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## ESTIMATION OF THE HAZARD OF LANDFILLS THROUGH TOXICITY TESTING OF LEACHATES

### 2. Comparison of physico-chemical characteristics of landfill leachates with their toxicity determined with a battery of tests.

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#### ABSTRACT

Twenty-two landfill leachates were tested on a battery of aquatic organisms (microalgae, daphnids, rotifers, crustaceans, protozoans, luminescent bacteria) and analysed for various physico-chemical parameters (pH, conductivity, alkalinity, chemical oxygen demand, dissolved organic carbon, organic nitrogen, total ammonia, K<sup>+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe, Zn, Cu). Principal Components Analyses (PCA) and regression analyses were performed on both types of variables in order to find possible explanations for the observed toxicity. The results of multivariate analyses showed a general relationship between both data sets, namely the most (or least) contaminated samples were also generally the most (or least) toxic. These analyses also suggested that ammonia, alkalinity, and chemical oxygen demand (COD) were associated with increasing toxicity. Simple and multiple regression analyses allowed to confirm the importance of ammonia and alkalinity for causing toxicity to most organisms. Luminescent bacteria, however, were found to be more sensitive to the organic load of the leachates.

The results are discussed in the context of risk assessment of various types of landfills.

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**Keywords:** landfill leachate, acute toxicity, physico-chemical composition, multivariate analysis.

#### INTRODUCTION

In France, discharge permits of effluents are mainly based on physico-chemical parameters, such as suspended solids, chemical oxygen demand (COD), total organic carbon (TOC), total and organic nitrogen, phenols, hydrocarbons, cyanide, heavy metals (Al, As, Cd, Pb, Cr, Cu, Zn, Hg). Biological assessment of effluents, however, is limited to the *Daphnia magna* acute toxicity test and biochemical oxygen demand (BOD<sub>5</sub>) measurements (Vasseur *et al.*, 1991). This approach has many drawbacks, especially for complex effluents, for which the above characteristics give but an incomplete idea of their actual composition and their potential effects (Persoone, 1992). Although in recent years it has become clear that discharge monitoring should be based on bioassays instead of -or in addition to- physico-chemical measurements, in France little has been done in this regard. Bioassays provide essential information on the ecotoxicological effects of chemicals and are especially useful when a battery of tests is applied (Giesy and Graney, 1989). The benefits of such an

approach have been recently demonstrated in a study of landfill leachates (Clément *et al.*, 1996). Landfill leachates can be considered as complex effluents which may contain organic compounds (fatty acids, humic substances, solvents, alcohols, phenols, aromatic compounds, pesticides,...), heavy metals (Cd, Zn, Cu, Pb, ...) and numerous other chemicals ( $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{S}^{2-}$ ,  $\text{HCO}_3^-$ , ...). In a recent review, Clément *et al.* (1993) stated that ammonia, heavy metals (such as Ag, Hg, Pb, Cd, Mn, Zn, Cu), and organic compounds (such as tanins, lignin and phenol) may individually or in combination be responsible for the observed toxicity of landfill leachates. The contribution of ammonia and alkalinity to the acute toxicity of landfill leachates to duckweed has been recently demonstrated by Clément and Merlin (1995). In the present paper, the results of 8 toxicity tests (from Clément *et al.*, 1996) performed on 22 landfill leachates and the corresponding 12 physico-chemical characteristics of these wastes have been analyzed using multivariate analysis.

## MATERIALS AND METHODS

### *Effluents*

Twenty two leachates were collected at 13 dumping sites receiving:

(i) municipal solid wastes alone (MSW samples L1a, L1b, L1c, L2, L5, L6a, L6b, L11a, L11b) or mixed with non hazardous industrial solid wastes (wood, paper, cardboards, wastewater treatment plant sludges) (MSW/ISW samples L3, L4),

(ii) non hazardous industrial solid wastes (ISW samples L8a, L8b),

(iii) hazardous industrial solid wastes (paint residues, waste water treatment sludges, fly ashes from incineration plants, etc.) alone (ISW\* samples L9a, L9b) or mixed with municipal solid wastes (I/MSW\* samples L10a, L10b, L15, L16a, L16b).

Two samples (L7a and L7b) were collected from 70 m<sup>3</sup> lysimeters filled with domestic solid wastes alone (L7a) or mixed with lime (L7b). Samples with the same number originate from the same site.

Details of the sampling procedures are given in Clément *et al.* (1996). From the 27 samples considered in Clément *et al.* (1996) only 22 were used in the present paper, due to the lack of physico-chemical or toxicological data for 5 samples.

### *Physico-chemical analyses*

The following parameters were measured: pH, conductivity, alkalinity, chemical oxygen demand, dissolved organic carbon, organic nitrogen, total ammonia,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , Fe, Zn, Cu. Analytical methods are reported in Clément and Merlin (1995).

### *Toxicity tests*

Tests were carried out on a battery of 8 organisms, including primary producers (*microalgae Scenedesmus subspicatus*, duckweed *Lemna minor*), micro-crustaceans (cladocera *Daphnia magna* and *Ceriodaphnia dubia*, and anostraca *Thamnocephalus platyurus*), rotifers (*Brachionus calyciflorus*), protozoans (*Spiristomum ambiguum*), and luminescent marine bacteria (*Vibrio fischeri*). The micro-algae test was performed according to

**Table 1.** Physico-chemical characteristics of the leachate samples (Cond: conductivity; Alk: alkalinity; COD: chemical oxygen demand; DOC: dissolved organic carbon; Norg: organic nitrogen; all values are in mg.l<sup>-1</sup> except Cond (mS.cm<sup>-1</sup>), Alk (mg CaCO<sub>3</sub>.l<sup>-1</sup>), COD (mg O<sub>2</sub>.l<sup>-1</sup>), DOC (mg C.l<sup>-1</sup>), Cu (µg.l<sup>-1</sup>); ND: not determined).

| Sample   | pH  | Cond | Alk   | COD | DOC | Norg | NH <sub>3</sub> | K <sup>+</sup> | Na <sup>+</sup> | Ca <sup>2+</sup> | Mg <sup>2+</sup> | Cl <sup>-</sup> | SO <sub>4</sub> <sup>--</sup> | Fe   | Zn  | Cu |
|--|-----|------|-------|-----|-----|------|-----------------|----------------|-----------------|------------------|------------------|-----------------|-------------------------------|------|-----|----|
| <b>Leachates from domestic wastes (MSW)</b>  |     |      |       |     |     |      |                 |                |                 |                  |                  |                 |                               |      |     |    |
| L1a  | 8.3 | 10.6 | 5450  | 3.5 | 1.1 | 177  | 1029            | 939            | 1135            | 82               | 101              | 1275            | 50                            | 7.0  | 0.7 | 20 |
| L1b  | 8.4 | 5.3  | 1820  | 1.7 | 0.4 | 32   | 142             | 627            | 577             | 48               | 51               | 750             | 11                            | 1.5  | 0.2 | 65 |
| L1c  | 8.0 | 7.4  | 3590  | 4.7 | 1.3 | 21   | 883             | 739            | 972             | 20               | 76               | 1165            | 42                            | 6.2  | 0.5 | 25 |
| L2   | 7.8 | 5.0  | 585   | 0.4 | 0.1 | 37   | 192             | 212            | 607             | 78               | 77               | 934             | 12                            | 0.0  | 0.0 | 30 |
| L5   | 8.6 | 13.6 | 5610  | 1.0 | 0.3 | 88   | 713             | 687            | 2760            | 17               | 217              | 829             | 10                            | 5.0  | 0.6 | 50 |
| L6a  | 7.9 | 10.4 | 4280  | 8.0 | 2.7 | 85   | 537             | 1612           | 788             | 158              | 295              | 1281            | 0                             | 7.0  | 0.5 | 15 |
| L6b  | 8.5 | 9.1  | 3960  | 1.8 | 0.5 | 85   | 396             | 1607           | 757             | 54               | 271              | 1301            | 58                            | 7.0  | 0.0 | 20 |
| L11a   | 7.9 | 18.2 | 6950  | 2.4 | 0.6 | 231  | 1231            | 864            | 2957            | 119              | 169              | 1710            | 25                            | 3.0  | 0.6 | ND |
| L11b   | 8.4 | 10.0 | 3700  | 1.2 | 0.3 | 146  | 628             | 523            | 1583            | 39               | 177              | 2185            | 148                           | 4.0  | 0.5 | ND |
| L3   | 8.0 | 5.2  | 2465  | 0.5 | 0.1 | 14   | 103             | 202            | 610             | 78               | 77               | 1326            | 12                            | 0.3  | 0.4 | 10 |
| L4   | 8.4 | 9.3  | 3650  | 0.8 | 0.3 | 81   | 627             | 1011           | 519             | 15               | 61               | 1953            | 11                            | 0.7  | 0.1 | 20 |
| <b>Leachates from lysimeters (domestic wastes)</b>                                 |     |      |       |     |     |      |                 |                |                 |                  |                  |                 |                               |      |     |    |
| L7a  | 8.0 | 11.7 | 10950 | 1.4 | 0.3 | 145  | 1247            | 1025           | 1419            | 190              | 151              | 1277            | 5                             | 10.0 | 0.0 | 60 |
| L7b  | 8.3 | 9.2  | 5990  | 1.5 | 0.3 | 113  | 935             | 683            | 1028            | 246              | 101              | 1103            | 506                           | ND   | ND  | ND |
| <b>Leachates from non-hazardous industrial solid wastes (ISW)</b>                  |     |      |       |     |     |      |                 |                |                 |                  |                  |                 |                               |      |     |    |
| L8a  | 8.0 | 9.7  | 2100  | 1.1 | 0.3 | 17   | 161             | 708            | 2592            | 50               | 35               | 1040            | 5                             | 1.0  | 1.3 | 60 |
| L8b  | 8.3 | 6.4  | 600   | 0.7 | 0.2 | 16   | 70              | 401            | 1547            | 29               | 21               | 922             | 137                           | 0.6  | 1.2 | 30 |
| <b>Leachates from hazardous industrial solid wastes (ISW*)</b>                     |     |      |       |     |     |      |                 |                |                 |                  |                  |                 |                               |      |     |    |
| L9a  | 6.2 | 3.2  | 620   | 2.0 | 0.7 | 24   | 27              | 49             | 146             | 657              | 41               | 611             | 9                             | 21.0 | 0.9 | 5  |
| L9b  | 6.9 | 7.9  | 1450  | 7.6 | 2.6 | 14   | 55              | 102            | 577             | 2026             | 0                | 949             | 32                            | 0.8  | 0.5 | 10 |
| <b>Leachates from hazardous industrial solid wastes + domestic wastes (I/MSW*)</b> |     |      |       |     |     |      |                 |                |                 |                  |                  |                 |                               |      |     |    |
| L10a   | 6.6 | 35.2 | 840   | 1.6 | 0.4 | 105  | 993             | 1881           | 3463            | 3319             | 343              | 9284            | 3                             | 65.0 | 2.5 | 20 |
| L10b   | 7.7 | 29.4 | 250   | 1.5 | 0.4 | 44   | 1010            | 1731           | 5032            | 3065             | 306              | 7916            | 22                            | 1.0  | 0.3 | 40 |
| L15  | 8.6 | 43.7 | 6500  | 8.8 | ND  | 100  | 4007            | 3000           | 6790            | 24               | 73               | 34200           | 522                           | ND   | ND  | ND |
| L16a   | 8.3 | 79.8 | 4200  | 5.2 | ND  | 146  | 1203            | 5900           | 22400           | 70               | 71               | 72400           | 3200                          | ND   | ND  | ND |
| L16b   | 8.4 | 82.1 | 3400  | 4.6 | ND  | 118  | 784             | 5000           | 19400           | 74               | 90               | ND              | 9170                          | ND   | ND  | ND |

the (experimental) AFNOR standard NT90-304 (AFNOR, 1980), modified in 1990 (AFNOR, 1990a).

The protocol used for the duckweed growth inhibition test, based on the measurement of frond increase after 5 days of exposure, is described in Clément and Bouvet (1993).

*Daphnia magna* assays were performed according to the AFNOR NF T 90-301 standard (AFNOR, 1990b).

**Table 2.** Toxicity results for the 22 landfill leachates tested. All results are expressed in toxic units (Bc: *Brachionus calyciflorus*; Dm: *Daphnia magna*; Tp: *Thamnocephalus platyurus*; Cd: *Ceriodaphnia dubia*; Sa: *Spiristomum ambiguum*; Lm: *Lemna minor*; Ss: *Scenedesmus subspicatus*; Vf: *Vibrio fisheri*).

|   | Ss   | Lm   | Vf   | Sa    | Bc   | Tp    | Dm   | Cd    |
|---|------|------|------|-------|------|-------|------|-------|
| Leachates from domestic wastes (MSW)  |      |      |      |       |      |       |      |       |
| L1a   | 8.3  | 20.8 | 6.9  | 37.0  | 10.1 | 200.0 | 13.5 | 17.5  |
| L1b   | 2.5  | 5.4  | 8.1  | 270.3 | 2.6  | 19.2  | 2.2  | 83.3  |
| L1c   | 5.0  | 12.0 | 14.5 | 370.4 | 2.0  | 100.0 | 9.4  | 111.1 |
| L2  | 1.2  | 3.4  | 1.1  | 3.9   | 2.7  | 2.1   | 1.9  | 3.0   |
| L5  | 4.3  | 26.3 | 5.4  | 117.6 | 13.1 | 52.6  | 15.7 | 33.3  |
| L6a   | 3.3  | 6.3  | 43.5 | 41.7  | 3.4  | 25    | 7.9  | 15.2  |
| L6b   | 2.6  | 10.0 | 3.0  | 83.3  | 7.2  | 43.5  | 4.6  | 25.6  |
| L11a  | 18.2 | 33.3 | 9.9  | 285.7 | 11.6 | 71.4  | 22.2 | 111.1 |
| L11b  | 16.7 | 16.1 | 2.4  | 135.1 | 7.4  | 66.7  | 14.5 | 83.3  |
| L3  | 1.0  | 4.3  | 2.5  | 29.4  | 1.7  | 7.3   | 2.8  | 10.0  |
| L4  | 7.7  | 13.5 | 18.2 | 83.3  | 16.8 | 111.1 | 9.4  | 43.5  |
| Leachates from lysimeters (domestic wastes)                                 |      |      |      |       |      |       |      |       |
| L7a   | 6.7  | 25.0 | 4.8  | 243.9 | 24.4 | 500.0 | 22.7 | 71.4  |
| L7b   | 5    | 14.5 | 2.5  | 90.9  | 5.7  | 30.3  | 13.0 | 18.5  |
| Leachates from non-hazardous industrial solid wastes (ISW)                  |      |      |      |       |      |       |      |       |
| L8a   | 1.6  | 6.8  | 8.8  | 100   | 4.4  | 9.3   | 3.1  | 13.7  |
| L8b   | 1.3  | 2.6  | 5.3  | 13.3  | 1.3  | 4.4   | 1.1  | 3.2   |
| Leachates from hazardous industrial solid wastes (ISW*)                     |      |      |      |       |      |       |      |       |
| L9a   | 3.7  | 2.2  | 14.7 | 1.0   | 1.0  | 1.0   | 1.3  | 1.7   |
| L9b   | 8.5  | 4.5  | 40   | 2.6   | 1.0  | 3.4   | 1.8  | 3.0   |
| Leachates from hazardous industrial solid wastes + domestic wastes (I/MSW*) |      |      |      |       |      |       |      |       |
| L10a  | 2.4  | 6.2  | 13.3 | 400.0 | 7.2  | 22.7  | 8.4  | 21.7  |
| L10b  | 33.3 | 2.8  | 10.6 | 222.2 | 5.5  | 12.5  | 4.6  | 29.4  |
| L15   | 16.7 | 40.0 | 37.0 | 400.0 | 19.6 | 200.0 | 41.7 | 125   |
| L16a  | 9.1  | 14.3 | 43.5 | 263.2 | 19.6 | 90.9  | 15.6 | 55.6  |
| L16b  | 6.8  | 15.6 | 9.7  | 208.3 | 16.4 | 35.7  | 28.6 | 76.9  |

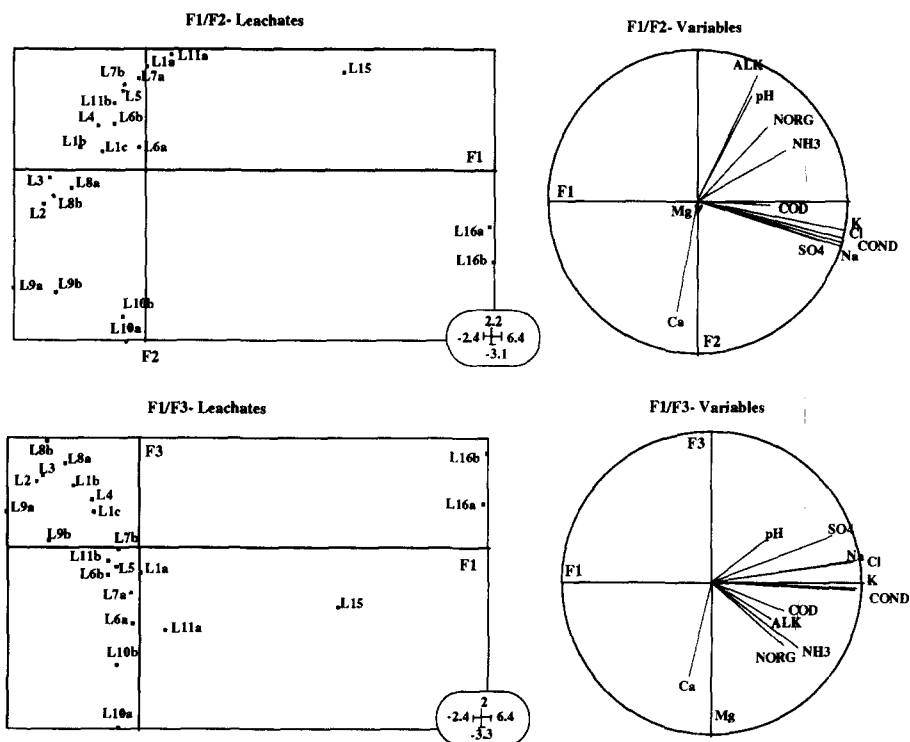
The microbiotests with the rotifer *Brachionus calyciflorus*, the ciliate *Spirostomum ambiguum*, and the crustaceans *Ceriodaphnia dubia* and *Thamnocephalus platyurus* were performed according to the Toxkit Standard Operational Procedures (Snell and Persoone, 1989; Van Steertegem and Persoone, 1993; Centeno *et al.*, 1994, Le Dû-Delepierre *et al.*, 1996).

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.

Results were expressed as toxic units, i.e. the inverse of the LC/EC50 expressed in %, according to the formula of Sprague and Ramsay (1965):  $TU = [1/L(E)C50] \times 100$ .

### Statistical analysis

Data were analyzed using simple and multi-linear regression analysis (software Statview®, Abacus Concepts, Inc, Berkeley, 1992) as well as Principal Components Analysis (software ADE 3.7 developed by D. Chessel and S. Dolédec, Université Claude Bernard Lyon 1, URA CNRS 1451, Ecologie des Eaux Douces et des Grands Fleuves).



**Figure 1.** Correlation diagrams of the variables (physico-chemical parameters) and factorial maps of the elements (leachates) in F1x2 and F1x3 planes of the PCA performed on the physico-chemical parameters of the 22 landfill leachates.

Principal Component Analysis (PCA) deals with two-dimensions tables where columns are represented by quantitative variables and rows by elements. The aim of PCA is to examine similarities between elements and

links between variables. The existence of links between variables leads to a reduction of the number of variables which are then called principal components. The data are projected onto Euclidian planes determined by principal components (Escofier and Pagès, 1990).

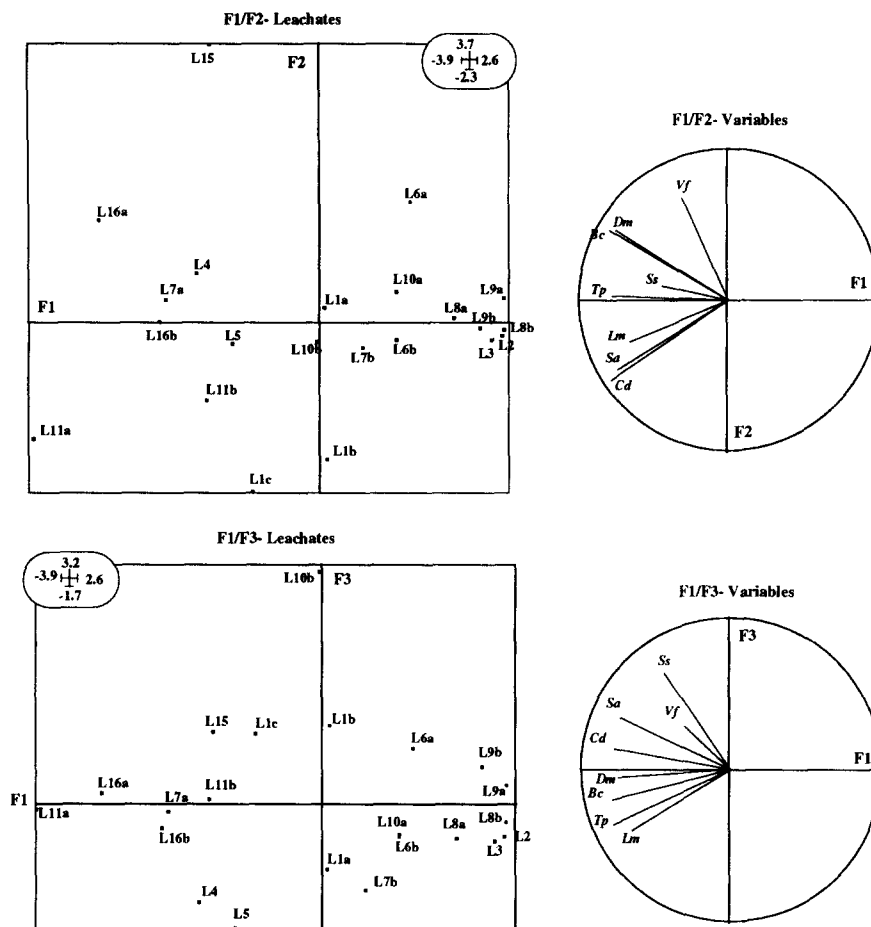
## RESULTS

### *Physico-chemical results*

Physico-chemical results are summarized in table 1 and are discussed in detail in Clément (1995). As a first step in the comparison of the ecotoxicological and physico-chemical data, a PCA was performed on the 16 physico-chemical variables (columns) and the 22 landfill leachates (rows). DOC was discarded due to its high correlation with COD ( $R=0.998$ ,  $p=0.0001$ ). Fe, Zn and Cu were discarded due to missing data and because they did not contribute much to total inertia. In the second analysis with the remaining 12 physico-chemical data, the first three axes, containing 78% of the information, were selected for the analysis. The results of the PCA are represented graphically in figure 1 and absolute contributions of the elements and variables to the selected factors are summarized in table 3.

The first axis is associated with parameters such as conductivity,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and mainly due to three leachates (L15, L16a and L16b) originating from landfills receiving household wastes and hazardous industrial wastes (high salinity). The second axis is characterized by the variables alkalinity, pH,  $\text{Ca}^{2+}$  and to some extent  $\text{NH}_3$ . Alkalinity, pH, and  $\text{NH}_3$  are linked together because landfill leachates with a high pH generally have a high alkalinity, and  $\text{NH}_3$  is a proton consumer, which thus contributes to the alkalinity of the samples. These three variables are characteristic of municipal solid wastes (MSW) landfill leachates, which are mainly located on the positive side of the second axis, whereas samples from landfills receiving industrial wastes (except L15) are located on the opposite side and characterized by a higher calcium content and a lower alkalinity. The third axis is explained by calcium and especially magnesium but only three samples (L8b, L10a, L10b) contribute significantly to this axis.

In a summary, it appears that the samples are distributed according to their origin (municipal solid wastes or industrial solid wastes) along the second axis, whereas the first axis characterizes their salinity. The factorial map F1xF2 (figure 1) clearly shows 6 groups: the MSW leachates on the upper left side, a group of 4 samples (L2, L3, L8a, L8b) close to the former, not very different by their origin but much less polluted, three couples of samples (L9a/L9b, L10a/L10b, L16a/L16b) which all have a typical profile due to specific industrial wastes, and sample L15 marked both by the domestic and the industrial components. Surprisingly, COD does not appear as a discriminating variable, probably because it is neither characteristic of the origin of landfill leachates nor linked to the mineral load. In addition, it can be observed that samples originating from the same location are close to each other on the PCA plot.



**Figure 2.** Correlation diagrams of the variables (test species) and factorial maps of the elements (leachates) in F1xF2 and F1xF3 planes of the PCA performed on the toxicological data of the 22 landfill leachates.

### Ecotoxicological results

The results of the toxicity tests (table 2) were presented and discussed in detail in Clément *et al.* (1996).

A PCA was performed on 8 ecotoxicological variables (columns) and 22 landfill leachates (rows). The first three axes contain 74.2% of the information and were consequently selected for the analysis. All the variables are located on the same side of correlation diagrams (figure 2), which means that in general all the tests respond in the same way, i.e. a sample is toxic (or not) for all organisms. The *Vibrio fischeri* (Vf) and *Scenedesmus subspicatus* (Ss) tests show a particular toxicity response since the former contributes mainly to the second axis (absolute contribution: 30.8%, table 4) and the latter contributes mainly to the third axis (absolute contribution: 42.2%). The structure of the data is nevertheless not simple because the other tests also have a significant contribution to the second and third axes. The position of the samples along the first axis

indicates that most tests give similar toxicity rankings. The position of L1b, L1c, L11a, L6a, L16a, L15 and L10b along the second and third axes, on the other hand, indicates that these samples may have different (compared to most other samples) toxicity characteristics. This observation is confirmed by the detailed analysis of ecotoxicological data discussed in Clément *et al.*, 1996.

#### *Ecotoxicological versus physico-chemical results*

In order to find possible relationships between the toxicity and physico-chemical data, we first projected (figure 3) the 12 physico-chemical variables as additional elements in the PCA of the ecotoxicological results (Jean and Fruget, 1994). Physico-chemical and toxicological variables are located on the same side, which shows a general relationship between both data sets, namely the most (or least) polluted samples are also generally the most (or least) toxic. This is the case for L1a, L7a, L11a, L11b, L15, L16a, and L16b on one hand, and for L2, L3, L8a, L8b on the other hand (see factorial maps in figure 2). Organic nitrogen, ammonia, alkalinity, conductivity, and chemical oxygen demand (COD) seem to be associated with increasing toxicity. It is nevertheless not possible to associate one particular test with one particular physico-chemical variable.

Considering all the variables simultaneously does not allow the identification of particular relationships between the toxicological results and the physico-chemical results. In particular, the relationship found by Clément and Merlin (1995) between toxicity to duckweed and ammonia associated with alkalinity or conductivity for 25 landfill leachates (including the 22 samples of the present study) is not confirmed by this type of analysis. However, these multivariate approaches indicate that ammonia and alkalinity, together with organic nitrogen and conductivity, might be « indicators » of potential toxicity. Moreover, a relationship between COD content and *Vibrio fischeri* responses is suggested. In order to clarify the existence of these possible relationships, simple and multiple regression analysis were performed. The results for the most significant relationships are displayed in table 3.

The contribution of ammonia and alkalinity to the observed toxicity in most tests is suggested. As un-ionized ammonia is generally suspected to be the toxic form of total ammonia (USEPA, 1985), a result which was confirmed by Clément and Merlin (1995) for duckweed, the un-ionized ammonia content of the leachate dilutions corresponding to EC50s for daphnids was compared with the 24hr-EC50 for un-ionized ammonia (Jean, 1991). Using the pH and temperature of the leachate dilutions (not available for samples L15, L16a and L16b), the un-ionized ammonia concentration can be calculated as follows (Emerson *et al.*, 1975):

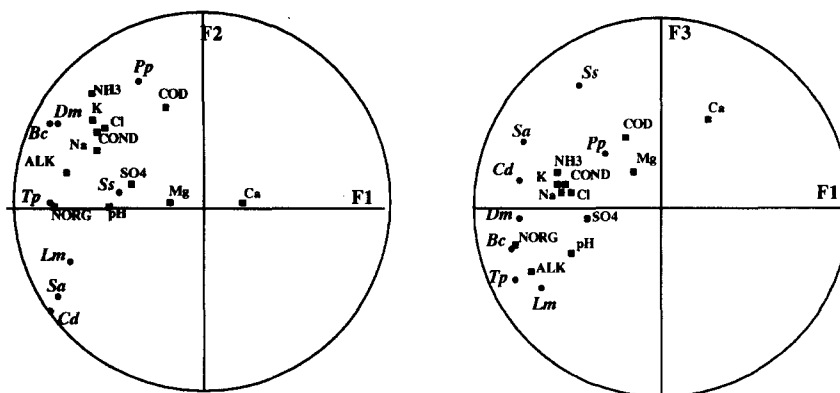
$$\% \text{ un-ionized NH}_3 = 100 / (1 + 10^{(\text{pKa} - \text{pH})})$$

where  $\text{pKa} = 0.09018 + 2729.92/T$

and T= temperature (°K)

It appears that the contribution of un-ionized ammonia to the observed toxicity could have been substantial for most samples (figure 4).

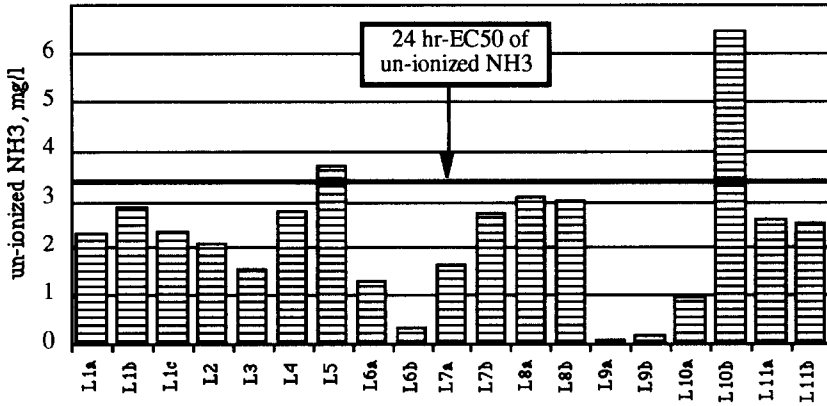




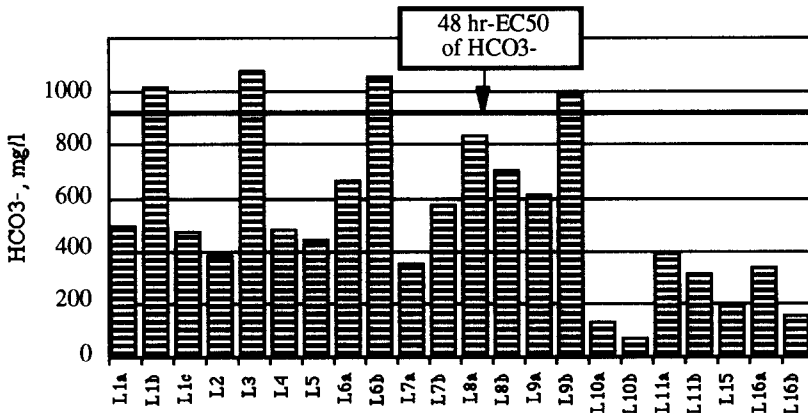
**Figure 3.** Correlation diagrams of the variables (test species) with projection of additional variables (physico-chemical parameters) in F1x2 and F1x3 planes. The vectors were not drawn in order to improve the legibility.

**Table 3.** Relationships between toxicity and physico-chemical parameters

| Organism            | Physico-chemical factors of toxicity   |
|---------------------|--|
| Duckweed (Lm)       | $EC50 = -23.3 \log(ALK) - 9.0 \log(NH_4^+) + 78.9$<br>( $R=0.95$ , $p=0.0001$ )                                |
| Microalgae (Ss)     | NH <sub>3</sub> with $\log(EC50)$ ( $R=0.56$ , $p=0.0069$ )<br>ALK with $EC50$ ( $R=0.50$ , $p=0.019$ )        |
| Daphnids (Dm)       | $EC50 = 2489(NH_3) - 0.62(ALK) - 0.386$<br>( $R=0.96$ , $p=0.0001$ )   |
| Rotifers (Bc)       | NH <sub>3</sub> with $\log(LC50)$ ( $R=0.68$ , $p=0.0015$ )<br>ALK with $\log(LC50)$ ( $R=0.59$ ; $p=0.0079$ ) |
| Bacteria (Pp)       | COD with $\log(EC50-5mn)$ ( $R=0.76$ , $p=0.0001$ )  |
| Ceriodaphnids (Cd)  | ALK with $\log(LC50)$ ( $R=0.62$ , $p=0.0023$ )<br>NH <sub>3</sub> with $\log(LC50)$ ( $R=0.58$ , $p=0.0048$ ) |
| Thamnocephalus (Tp) | ALK with $\log(LC50)$ ( $R=0.80$ , $p=0.0001$ )<br>$\log(NH_3)$ with $\log(LC50)$ ( $R=0.84$ , $p=0.0001$ )    |
| Protozoan (Sa)      | $\log(NH_3)$ with $\log(LC50)$ ( $R=0.81$ , $p=0.0001$ )   |



**Figure 4.** Un-ionized ammonia concentrations of EC50s of 19 samples for *D. magna* tests. The 24 hr-EC50 of 3.3 mg un-ionized ammonia.l<sup>-1</sup> was taken from Jean (1991).



**Figure 5.** Bicarbonate concentrations of EC50s of 22 samples for *D. magna* tests. The 48 hr-EC50 of 921 mg HCO<sub>3</sub><sup>-</sup>.l<sup>-1</sup> was taken from Hoke *et al.* (1992).

In addition, alkalinity, expressed as bicarbonate, seems to be an important factor determining the toxicity of the leachates to daphnids. The comparison of bicarbonate concentrations for leachate dilutions corresponding to EC50s and the 48 hr-EC50 for bicarbonate (Hoke *et al.*, 1992) indicates that for quite a number of samples the bicarbonate concentrations might be high enough to adversely affect *Daphnia magna* (figure 5). For other organisms, the lack of data regarding toxicity of un-ionized ammonia and alkalinity prevented similar types of analysis.

A significant relationship between the response of *Vibrio fischeri* and the organic load of the samples was also observed (table 3). This result is consistent with the reported high sensitivity of *Vibrio fischeri* to organic

contaminants (Munkittrick *et al.*, 1991) and the good correlation ( $R=0.68$ ,  $p=0.0001$ ) obtained between  $\log(\text{EC}_{50})$  and  $\log(\text{COD})$  after addition to our data of 33 couples of data found in Plotkin and Ram (1984), Deneuvy (1987), Jean (1991), Lambomez *et al.* (1993). However, in addition to the COD, ammonia might also have contributed to the observed toxicity. The 5min-EC<sub>50</sub> for un-ionized ammonia is  $1.5 \text{ mg NH}_3 \cdot \text{l}^{-1}$  (Qureshi *et al.*, 1982), and for some samples (L1a, L7a, L5, L6b, L11a, L11b) the concentration of un-ionized ammonia concentration at the dilution corresponding to the 5min-EC<sub>50</sub> ranges from 0.33 to  $1.38 \text{ mg} \cdot \text{l}^{-1}$ .

## DISCUSSION AND CONCLUSION

Multivariate analyses of toxicological and physico-chemical data suggested the existence of a general relationship between toxicity and the pollutant load. However, to better identify possible toxicants, regression analyses were performed. For most ecotoxicity tests, ammonia and alkalinity appeared to be the most probable factors contributing to the observed toxicity, whereas luminescent bacteria were more sensitive to organic loading measured as COD. The observed relationships need to be evaluated with caution as (i) relationships are not perfect and (ii) only a small number of physico-chemical parameters have been measured in this study. Analyses of additional compounds such as phenols, hydrocarbons, sulfur, cyanide, fatty acids, and other heavy metals, might have allowed the identification of the residual toxicity which cannot be explained by ammonia and alkalinity. The role of ammonia as a major toxicant was to some extent confirmed for duckweed (Clément and Merlin, 1995) and in this study for daphnids. The role of ammonia as potential toxicant in landfill leachates has been hypothesized by several authors (see references in Clément *et al.*, 1993). In landfill leachates the toxicity of ammonia is augmented by: (1) the (generally) high pH which results in a higher proportion of un-ionized ammonia, and (2) their high alkalinity which helps keeping pH constant after sample dilution (Clément and Merlin, 1995). As previously suggested by Clément and Merlin (1995), alkalinity seems to enhance ammonia toxicity, as reflected by its co-occurrence in the multiple regressions performed in the present study. In some cases, alkalinity could exert a direct toxic stress as suggested for the daphnids. Hoke *et al.* (1992) came to similar conclusion examining the alkalinity-toxicity relationships of sediment pore waters tested with *Daphnia magna*. These authors recommended to consider this parameter when interpreting the results of sediment pore waters and effluent toxicity tests.

Fatty acids and phenols, organic compounds currently found in landfill leachates (Clément *et al.*, 1993), might have been contributing to the toxicity of the samples towards luminescent bacteria. In addition, many other organic contaminants of landfill leachates such as benzene, naphthalene, chlorobenzene, trichloroethane, are often found at concentration levels harmful to luminescent bacteria (Brown and Donnelly, 1988; Kaiser and Palabrica, 1991).

The importance of ammonia and alkalinity as a responsible/contributing factor of the landfill leachate toxicity highlights some of the results found by Clément *et al.* (1996), namely:

- the significant relationships found between these physico-chemical parameters and the toxicity test results for all species except the luminescent bacteria test which appeared to be more sensitive to organic matters,
- the low acute toxicity (except to luminescent bacteria) of leachates from landfills receiving only hazardous wastes. This might be due to the low ammonia content and the low alkalinity of this type of leachates,

- the (generally) high toxicity of leachates from landfills receiving municipal solid wastes. This might be attributed to the high ammonia content and alkalinity of these samples,
- the very high toxicity of leachates from mixed landfills (receiving municipal solid wastes and hazardous wastes), which might be due to their high inorganic and organic pollutant loads (including ammonia and alkalinity).

Finally, the results of the present study underline the need for relating ecotoxicological and physico-chemical parameters in the risk assessment of environmental wastes. For landfill leachates, the french regulatory framework only focuses on parameters such as pH, hydrocarbons, chemical oxygen demand, phenols, cyanide, fluoride and heavy metals (Ministère de l'Environnement, 1992, 1993a, 1993b) but does not consider possible toxic effects of landfill leachates due to ammonia and alkalinity, and the risk for eutrophication due to nitrogen discharge into rivers. In the light of the present study, this approach might at best be inadequate.

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