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COMPARISON OF ECOTOXICOLOGICAL AND PHYSICO-CHEMICAL DATA BY USE OF MULTIVARIATE ANALYSES AND GRAPHICAL DISPLAYS

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ABSTRACT

Bioassays allow the appreciation of the toxicity of complex effluents, which is difficult to characterize by the sole measurement of some physico-chemical parameters. We have developed a macroinvertebrate multi-test (M.M.T.) with species characteristic of different trophic levels in order to appreciate the ecotoxicological impact of various effluents (especially landfill leachates) and chemicals on aquatic environments. Our aim was: (i) to compare the sensitivity of the macroinvertebrates among themselves and with regard to those of three standard bioassays; (ii) to characterize the effluents according to their toxicity on one hand and according to their physico-chemical composition on the other hand; (iii) to search for possible correlations between the physico-chemical composition of an effluent and its toxicity. In order to achieve these objectives we used first a combination of graphical displays of the raw data, and secondly multivariate analyses: clustering techniques and scaling (ordination) techniques (Principal Components Analysis).

Keywords: macroinvertebrates, bioassay, physico-chemistry, multivariate analysis, graphical display.

INTRODUCTION

The species used in standard bioassays (such as *Photobacterium phosphoreum*, *Daphnia magna*, *Brachydanio rerio*) to appreciate the ecotoxicological impact of complex effluents often do not appear as sufficiently representative of rivers and streams (1-5). Thus, we have developed a macroinvertebrate multi-test (M.M.T.) using 10 species characteristic of different trophic levels (6-8). This test has been validated on 17 different effluents. The results of the M.M.T. have been compared with those of three standard bioassays (6, 9).

Bioassays were often completed by the measurement of some physico-chemical parameters of the effluents tested in order to investigate the correlation between the physico-chemical composition of an effluent and its toxicity. However, an element at a high concentration in an effluent is not necessarily toxic (because of antagonism, availability, ...). Thus, the interpretation of bioassay results is not always easy.

Multivariate analysis allows the examination of latent structures between objects (effluents and species or chemicals in our case) in terms of groups of similar elements, or of hierarchy between these groups. It also provides assistance in interpretation, such as information on the contribution of each variable to the composition of the structures between the objects (10-12). The combined use of various numerical techniques is recommended by different authors (12-17).

Thus, in our study, in order (i) to compare the sensitivity of the macroinvertebrates among themselves and with regard to that of three standard bioassays; (ii) to characterize the effluents according to their toxicity on one hand and according to their physico-chemical composition on the other hand; (iii) to point out a synthetic relationship between the toxic and physico-chemical results (efficiency of treatments, possible relationship between the physico-chemical composition of an effluent and its toxicity, ...) we have used a combination of multivariate analyses: first clustering techniques, and next scaling (ordination) techniques (Principal Components Analysis, PCA).

MATERIAL AND METHODS

The bioassay

The multi-test was an acute 96 hours toxicity test. The macroinvertebrates used were: *Dinocras cephalotes* (Plecoptera); *Ecdyonurus venosus* and *Ephemera danica* (Ephemeroptera); *Sericostoma personatum* and *Hydropsyche dinarica* (Trichoptera); *Gammarus fossarum* (Crustacea); *Radix* group *peregra-ovata* (Gastropod Mollusc); *Eiseniella tetraedra* (Oligochaeta); *Erpobdella octoculata* and *Glossiphonia complanata* (Achaeta). Apart from ecological diversity, one of the criteria for the choice of these species was their sensitivity to organic pollution (classification according to the grid of Verneaux's biological index) in order to compare the results of organic and toxic pollutions. All these organisms were sampled in the River Seille (Jura, France), a tributary of the River Saône. The physico-chemistry and the hydrobiology of this river were well known. The invertebrates were kept in the laboratory in a large aquarium with artificial ecological conditions as close as possible to the natural ones. The bioassay was carried out in plexiglass containers (one per dilution) divided into 10 compartments in which 5 litres of solution were circulated. Each compartment sheltered the individuals of a species and their specific substrate (sand, gravel,...). The physico-chemical quality of the water was controlled each day and dead individuals were removed.

Lethal concentrations were calculated for each species from percent mortality after 96 hours assay (LC50 96h). The reproductibility of the M.M.T. was confirmed with assays carried out on $K_2Cr_2O_7$, NaCl and CH_3COONa, and with invertebrates sampled in the same river at different times, and then in another river.

Ecotoxicological and physico-chemical data

Two data tables have been produced from the results of the M.M.T. and of the three standard bioassays.

The first one shows the results of the toxicity tests of the 17 effluents (elements) on the 13 species (variables) (Tables I, II and III).

Table I. List of the species of the M.M.T. and of the three standard bioassays.

N°	Code	Species	Taxonomic level	Bioassay
1	Dc	*Dinocras cephalotes*	Insect Plecoptera	MMT
2	Ev	*Ecdyonurus venosus*	Insect Ephemeroptera	MMT
3	Ed	*Ephemera danica*	Insect Ephemeroptera	MMT
4	Sp	*Sericostoma personatum*	Insect Trichoptera	MMT
5	Hd	*Hydropsyche dinarica*	Insect Trichoptera	MMT
6	Gf	*Gammarus fossarum*	Crustacea Amphipoda	MMT
7	Rp	*Radix group peregra-ovata*	Mollusc Gastropoda	MMT
8	Et	*Eiseniella tetraedra*	Worm Oligochaeta	MMT
9	Eo	*Erpobdella octoculata*	Worm Achaeta	MMT
10	Gc	*Glossiphonia complanata*	Worm Achaeta	MMT
11	Pp	*Photobacterium phosphoreum*	Bacteria	Microtox bioassay
12	Dm	*Daphnia magna*	Crustacea Cladocera	Daphnia bioassay
13	Br	*Brachydanio rerio*	Fish	Brachydanio bioassay

Table II. List of the effluents tested.
* : the mark 1 means that the effluent is raw; the other marks correspond to different treatments.

N°	Code	Effluents *	Category
1	L1	LIV1'	Landfill leachate
2	L2	LIV1	Landfill leachate
3	L3	LIV2	Landfill leachate
4	L4	LIV3	Landfill leachate
5	L5	LIV4	Landfill leachate
6	L6	LIM1	Landfill leachate
7	L7	LIM2	Landfill leachate
8	L8	LIF1	Landfill leachate
9	L9	LIF2	Landfill leachate
10	LA	LIA1	Landfill leachate
11	LB	LIC1	Landfill leachate
12	I1	IND1	Industrial
13	I2	IND2	Industrial
14	I3	IND3	Industrial
15	T1	TAN1	Tannery
16	T2	TAN2	Tannery
17	T3	TAN3	Tannery

The values were expressed by the medium lethal concentration (LC50 in %), then transformed as the decimal logarithms of the ratio 100/C, which represents the equitox number, that is to say the number of dilutions of an effluent that kills 50% of the organisms. This ratio varies in the same way as toxicity, which justifies its use.

Table III. Raw data matrix of toxicity (LC50 expressed as log100/C, C in %).

	D.c.	E. v.	E.d.	S.p.	H.d.	G.f.	R.p.	E.t.	E.o.	G.c.	P.p.	D.m.	B.r.
LIV1'	0.12	0.68	0.22	0.00	0.25	0.57	1.10	1.10	0.89	0.68	0.80	0.69	0.77
LIV1	0.49	0.96	0.60	0.00	0.64	0.96	1.17	1.25	1.25	0.77	0.89	0.96	1.00
LIV2	0.38	1.10	0.57	0.00	0.12	0.62	1.55	1.55	1.36	0.72	1.10	0.87	1.22
LIV3	0.00	0.77	0.75	0.00	0.25	0.52	0.77	1.08	0.89	0.36	0.62	0.56	0.64
LIV4	0.00	0.48	0.19	0.00	0.27	0.82	0.09	0.33	0.07	0.00	0.00	0.24	0.49
LIM1	0.25	0.42	0.17	0.00	0.00	0.62	0.77	0.62	0.17	0.52	0.77	0.60	0.80
LIM2	0.00	0.59	0.75	0.00	0.00	0.38	0.89	0.89	0.55	0.28	0.02	0.18	0.55
LIF1	0.49	1.12	0.77	0.00	0.57	0.96	1.52	1.46	1.46	0.92	0.74	1.04	1.33
LIF2	0.00	0.44	0.21	0.00	0.00	0.32	0.19	0.00	0.00	0.00	0.00	0.00	0.00
LIA1	0.23	0.77	0.62	0.00	0.12	0.62	1.38	1.12	0.68	0.38	0.52	0.32	0.46
LIC1	0.00	0.52	0.22	0.00	0.00	0.62	0.72	0.82	0.59	0.38	0.82	0.51	0.49
IND1	0.82	1.15	0.85	0.82	0.82	0.92	1.05	1.52	0.82	0.82	1.74	1.32	1.00
IND2	0.28	1.08	0.33	0.22	0.28	0.68	0.89	1.17	0.62	0.62	1.15	0.66	0.94
IND3	0.12	0.82	0.28	0.00	0.12	0.38	1.46	1.74	0.82	0.62	0.92	0.28	0.74
TAN1	0.62	1.42	0.38	0.38	0.96	1.42	1.12	1.38	1.12	1.08	2.40	2.39	2.22
TAN2	1.38	1.62	1.48	1.12	1.52	2.10	1.62	1.62	1.62	1.68	2.52	2.22	1.89
TAN3	0.38	1.38	0.89	0.38	0.52	0.62	0.68	1.48	0.38	0.38	1.00	1.00	2.00
mean	0.33	0.90	0.55	0.17	0.38	0.77	1.00	1.13	0.78	0.60	0.94	0.81	0.97
variance	0.13	0.13	0.12	0.11	0.17	0.19	0.20	0.22	0.23	0.16	0.52	0.44	0.36

The second table contains the measurements of 20 physico-chemical parameters (variables) of the 17 effluents (elements) (Tables IV and V). All the chemical analyses were carried out with classical methods such as ionic chromatography for the inorganic and metallic compounds and chemical oxydation and purple-ultra detection for total organic carbon. Urban effluents were removed from the original tables because they did not prove toxic during the assays.

Table IV. List of the physico-chemical parameters analysed in the effluents.

N°	Code	Physico-chemical parameters	Unit
1	pH	pH	pH units
2	CD	COD	$mg.l^{-1}$
3	TC	TOC	$mg.l^{-1}$
4	Cy	Conductivity	mS
5	N4	NH_4^+	$mg.l^{-1}$
6	N2	NO_2^-	$mg.l^{-1}$
7	N3	NO_3^-	$mg.l^{-1}$
8	PO	PO_4^{3-}	$mg.l^{-1}$
9	SO	SO_4^{2-}	$mg.l^{-1}$
10	Cl	Cl^-	$mg.l^{-1}$
11	Cr	Cr_t	$mg.l^{-1}$
12	Cu	Cu^{2+}	$mg.l^{-1}$

(Table IV continued)

N°	Code	Physico-chemical parameters	Unit
13	Fe	Fe_t	$mg.l^{-1}$
14	Mn	Mn^{2+}	$mg.l^{-1}$
15	Cd	Cd^{2+}	$mg.l^{-1}$
16	Zn	Zn^{2+}	$mg.l^{-1}$
17	Ca	Ca^{2+}	$mg.l^{-1}$
18	Mg	Mg^{2+}	$mg.l^{-1}$
19	Na	Na^+	$mg.l^{-1}$
20	K+	K^+	$mg.l^{-1}$

The results were plotted for a preliminary examination.

Statistical analyses

First of all, the data were processed with a hierarchical cluster analysis. Clustering methods are widely used to identify either species associations or groups of similar objects (10, 17-19). The hierarchical tree of these objects is a schematization of the connections between them and their distances quantify their similarity. The display given by the dendrogram is presumed to be a good approximation of the data (12). The choice of the distance computation method and of the agglomeration method was made according to (12) and (20). First, the chi-square distance was applied to the ecotoxicological results to equalize the variations in the components of the data matrix and the Euclidean distance was applied to the physico-chemical results because the normalization of the data. Secondly, hierarchies were computed with Ward's agglomerative method (also called Second Order Moment Method) for the ecotoxicological set of data and with the Average Link Agglomerative Method (also called UPGMA method) for the physico-chemical set of data. According to the means chosen in the first step to measure similarity between the objects, these agglomeration methods seem to be good compromises (12). The calculations and the graphical displays were made with MacDendro and GraphMu softwares developed by J. Thioulouse (URA CNRS 243, Lab. of Biometry, Univ. Lyon I).

Next, some normalized PCA (nPCA) were performed. This is a common and frequently used multivariate analysis method. It allows the classification of structures in a table with quantitative data (11, 21). It analyses the correlation or covariance structure among variables (e.g. species) and allows to project the data onto Euclidean planes, that is to say to convert a data matrix into graphical displays (22). PCA helps to reduce the dimensionality of the data as inertia methods concentrate the total variation within the first components (axes) (15). The examination was done according to the instructions and aids to interpretation advocated by (21), (23) and (24): distribution of inertia within the factorial axes (component scores, or eigenvalues), part of variability of a factor due to an element (absolute contribution), examination of the factorial maps. The calculations and the graphical displays were made with MacMul and GraphMu softwares (25, 26). A preliminary normalization of the raw data was performed in the case of the physico-

Table V. Physico-chemical composition of the effluents.

	pH	COD	TOC	Cond.	NH4	NO2	NO3	PO4	SO4	Cl	Cr	Cu	Fe	Mn	Cd	Zn	Ca	Mg	Na	K
LIV1'	8.1	9500	2550	10	500	0.1	45	8.9	183	2500	0.07	0.02	222	4.5	0.02	3.6	661	183	540	380
LIV1	7.9	9100	2600	11	1400	0.15	58	9.5	360	1668	0.11	0.02	1.97	2.39	0.02	0.18	333	190	900	400
LIV2	8.2	4500	1400	8.5	1600	0.25	19	13	80	1300	0.05	0.02	0.85	0.08	0.02	0.04	41	170	825	380
LIV3	8.4	1200	180	5.6	450	0.15	93	1.1	616	1015	0.05	0.02	0.17	0.37	0.02	0.02	75	100	550	210
LIV4	8.9	80	9	4.8	160	0.6	68	0	366	852	0.05	0.02	0.02	0.05	0.02	0.02	28	100	725	500
LIM1	8.5	2720	900	6.1	150	0.15	410	2	95	916	0.07	0.02	0.57	0.48	0.02	0.07	80	145	1050	425
LIM2	8.6	750	200	4.6	100	0.45	80	9	224	660	0.05	0.02	0.11	0.32	0.02	0.02	50	90	650	150
LIF1	8.9	2800	1000	5	1900	0.5	129	10	723	902	0.06	0.02	2.18	0.3	0.02	0.12	50	0.58	800	350
LIF2	8.2	80	22	1.7	55	0.2	303	0.1	170	1170	0.05	0.17	0.03	0.22	0.04	0.85	395	250	1425	275
LIA1	8.4	600	325	5.6	135	0.01	154	2	96	1207	0.05	0.02	0.05	0.31	0.02	0.02	89	88	1300	240
LIC1	8.7	700	287	0.8	18	0.12	2.6	1.5	95	469	0.05	0.02	0.12	0.31	0.02	0.08	50	29	400	91
IND1	7.3	4000	2300	17.4	590	0.25	19	119	15000	6000	25.5	0.12	3.4	0.18	0.04	0.24	263	10	9100	385
IND2	8.7	1700	1450	10	550	0.01	30	51	11000	6100	47.5	0.02	2.07	0.05	0.04	0.14	133	10	8800	405
IND3	8.7	1000	220	8.7	26	0.12	2.7	67	6300	1917	10.6	0.02	0.92	0.06	0.02	0.17	114	8	4100	225
TAN1	11.4	18000	6800	15	116	7.3	1320	16	330	6436	12	1.4	0.76	0.19	0.03	0.9	870	110	4700	100
TAN2	3.6	12400	5200	20	297	15	5280	8	8000	31050	7500	0.3	12.5	2.08	0.19	1.9	1240	320	29500	72
TAN3	6.2	4000	1400	13	1225	2.6	1100	2.5	1400	1988	0.5	0.04	0.06	0.62	0.02	0.05	490	21	2000	2800

chemical data: this initial transformation is $x_{ij} = (z_{ij} - \bar{z}_j)/s_j$ where z_{ij} is the value of variable j in sample i, $\bar{z}_j$ is the mean of variable j, s_j is the standard deviation of variable j).

EXPERIMENTAL RESULTS

Toxicological results

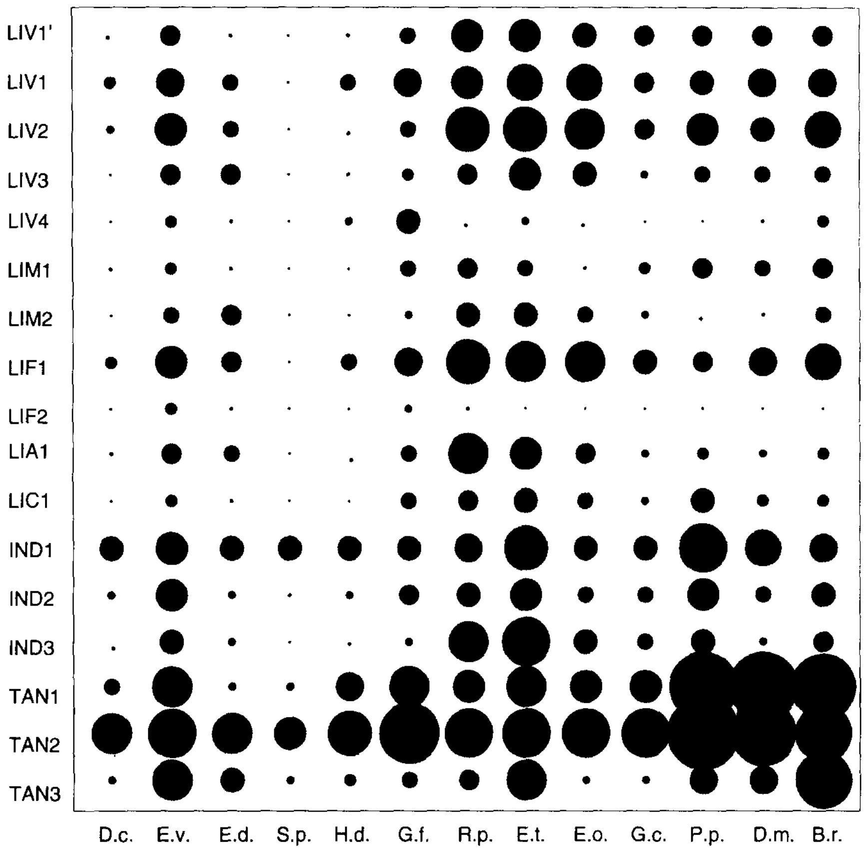

Figure 1. Graphical display of the raw data (LC50 expressed as log100/C) of species sensitivity and of effluents toxicity (17 effluents in rows, 13 species in columns). The size of the circles is proportionnal to the values.

The sensitivity of the species appears immediatly on a graphical display (Fig. 1): the less sensitive macroinvertebrate species (lowest means in Table III) appear in the columns with small circles (*Dinocras cephalotes*, *Sericostoma personatum*, *Hydropsyche dinarica*), in contrast the most sensitive species appear in the columns with large circles (*Radix peregra-ovata*, *Eiseniella tetraedra*, and to a lesser extent *Ecdyonurus venosus*, *Erpobdella octoculata*, *Gammarus fossarum*, *Glossiphonia complanata*). *Ephemera danica* is intermediate between the two groups of species with a general moderate tendancy. *Photobacterium* *phosphoreum*, *Daphnia magna* and *Brachydanio rerio* are also sensitive species, they show some of the highest means, but especially the highest differences among the effluents (Table III). Similar contrasts appear between some of the effluents: effluents LIV3 and LIF2 appear less toxic (small circles), whereas effluents LIV2, LIF1, IND2, TAN1 and TAN2 are the most toxic (larger circles).

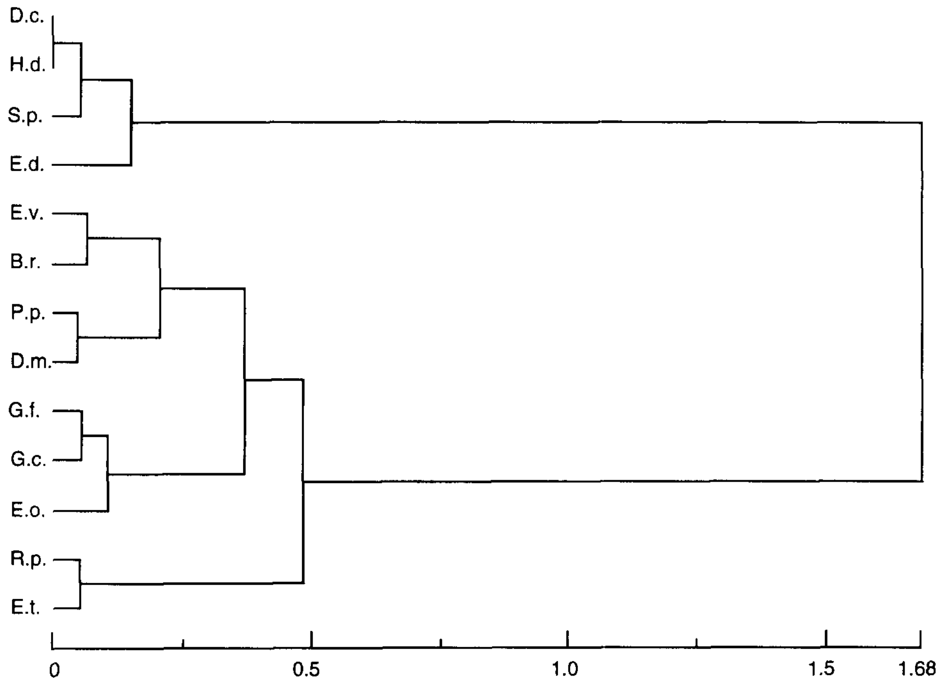

Figure 2. Dendrogram of the sensitivity of the species to the effluents using Ward's method. The X axis represents distance units.

The dendrogram obtained with the raw data shows four distinct groups of species (Fig. 2): 1/ *Dinocras cephalotes* and *Hydropsyche dinarica* associated with *Sericostoma personatum* and *Ephemera danica*, i.e. three of the less sensitive species and an intermediate species identified by the graphical display of the previous raw data. This group appears the most distant from the three other ones, due to great differences of sensitivity; 2/ *Ecdyonurus venosus* and *Brachydanio rerio* associated with *Photobacterium phosphoreum* and *Daphnia magna*, i.e. the three species of the standard tests of acute toxicity and one of the previous sensitive secondary species; 3/ *Gammarus fossarum*, *Glossiphonia complanata* and *Erpobdella octoculata*, i.e. three sensitive species, 4/ finally, *Radix peregra-ovata* and *Eiseniella tetraedra*, which appear to be the most sensitive species of the M.M.T.

If the hierarchical cluster analysis is easy to use, the classification achieved presents some weaknesses, including the lack of information on the contribution of the variables (effluents in our case) in the determination of the different connections, that is to say the lack of explanatory criteria. Thus, after simplifying the problem with this method, the results can be improved with scaling (ordination) techniques.

A nPCA was performed on the table of the raw data with 17 rows (effluents) and 13 columns (species) expressed as log100/LC50. The graphical displays are drawn on Fig. 3.

Table VI. Absolute contributions, expressed in ‰o variability of a factor due to A/ the elements (effluents), and B/ the variables (species) for the nPCA 17x13.

A/ Absolute contributions of rows.
Factors are in columns.

	1	2	3	4
1 |	64.	234.	365.	315.
2 |	63.	156.	1.	417.
3 |	53.	1653.	61.	37.
4 |	110.	51.	377.	84.
5 |	857.	1722.	4.	65.
6 |	328.	203.	477.	323.
7 |	397.	69.	1176.	94.
8 |	216.	922.	46.	99.
9 |	1417.	1232.	136.	183.
10 |	99.	424.	547.	45.
11 |	345.	5.	209.	271.
12 |	493.	369.	485.	77.
13 |	4.	6.	220.	131.
14 |	39.	1675.	99.	94.
15 |	1152.	327.	4543.	10.
16 |	4289.	648.	1242.	902.
17 |	74.	301.	13.	6854.

B/ Absolute contributions of columns.
Factors are in columns.

	1	2	3	4
1 |	935.	198.	246.	237.
2 |	901.	2.	15.	1702.
3 |	567.	2.	5487.	463.
4 |	688.	1297.	714.	75.
5 |	897.	593.	44.	76.
6 |	801.	663.	8.	1488.
7 |	478.	3210.	48.	391.
8 |	607.	1902.	2.	1479.
9 |	654.	1676.	18.	885.
10 |	936.	111.	83.	1040.
11 |	880.	76.	1042.	7.
12 |	905.	248.	1008.	14.
13 |	750.	24.	1286.	2144.

The eigenvalues diagram shows that the first axis contains more than 70% of the information (94.5% for the first four axes). Since the species are highly correlated along the first axis, factorial maps F1xF2, F1xF3 and F1xF4 will only be considered.

All the species are located on the negative side of axis 1 (see correlation diagram of the variables). This fact represents the general tendancy of the species to react in a same way: an effluent is toxic for all the species, and the reverse for a non-toxic effluent. The first axis of the factorial map of the elements is an axis of general toxicity which classes the effluents according to a gradient of increasing toxicity. The most toxic effluents are characterized by negative coordinates (effluents L8, I1, T1 and T2, i.e. LIF1, IND1, TAN1 and TAN2), and the less toxic ones by positive coordinates (effluents L5 and L9, i.e. LIV4 and LIF2).

On the second axis, the opposition between very sensitive species, such as *Eiseniella tetraedra*, *Radix peregra-ovata* and *Erpobdella octoculata*, and species with various sensitivities, such as *Sericostoma personatum*, *Hydropsyche dinarica* and *Gammarus fossarum*, is observed for most effluents. However, the highest differences in sensitivity are clearly marked between effluents L3 and I3 (i.e. LIV2 and IND3). In contrast, *Gammarus fossarum* is more sensitive to effluent L5 (LIV4) than the species considered as most sensitive.

The factorial map F1xF3 opposes on one hand *Ephemera danica* to *Photobacterium phosphoreum*, *Daphnia magna*, *Brachydanio rerio*, i.e. the 3 species of the standard bioassays, and on the other hand

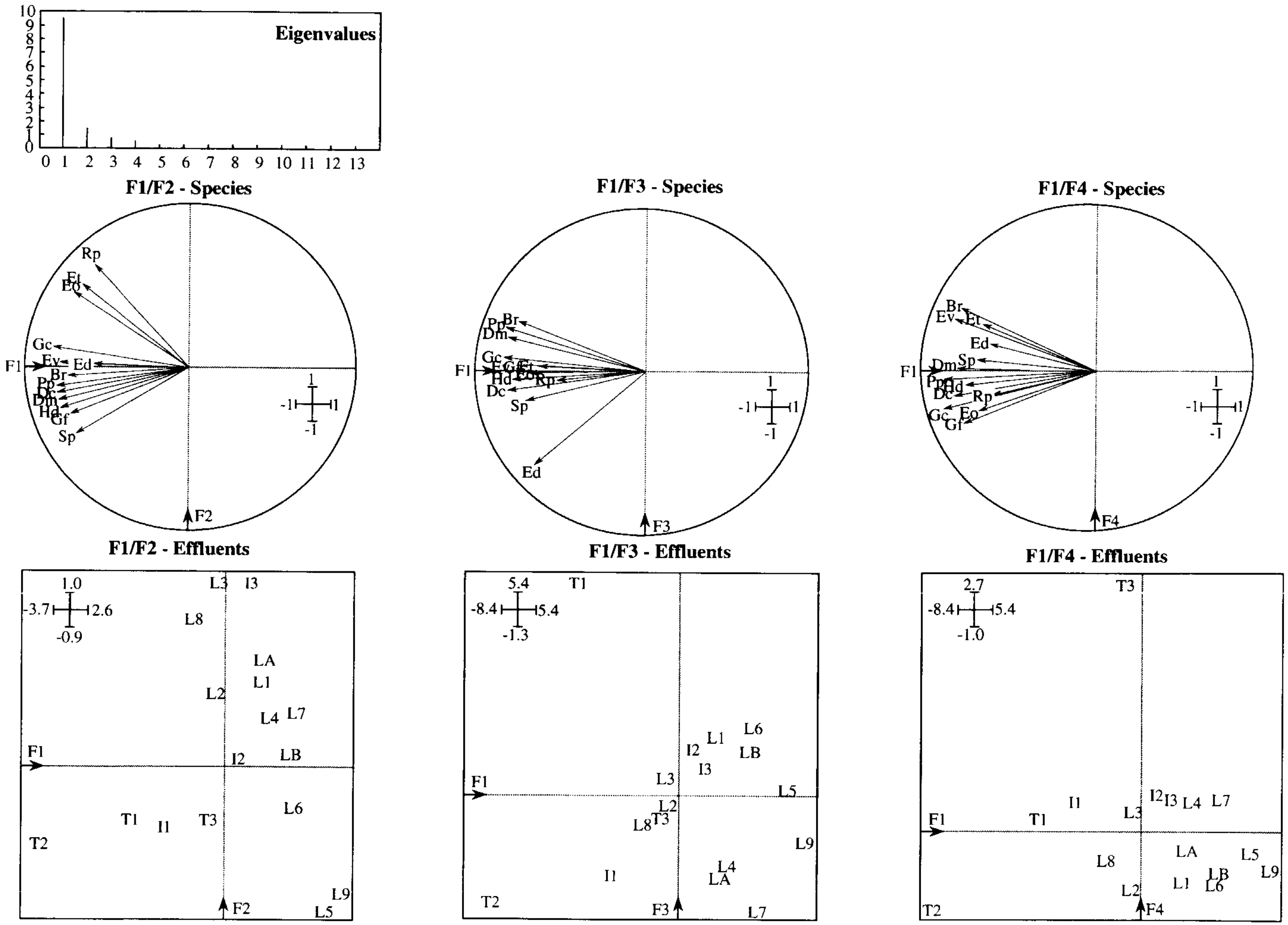

Figure 3. Eigenvalues, correlation diagrams of the variables (species) and factorial maps of the elements (effluents) in F1xF2, F1xF3 and F1xF4 planes of the nPCA 17x13.

effluent T1 to effluent L7. In fact, T1 (TAN1) is very toxic for the "standard" species and non-toxic for *Ephemera danica*. In contrast, L7 (LIM2) is quite toxic for *Ephemera danica* and less toxic for *Photobacterium phosphoreum* and *Daphnia magna* in particular. Except for the previous effluent, these latter species are always more sensitive than *Ephemera danica*.

The fourth axis is mainly described by effluent T3 (TAN3) (see in Table VI-A its absolute contribution close to 68.5%). *Glossiphonia complanata* and *Gammarus fossarum* are opposed to *Brachydanio rerio* and *Ecdyonurus venosus* because these two latter species are much more sensitive to this effluent, whereas in general they have quite similar reactions.

Physico-chemical results

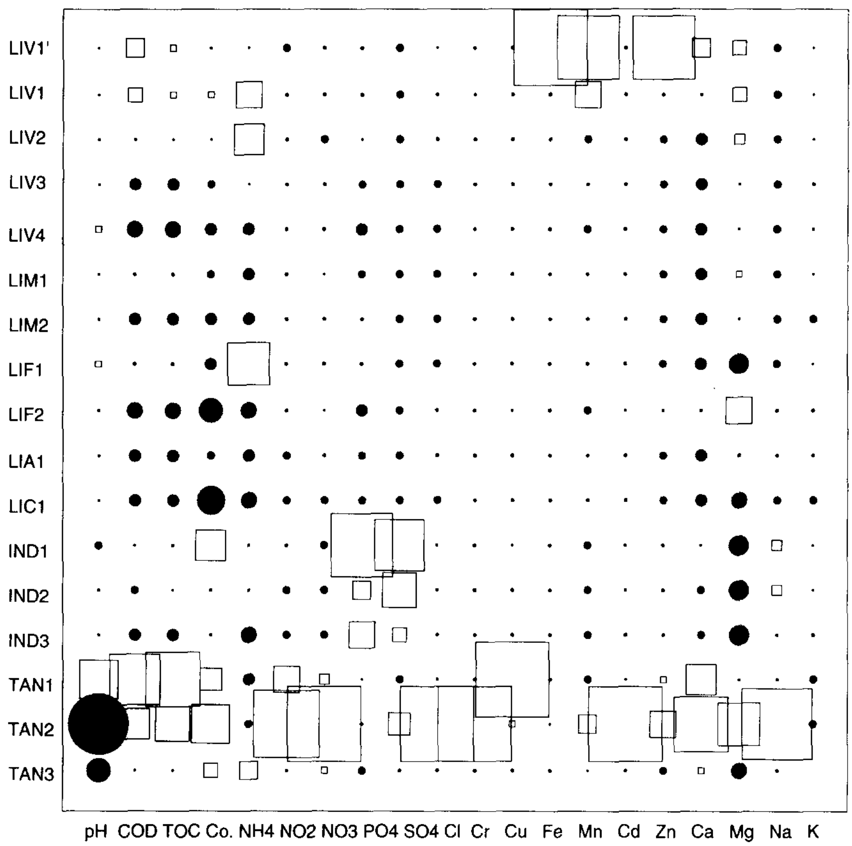

Figure 4. Graphical display of the physico-chemical composition of the effluents (17 effluents as rows, 20 physico-chemical parameters as columns). The data are normalized. The circles are proportional to negative values, the squares to positive ones.

The graphical display of the normalized physico-chemical data (Fig. 4) shows the relative concentration of the chemical elements in the different effluents. The squares indicate positive values (i.e. above the mean). For example, effluents LIV1' and LIF1 (raw leachates) were loaded in iron, manganese and zinc for the first one, and in ammonia for the second one; the raw industrial effluent IND1 had high conductivity, high concentrations in phosphate and sulfate and the effluents of tannery TAN1 and TAN2 were loaded in various compounds except ammonia and potassium. In contrast, the circles indicate negative values (i.e. below the mean). Low values were typical of numerous leachates after various treatments, as well as of the industrial effluent IND3 and the effluents of tannery TAN2 and TAN3 for pH.

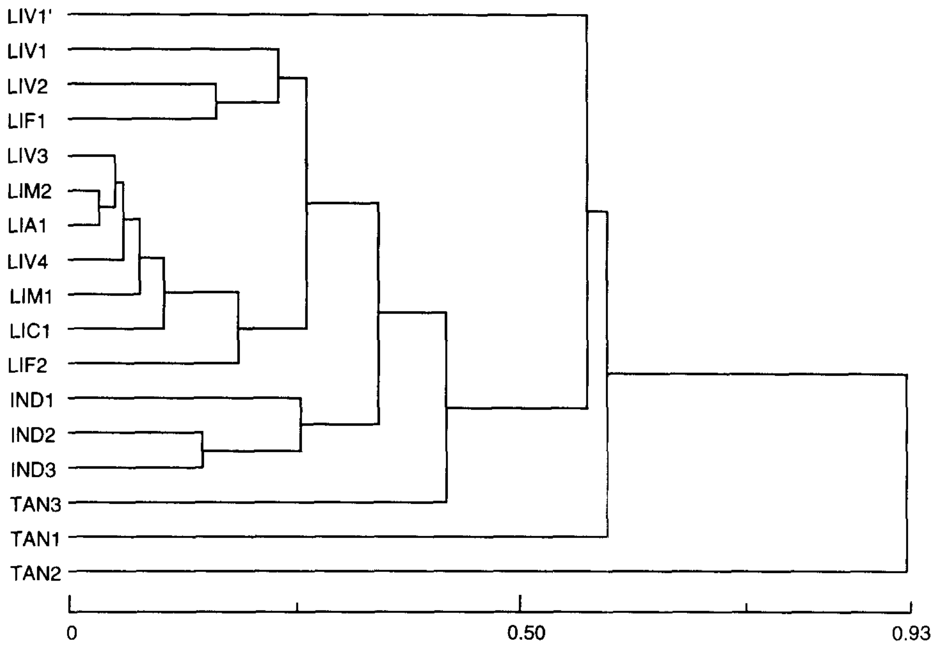

Figure 5. Dendrogram of the effluents according to their physico-chemical composition using the Average Link Method (UPGMA method). The X axis represents distance units.

The dendrogram obtained for the effluents separates 1/ distinct entities which are not linked with other effluents (LIV1', each of the three effluents of tannery); 2/ the group of the industrial effluents; 3/ the leachates (excluding LIV1') which group together graduadly (Fig. 5). The clear separation among the effluents of tannery, that is to say their particular ecotoxicological behaviour, can be explained by their three levels of treatment corresponding to three different levels of toxicity.

The eigenvalues diagram of the nPCA of table 17x20 (effluentsxphysico-chemical parameters) shows that the information is distributed between several axes (Fig. 6). However, the first one clearly dominates (46.7% of the information). We will consider the first six axes (near 97% of the information).

Table VII. Absolute contributions, expressed in ⁰/₀₀₀ variability of a factor due to A/ the elements (effluents), and B/ the variables (physico-chemical parameters) for the nPCA 17x20.

A/ Absolute contributions of rows.
Factors are in columns.

	1	2	3	4	5	6
1 |	158.	5371.	161.	2738.	513.	135.
2 |	0.	322.	1.	76.	590.	1980.
3 |	83.	2.	16.	9.	507.	2077.
4 |	178.	0.	87.	167.	17.	15.
5 |	233.	4.	83.	298.	31.	188.
6 |	103.	11.	56.	242.	17.	39.
7 |	205.	2.	56.	238.	180.	33.
8 |	212.	5.	0.	6.	626.	1766.
9 |	44.	102.	289.	650.	311.	123.
10 |	175.	2.	78.	230.	81.	67.
11 |	300.	0.	57.	350.	277.	123.
12 |	26.	1884.	625.	2828.	295.	18.
13 |	15.	847.	84.	470.	190.	2.
14 |	122.	511.	66.	203.	614.	195.
15 |	571.	533.	7234.	891.	41.	101.
16 |	7574.	350.	987.	288.	23.	35.
17 |	0.	55.	120.	315.	5686.	3103.

B/ Absolute contributions of columns.
Factors are in columns.

	1	2	3	4	5	6
1 |	404.	247.	1811.	202.	234.	52.
2 |	570.	468.	1071.	5.	207.	217.
3 |	671.	106.	1262.	2.	119.	120.
4 |	647.	127.	414.	770.	280.	0.
5 |	7.	2.	23.	511.	3109.	4424.
6 |	935.	24.	21.	376.	71.	63.
7 |	929.	60.	96.	285.	81.	93.
8 |	1.	1020.	593.	1972.	539.	32.
9 |	131.	1492.	91.	1422.	432.	0.
10 |	972.	188.	54.	17.	34.	19.
11 |	863.	123.	433.	142.	16.	48.
12 |	193.	100.	2741.	521.	7.	155.
13 |	35.	1756.	85.	1419.	344.	151.
14 |	216.	1512.	312.	899.	13.	66.
15 |	890.	210.	269.	87.	68.	31.
16 |	368.	1330.	42.	531.	438.	149.
17 |	912.	189.	85.	1.	51.	409.
18 |	386.	526.	477.	475.	67.	526.
19 |	863.	505.	41.	2.	95.	7.
20 |	8.	13.	80.	362.	3793.	3440.

According to the absolute contributions of elements and variables to the axes (Table VII-A and VII-B), we have drawn the graphical displays of the factorial maps F1xF2, F1xF3, F2xF4, F5xF6.

Most physico-chemical parameters are located on the negative side of the first axis of the correlation diagram of the variables F1xF2. However, pH has clear positive coordinates and appears opposed to sodium. The other significant parameters are nitrite, nitrate, chloride, chromium, cadmium, calcium and sodium. Axis F1 of the elements mainly characterizes effluent T2 (TAN2) that is highly loaded in the compounds mentioned and especially with a high acidity (very low pH), and to a lesser extent effluent T1

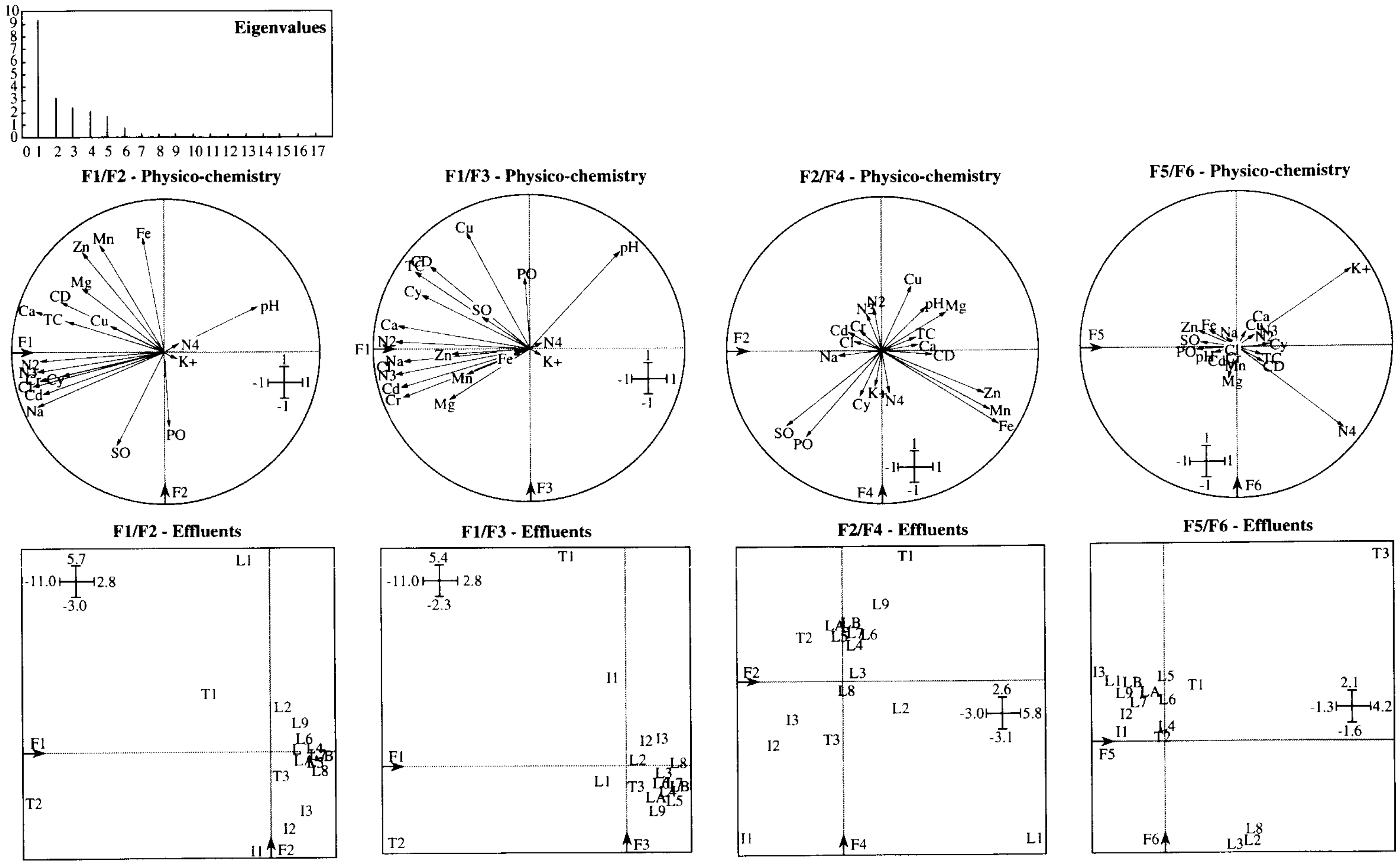

Figure 6. Eigenvalues, correlation diagrams of the variables (physico-chemical parameters) and factorial maps of the elements (effluents) in F1xF2, F1xF3, F2xF4 and F5xF6 planes of the nPCA 17x20.

(TAN1), with a very basic pH. At the opposite end of this axis, we find effluents L4, L5, L7, LA and LB (i.e. effluents LIV3, LIV4, LIM2, LIA1 and LIC1) that are less loaded in the previously mentioned compounds. All are leachates of various origins, treated or not. The two effluents of tannery T1 and T2 are located according to their pH values, which highly differ from the mean.

The second axis is characterized first by the variables iron, manganese, zinc and sulfate, and secondly by leachate L1 (LIV1') and industrial effluents I1 and I2 (IND1 and IND2). The first three compounds are especially present in the leachate, which is a raw effluent, whereas the two industrial effluents have high sulfate content.

The third axis is mainly characterized by effluent T1 (TAN1) which differs from the others by its basic pH and its higher concentration in copper.

The physico-chemical parameters phosphate, sulfate and iron and the three industrial effluents, that are highly concentrated in phosphate distinguish axis 4.

Finally, the high content in potassium of effluent T3 (TAN3) and the high concentrations in ammonia of effluents L2, L3 and L6 (i.e. leachates LIV1, LIV2 and LIM1) characterize the factorial map F5xF6.

Toxicity versus physico-chemical results

The last step of our study was the research of possible correlations between the physico-chemical composition of an effluent and its toxicity, that is to say to appreciate the ecotoxicological risk associated with the discharge of an effluent on the basis of its chemistry. In order to achieve this aim we projected the 20 physico-chemical parameters analyzed on the 17 effluents as additional elements (additional columns) in the nPCA of the ecotoxicological results. We only considered, as an example, the factorial maps F1xF2 and F1xF3 (Fig. 7) and we will discuss the case of the more toxic effluents.

We have seen previously that: first, axis 1 is an axis of general toxicity, from the less toxic effluents (L5 and L9, i.e. LIV4 and LIF2) to the more toxic ones (L8, I1, T1 and T2, i.e. LIF1, IND1, TAN1 and TAN2) and, secondly, the species tend to react in a same way, i.e. an effluent is toxic for all species or none. The projection of the physico-chemical parameters as additional elements on the correlation circle of the variables (species) shows that NH_4 is more particularly involved in the toxicity of LIF1, SO_4 and PO_4 in the toxicity of IND1, Cu in the toxicity of TAN1, and various compounds such as NO_2, NO_3, Cl^-, Cr, Cd, Ca, Na in the toxicity of TAN2. The toxicity of TAN1 and TAN2 is also highly due to their specific pH which explains their location on the factorial map with regard to this parameter: high alcalinity for TAN1 and high acidity for TAN2.

The second axis is mainly characteristic of the toxicity of NH_4, which partly justifies the toxicity of the leachates L2, L3 and L8 (LIV1, LIV2 and LIF1). This is especially true for LIV2, the toxicity of which is clearly marked for *Radix peregra-ovata*, *Eiseniella tetraedra* and *Erpobdella octoculata*.

The third axis is correlated to the effluent of tannery TAN1, which is toxic for all species except *Ephemera danica*. This toxicity is essentially due to pH and Cu.

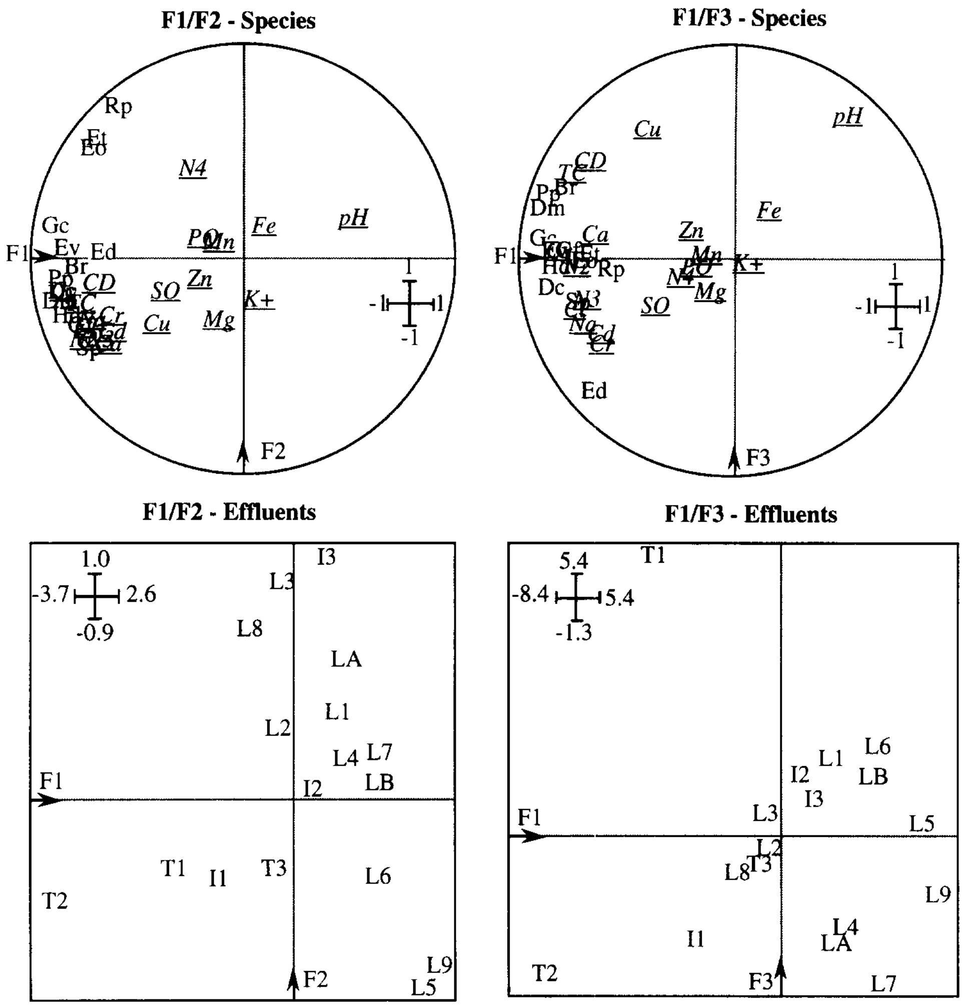

Figure 7. Correlation diagrams of the variables (species) with projection of additional elements (physico-chemical parameters), and factorial maps of the elements (effluents) in F1xF2 and F1xF3 planes of the nPCA 17x13. The vectors were not drawn on the correlation diagrams in order to facilitate the legibility.

However, the search for correlations between the physico-chemical composition of effluents and their toxicity appears to be quite difficult, not due to the statistical methods but to the complex composition of most effluents. In fact, their exact physico-chemical composition, in particular the one of landfill leachates, is rarely exactly kwown (27) and the results are very variable according to the bioassays. Thus, in our study we only analyzed 20 "standard" inorganic and metallic compounds, but no organic compounds or micropollutants, for example.

CONCLUSION

From a biological point of view, although the standard bioassays allow estimation of the concentration which will eliminate the most sensitive species of the multi-test in most cases, they have shown very different reactions of some macroinvertebrates, which justifies the interest of the M.M.T. This test also confirms the clear difference in sensitivity of the macroinvertebrates between toxic and organic or biodegradable pollutions: the most sensitive species of the M.M.T. (especially *Eiseniella tetraedra*, *Radix peregra-ovata* and *Erpobdella octoculata*) belong to the taxonomic groups that are less sensitive to organic pollutions (Molluscs, Oligochaeta, Achaeta) according to the grid of the French biological index called IBGN (28).

From a statistical point of view, the different multivariate analyses and graphical displays of the data appear to be good tools to interpret ecotoxicological and physico-chemical results obtained in aquatic studies. The combination of several approaches of increasing complexity and sensitivity provide explanations and validation of most results. It is possible to extract more information from the data by use of various statistical analyses. Consequently, cluster and ordination methods appear complementary. In fact, the main problem with the clustering techniques is to explain the formation of clusters. The different clustering algorithms implicitly or explicitly favour different cluster shapes (however, in our study, the hierarchy obtained with Ward's and UPGMA methods applied to the ecotoxicological data was quite similar - the second one was not represented here). The combination with an ordination method allows validation of the choice of the clustering procedure.

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