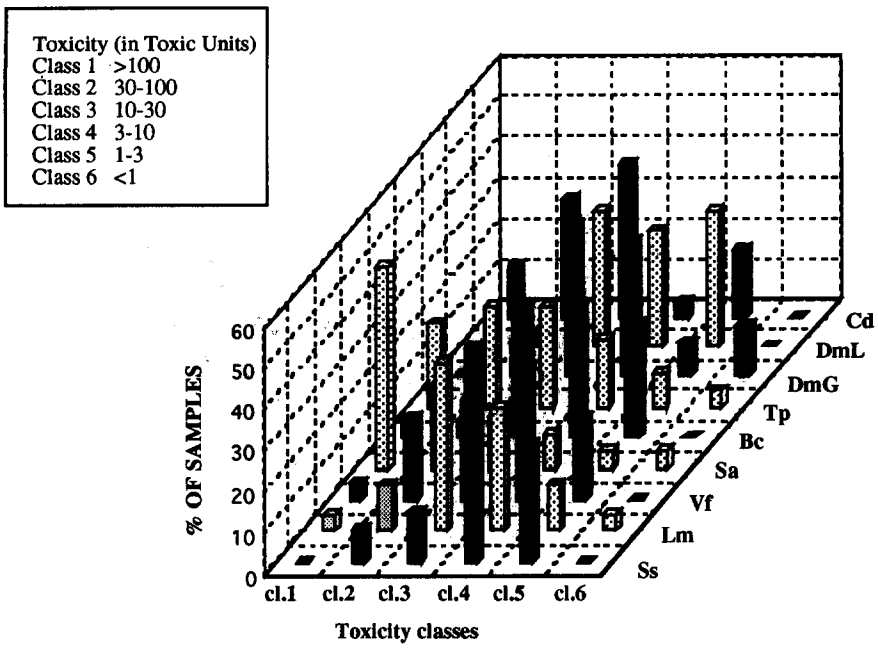

Figure 5. Toxicity ranking of 23 landfill leachates according to the classification of Bulich (1982)

An alternative way to compare the sensitivity of the different tests is to use the procedure worked out by Slooff (1983). Slooff's method calculates, for each sample, the arithmetic mean of all test results (expressed as L(E)C50's), then divides each test result by this mean, and subsequently calculates the geometric mean of the ratios for each assay. The smaller the final value, the more sensitive the test. The outcome of these calculations for the landfill leachates is presented in Table 3 by order of decreasing sensitivity; the sequence corroborates the ranking in toxicity classes according to Bulich (1982) shown in Figure 5, namely the highest sensitivity of the protozoan assay (0.12), followed by the crustacean microbiotests with *Thamnocephalus platyurus* (0.27) and *Ceriodaphnia dubia* (0.33). All other types of bioassays appeared to be substantially less sensitive to the toxicants present in the landfill leachates (from 0.8 to 1.56).

Both Table 3 and Figure 5 also show that from the two tests with plant biota, the duckweed assay is substantially more sensitive to the leachate toxicants than the micro-algal test. As can be seen from Table 2, there are in fact only three samples (L9a, L9b, L10b) for which the *S. subspicatus* assay showed a higher toxicity than the *L. minor* test.

Table 3. Relative tolerance of bioassays performed on landfill leachates on the basis of their LC50 or EC50 calculated according to the method of Slooff (1983)

S.s.: *Scenedesmus subspicatus*; *L.m.*: *Lemna minor*; *V.f.*: *Vibrio fisheri*; *S.a.*: *Spirostomum ambiguum*; *B.c.*: *Brachionus calyciflorus*; *T.p.*: *Thamnocephalus platyurus*; *D.m.*: *Daphnia magna*; *C.d.*: *Ceriodaphnia dubia*.

Organism	S.s.	L.m.	V.f.	S.a.	B.c.	T.p.	D.m.(G)	D.m.(L)	C.d.
Slooff value	1.56	0.81	0.8	0.12	1.27	0.27	0.96	1.1	0.33

Comparison of the results of the *D. magna* tests performed in France, with those of the assays carried out in the laboratory in Belgium on the same test species, revealed that for the majority of the leachates the toxicity had not changed significantly during the (very) long period of (frozen) storage. Indeed for 11 out of the 19 samples for which data pairs were available, the ratio of the numbers of TU's was less than 2, and for 5 other samples less than a factor 3; however, for one leachate (L1b) the ratio was 13 and for two others (L2 and L9a) no acute toxicity was found with the *D. magna* assay in Ghent, whereas the same leachate was quite toxic (50 and 80 TU respectively) to this crustacean test species, in the assays performed in France.

Selection of minimum test battery

Principal Component Analysis was eventually performed on 18 samples for which data were available for all the different bioassays; for the *D. magna* tests, only the results of the French laboratory were taken into consideration for this type of statistical analysis.

The calculations were carried out on the toxicity data from leachates of 11 domestic waste landfills (L1a, L1b, L1C, L2, L5, L6a, L6b, L7a, L7b, L11a, L11b), 4 domestic waste + hazardous or non-hazardous waste landfills (L3, L4, L10a, L10b) and 3 industrial waste landfills (L8a, L8b and L9b).

The outcome of the PCA is given in Table 4. Table 4 reveals that, when taking 0.7 as threshold value, the first axis contributes for approximately 50% to the total variation, with 5 tests based on the following species: *L. minor*, *B. calyciflorus*, *T. platyurus*, *D. magna* and *C. dubia*.

Axis 3 contributes for 12% to the variation with the *V. fisheri* assay which showed a particular pattern. Indeed, some samples (L5, L6b, L7a, L7b, L11b) which were generally very toxic to most organisms, were only slightly toxic to the luminescent bacteria, whereas others (L6a, L9a, L9b) showed the opposite.

A substantial gain in toxicity detection capacity of the battery can be made by adding axis 2 and 4, which account for 17% and 10% respectively of the total variation. Both the latter axes indeed have values which are only slightly below the 0.7 threshold for the *S. ambiguum* and the *S. subspicatus* assays respectively. The latter choice eventually means that in approximately 90% of the cases the toxicity of the samples can be

assessed by applying a test battery composed of a bacterial assay, a protozoan test and an assay with micro-algae, jointly with one of the following 5 bioassays: higher plants, rotifers or crustaceans (either *T. platyurus*, *D. magna*, or *C. dubia*).

Table 4. Principal Components Analysis on the toxicity data of 18 leachates for 8 types of tests
S.s.: *Scenedesmus subspicatus*; *L.m.*: *Lemna minor*; *V.f.*: *Vibrio fisheri*; *S.a.*: *Spirostomum ambiguum*; *B.c.*: *Brachionus calyciflorus*; *T.p.*: *Thamnocephalus platyurus*; *D.m.*: *Daphnia magna*; *C.d.*: *Ceriodaphnia dubia*.

Axes	1	2	3	4
Variables	Principal components			
S.s.	-0.3253	0.5151	0.3772	0.6744
L.m.	-0.8840	-0.1636	0.0504	0.0705
V.f.	0.2750	0.1377	0.8579	-0.4072
S.a.	-0.5247	0.6664	-0.2126	-0.3331
B.c.	-0.8397	-0.3864	0.1206	0.0287
T.p.	-0.7660	-0.3863	0.0778	-0.1714
D.m.	-0.9366	-0.1084	0.1211	0.0320
C.d.	-0.7077	0.5470	-0.1330	-0.2002
Contribution to total variation (in %)				
	48.9	17.1	12.2	10.1

The underlined figures are values above or near 0.70 threshold, and taken into consideration for the selection of the test battery

DISCUSSION

The present study again confirms the species-specific character of toxic impacts. The effect ratio between the most sensitive species and the least sensitive one of the 8 bioassays for each individual leachate, indeed ranged from as low as a factor 2 to as high as a factor 187. The samples with the highest ratios were from landfills L1b, L1c, L7a, L10a and L10b. Those with the lowest ratios originate from landfills L2, L12, L17a and L17b. It is interesting to note that the latter case comprises samples that were either very toxic (L12) or only slightly toxic (L2, L17a and L17b).

There does not seem to be any relationship between the large or small differences in sensitivity of the test organisms and the nature of the landfills; the high or low ratios mentioned above indeed originate from each of the 4 classes of landfills indicated in Figure 1. Consequently, the classification of the leachates on the basis of their toxicity does not reflect a categorization according to the type of wastes which the landfills are

receiving, except for the non-mixed industrial wastes which appeared to be the least toxic. It ensues that landfills receiving domestic solid waste must be considered as as dangerous as industrial waste landfills, a result also found out by Schrab *et al.* (1993) who measured the toxicity and the mutagenicity of various landfill leachates. Moreover our results show that co-disposal of industrial and domestic wastes increases the risks for the environment. In France, where most industrial landfills have received or are still receiving household refuse at an average rate of 40%, co-disposal is now considered as a waste of space and volume, due to the scarcity of sites which are geologically suited for waste disposal.

A (conservative) factor of 100 has been applied to the highest toxicity figure for each landfill leachate to extrapolate a Predicted No-Effect Concentration (PNEC) which would be protective for all environmental biota. The outcome of these calculations revealed that in quite a number of cases, the leachates need to be diluted by more than 10.000 times to make them innocuous for environmental biota. For the 25 landfill leachates concerned, the dilution of the leachates in nearby watersheds appears to be much lower in most cases, not the least because of the relatively high flow rate of particular leachates (from a few liters/sec up to over 10 liters/sec). For example, the discharge of leachate from landfill L11b into a small stream resulted in a very low dilution (2 to 3 fold) and samples taken 2 km from the point of inflow were still highly toxic to duckweed (>13 TU).

Although on the basis of the outcome of the sensitivity calculations according to the method of Slooff (1983) one would be tempted to take the ciliate microbiotest and one crustacean microbiotest as "the best" tools for assessing the hazard of landfill leachates, the outcome of the PCA clearly showed that for a reliable hazard estimation (covering 90% of the cases), one needs 4 out of the 8 bioassays that were applied in this study.

Interestingly, the battery emerging from these considerations consists of test species belonging to 4 phylogenetically different groups of biota: procaryotes (*V. fisheri*), unicellular animal eucaryotes (*S. ambiguum*), unicellular plant eucaryotes (*S.subspicatus*) and one representative of either a multicellular plant, or various groups of animal eucaryotes.

Caution has, however, to be expressed for a too strict application of the PCA data. The PCA was indeed made with the data of 18 out of the 25 samples and hence does not reflect the full set of results. As indicated in the section on sensitivity of the test organisms, it appeared, for example, that the assay on the growth of fronds of the duckweed *L.minor* showed in all cases but two, to be more sensitive than the test with the microalgae. Consequently it seems logical to also consider the former test (instead of the microalgal test), for incorporation in the minimum battery of assays for detection of the toxic effects of solid waste leachates on plants.

CONCLUSIONS

The assessment of the toxic hazard of the 25 landfill leachates with a battery of 8 tests comprising biota of different phylogenetic origins, revealed that many leachates were highly toxic and hence should be submitted to an efficient treatment before discharge.

The toxicological approach allowed to earmark the high toxicity of the leachates of landfills receiving domestic wastes either or not combined with industrial wastes. The latter finding gives solid ground to opposing to the present common procedure of co-disposal of different types of wastes.

PCA revealed that several of the tests applied gave redundant information and that a selected battery of only 4 tests suffices to assess the toxic impact of landfills in 90% of the cases.

Finally, with regard to the financial implications resulting from application of 4 different types of assays on each waste sample, it is interesting to mention that low cost microbiotests are now available (or will become available very shortly) for most of the tests needed in the battery. Indeed, besides the bacterial luminescence inhibition test, low cost "Toxkit" microbiotests with crustaceans, micro-algae and protozoan test species are either already available commercially or are nearing completion in the Laboratory for Biological Research in Aquatic Pollution at the University of Ghent in Belgium.

Like the bacterial luminescence inhibition test, each of these new microbiotests is based on the use of "non-active" (dormant) stages of particular test species, and hence is fully independent of the culturing/maintenance burden of live stocks.

It ensues that cost-effective large scale hazard monitoring of landfills by application of simple and rapid microbiotests has now become possible, which should allow to earmark the most dangerous sites where urgent action is needed.

ACKNOWLEDGEMENTS

Part of this study was carried out in the Laboratory of Environmental Sciences, at the Ecole Nationale des Travaux Publics de l'Etat, in Lyon (France). Fundings were provided by the French Ministry of Public Works.

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