



Waste ecocompatibility in storage and reuse scenarios: global methodology and detailed presentation of the impact study on the recipient environments

Y. Perrodin^{a,*}, A. Gobbey^b, L. Grelier-Volatier^a, V. Canivet^c,
J.F. Fruget^d, J. Gibert^c, C. Texier^e, D. Cluzeau^e, Raphaël Gros^g,
F. Poly^f, L. Jocteur-Monrozier^f

^a*POLDEN, National Institute of Applied Sciences of Lyon, France*

^b*ADEME, Industry Dept., Facilities and Technologies, Angers, France*

^c*Ecology of Fluvial Hydrosystem, ESA CNRS 5023, Lyon, France*

^d*ARALEPBP, Freshwater Ecology, University of Lyon I, France*

^e*Station Biologique de Paimpont, University of Rennes I, France*

^f*Laboratory for Soil Microbial Ecology, CNRS-University of Lyon I, France*

^g*CISM, Soil Science, University of Savoie, France*

Abstract

In 1995, the ADEME launched a research program called “Waste Ecocompatibility” in order to define a reliable methodology for measuring the impact of waste in storage or reuse scenarios. The French concept of “Ecocompatibility” is defined as the situation where the pollutant flux from waste disposed of or used in specified conditions is compatible with the environmental acceptance of the receiving environments. The chief feature of this definition is to integrate the evaluation of the three following terms: pollutants emission from the waste, transport of the pollutants from the waste to the receptor cells and the environmental acceptance of the receiving environments. The “Waste Ecocompatibility” program consisted of a literature survey and an experimental part. The literature study aimed to determine factors and waste characteristics to be considered for a reliable ecocompatibility assessment, to provide an overview of the available tools for measuring those factors and characteristics and to propose a first approach of the methodology. In the framework of the experimental program, this approach was then applied to three theoretical scenarios to validate the laboratory tools (comparative study of laboratory and field results) and to calibrate the global methodology. This paper deals with the results of the experimental program concerning the impact study on receiving environments: impact on plants and microorganisms living in soil, impacts on soil fauna and aquatic fauna. In other papers we intend to present the operational methodology for the assessment of waste ecocompatibility. It includes bio-assays at laboratory scale (microcosms), pilot scale (mesocosms) and in situ experiments (experimental prairie). To limit the use of in situ experiments other research works are necessary to validate bio-assays at laboratory or pilot scale. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Storage or reuse conditions of waste are still currently defined on a regulatory or technical basis which does not take into account the impact of the waste deposit on the environment, due to lack of technical data in this domain.

Since waste management according to the Best Available Technology approach can no longer constitute the only response as regards environmental protection, ADEME (French Agency for Environmental Management and Energy Conservation) has launched a research program called “Waste Ecocompatibility”.

This program aims to define a reliable methodology for measuring the impacts of different storage and reuse scenarios, when waste comes into direct contact with the natural environment.

First, this paper deals with a general presentation of the research program; then, it focuses on the impact study on the recipient environment.

* Corresponding author. Tel.: +33-472-438-386; fax: +33-472-439-866.

E-mail address: polden@insa-lyon.fr (Y. Perrodin).

2. Part I: General presentation of the research program on waste ecocompatibility

Ecocompatibility can be defined as a situation where the pollutant release from the waste, when deposited in a specific physical, hydrogeological, physico-chemical and biological context, is in keeping with the acceptable pollutant level of receiving environments [1].

Pollutant emission into the environment depends on:

1. the pollutant flux from waste either disposed of or used in specified conditions, called “source term”; and
2. the transport of the pollutants from the envelope to the surrounding environment, called “transport term”.

The acceptable pollutant level of the surrounding environment (called “impact term”) mainly depends on the target concerned. The two most relevant targets for our study are:

1. the aquatic environment (surface water and groundwater); and
2. the soil.

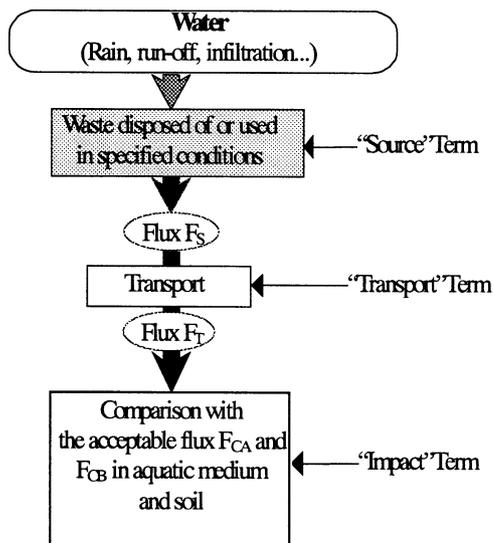
Furthermore, given the type of wastes for which an ecocompatible situation can be expected (final wastes with mainly inorganic constituents), water was considered to be the main vector for transporting the pollutants from the source term towards the environment. A summary of this methodological approach is presented in Fig. 1.

2.1. Literature survey

The survey carried out by the nine different research teams involved in the program first consisted of a general survey on the knowledge and tools available for the study of each term retained in the global methodology. Each team then made a summary and a critical study of these tools in order to determine which were

Table 1
Guidelines for the global assessment methodology

Main methodology steps	Examples for term A (waste)
Each storage or reuse scenario must be described as a combination of sub scenarios referring to the different terms	Granular porous waste subjected to percolation
Determination of the set of factors having an influence on the different terms	Physical state of the waste Nature of contact between water and waste
For each term, determination of the characterizing parameters to be taken into account for Ecocompatibility assessment	Waste porosity Dynamic leaching behavior Waste biodegradability Pollutant solubility with pH Porosity measurement Percolation test in a column Biodegradability test Test of influence of pH on pollutant solubility
Implementation of laboratory assays in order to evaluate these parameters	



Let :
 F_S the pollutant flux of the source term
 F_T the pollutant flux reaching the surrounding environment after transfer and transport
 F_{CA} acceptable flux in the aquatic medium
 F_{CB} acceptable flux in soil

Waste in storage or reuse scenarios are considered as ecocompatible if :
 $F_T \leq F_{CA}$ and $F_T \leq F_{CB}$

Fig. 1. Simplified global scheme of the waste ecocompatibility assessment.

the most relevant for the Waste Ecocompatibility Assessment.

Therefore a first approach of the global assessment methodology was able to be designed along the main guidelines. These are presented in Table 1.

2.2. Experimental program

2.2.1. Scenarios and wastes studied

In order to develop, validate and calibrate the methodology previously designed, three scenarios were used for the experimental studies. These three scenarios were chosen with quite a high exposure so that a measurable effect on the environment could be observed, which was necessary for the validation and calibration of the methodology.

These are therefore virtual scenarios which do not necessarily correspond to the real conditions in which the wastes are stored or reused. The first two scenarios correspond to percolation of water through a granular waste deposit, with a high content of metals and salts. The third scenario corresponds to immersion of a porous material in water.

For the three scenarios, the different mass or volume ratios (percolate mass/mass of transfer soil concerned, fraction of percolates directed towards grassland...) were accurately defined. These scenarios are represented in Figs. 2–4.

2.2.1.1. Scenario 1: bottom ash from municipal solid waste incineration (MSWI).

1. A granular material is used as road embankment in a mountainous region.
2. The waste receives rain and run-off water leading to the production of 2 m³ of percolate per ton of dry waste every four months.
3. Below the roadside, grassland receives the run-off effluents having percolated through the waste.
4. A river, also below the roadside, is supplied by groundwater which receives effluents having percolated through the waste and then through a permeable subsoil.

2.2.1.2. Scenario 2: matured slags from the second smelting of lead.

1. A granular material is deposited in heap just below an industrial site.
2. The waste receives rain and run-off water leading to the production 7.5 m³ of percolates per ton of dry waste every 4 months.
3. Below the waste heap, grassland receives the run-off effluents having percolated through the waste.
4. A river, also below, is supplied by groundwater which receives effluents having percolated through the waste and then through the semi-permeable subsoil.

2.2.1.3. Scenario 3: solidified air pollution control (APC) residues from MSWI.

1. A waste solidified with hydraulic binders is used to construct a fire reservoir on an industrial site.
2. The reservoir filled with water, leaks through one of the walls after 4 months storage (leachate produced: 0.5 m³ per m² of the water/wall interface for 4 months).
3. Grassland receives the reservoir run-off water.
4. A river downstream is supplied by groundwater which receives percolated reservoir water through an unsaturated permeable subsoil.

2.2.2. Experimental assays

The program consisted of assays carried out in the laboratory, aimed at developing the different tools necessary for the Waste Ecocompatibility Assessment; and assays in the field, to calibrate and validate these tools. The experimental site was located at Vernon in Normandie (France).

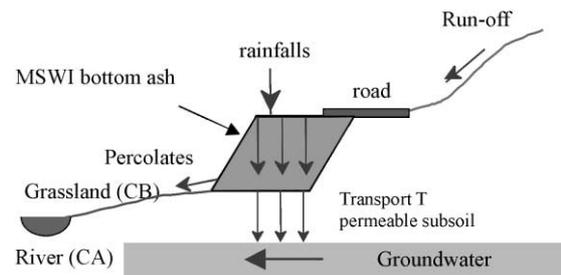


Fig. 2. Scenario 1.

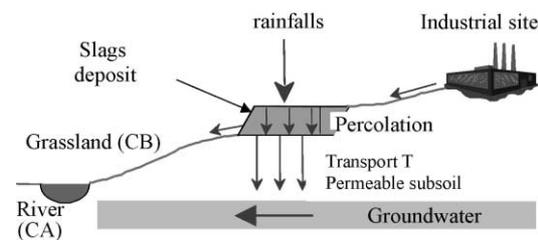


Fig. 3. Scenario 2.

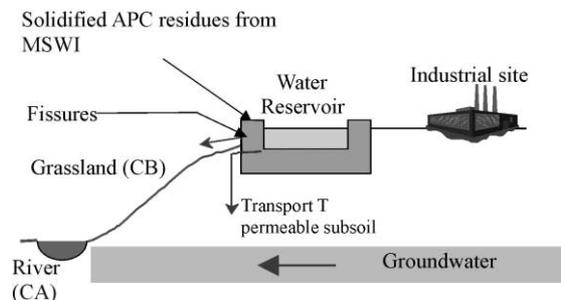


Fig. 4. Scenario 3.

Field experiments were carried out in two steps:

1. firstly considering the three scenarios but only for the source term; and
2. secondly considering the same scenarios but for all the terms (source, transport and impact terms): following the laboratory results, it was decided to study only the scenarios presenting “average” impact (a scenario with very high or low impacts would make comparison between laboratory and field results difficult).

This paper only focuses on the terms CA (aquatic environment) and CB (soil environment) but all the assays carried out during the program are summarized in Fig. 5.

2.2.3. Information concerning percolates and leachates produced

Different types of leachates (scenario 3) or percolates (scenarios 1 and 2) were produced and characterised in the program. They can be globally classified into two categories:

1. leachates and percolates produced during *parametric tests*, designed for the elaboration of a release mode; and
2. leachates and percolates produced during *simulation tests*, designed to approach the actual conditions of waste deposit in the scenario.

The *parametric tests* are used to measure an intrinsic property or to evaluate the effect of a given external

parameter. The implementation of such tests enables us to supplement data from simulation tests and to explain the observed phenomena for a long term prediction.

The *simulation tests*, taking into account the influence of the several parameters, simulate the combined effect of various parameters. They enable us to reproduce at laboratory scale the field phenomena observed over a specific period.

Only leachates and percolates from simulation tests were supplied to the teams dealing with the transport and impact terms, so that they worked with effluents as similar as possible to real effluents. Furthermore, it is now well known that the nature of the percolates from waste deposit evolves with time, so that the environment is subjected to successively different effluents. In order to integrate this notion when measuring potential impact, it was decided to sample percolates from scenarios 1 and 2 at three different times (percolates P1, P2 and P3), and then to study the migration in the soil and the impact on the environment of these three percolates successively used.

Scenario 3 which corresponds to an accident, is different in so far as it generates only one type of leachate, released when the reservoir side wall is fissured.

3. Part II: Detailed presentation of the impact study on recipient environments (impact term)

Laboratory experiments concerned the three scenarios studied but as we said before the field experiments were

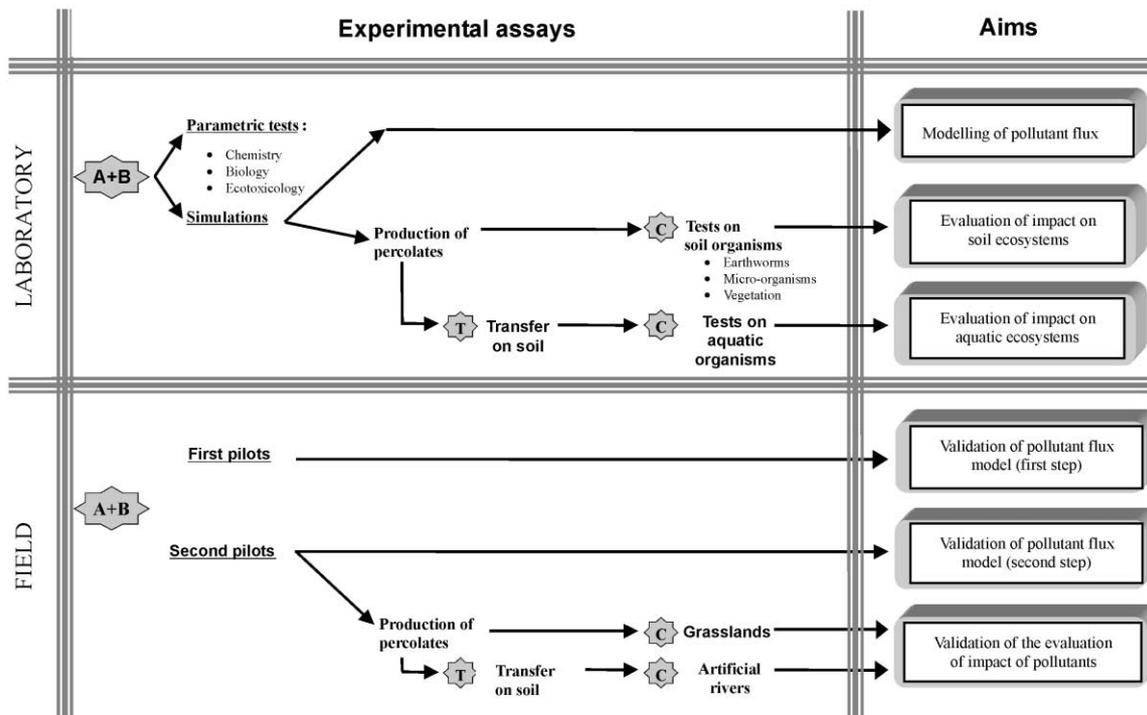


Fig. 5. Assays and aims.

only carried out on percolates or leachates coming from two of them (Table 2).

3.1. Aims and principles

3.1.1. Impact study on plants and microorganisms

Living organisms in soils belong to procaryotes (bacteria) and to eucaryotes (plants and animals). Plants are the source of organic matter which sustains life of heterotrophic organisms like animals, fungi and bacteria inhabiting soil as well as the life of higher animals. Bacteria using the energy from plant detritus or exsudates are responsible for the introduction of many elements in the life cycle: they can fix atmospheric nitrogen, solubilize minerals, in particular they can release nutrients from mineral matrix and they degrade organic matter in simple products like CO₂, H₂O, and NH₄ that some of them transform into nitrite and nitrate. Through rhizosphere, soil micro-organism communities and plants associate to their mutual benefit. Then deleterious effects on one of the partners or on the communication between partners, i.e. on the soils physical properties, may affect the whole soil ecosystem. Then the effects of complex pollutants, like percolates from MSWI bottom ash, on soil ecosystems were assessed through the analysis of metabolic (individual) and population reactions of plant and bacteria to the introduction of percolates from MSWI bottom ash as well as leachates from solidified APC residues from MSWI.

Modifications of soil micro-organisms and plants could be studied using quantitative approaches on organism demography (number of cultivable bacteria per gram of soil, number of germinating seeds per surface unit, etc.) or on metabolic process (N transformations by bacteria, photosynthesis or root development for plants). Bacteria communities may be characterized on genetic molecular basis using bacterial DNA extracted from soil when the previous techniques of cultivation and isolation could reach only a few percent of the soil populations [2]. The objective of this study was to

evidence the impact of the pollutants brought by the percolates or the leachates from solid waste (MSWI bottom ash, solidified APC residues from MSWI) on a reference soil by the analysis of indigenous microbial communities and activities and on seed germination as well as root and aerial biomass of grasses [3] which grew on this reference soil.

3.1.2. Impact study on soil fauna

The aim of this study was to assess the impact of pollutants flows on soil environment through biological parameters of earthworms. The integration of laboratory data to field observations is necessary to define environmental risks of pollution [4,5]. So, two approaches were needed: a laboratory one to evaluate earthworm reactions and an other semi-experimental in the field to describe density and structure modifications of communities.

3.1.3. Impact study on aquatic receiving environments

Two methodologies were applied in the study of pollution impact on aquatic biological communities:

1. an ecotoxicological approach on experimental communities in the laboratory, to evaluate the risks; and
2. an ecological approach in outdoor artificial streams, to describe in situ effects on the biocenoses. The second approach was more realistic from an ecological point of view.

Léglize and Nourisson [6] considered that the rate of toxicity of invertebrates reflected not only water contamination but also that of the whole aquatic system (sediments, etc.). According to numerous criteria (taxonomic, ecological, physiological, etc.), the invertebrates could be considered as good test-organisms in ecotoxicological assays [7]. Thus, benthic and interstitial invertebrate assemblages were reconstituted in experimental systems to test and to predict the effects of some pollutants on different community variables (faunistic composition, abundance, diversity, etc.).

Table 2
Field pilots

Waste	European code ^a	Scenario	Transfer on soil ^b	Pilot volume	Recipient environment
MSWI ^c bottom ash	19 01 01	Scenario 1	–	30 m ³	Plants and soil microorganisms Soil fauna
MSWI bottom ash	19 01 01	Scenario 1	X	30 m ³	Aquatic fauna
Slags from second smelting of lead	10 04 01	Scenario 2	–	2 m ³	Soil fauna
Slags from second smelting of lead	10 04 01	Scenario 2	X	30 m ³	Aquatic fauna
Solidified APC ^d residues from MSWI	19 03 01	Scenario 3	–	30 m ³	Plants and soil microorganisms

^a Commission Decision of 20 December 1993 (94/3/EC) establishing a list of wastes pursuant to Article 1 (a) of Council Directive 75/442/EEC on waste.

^b X, transfer on soil (i.e. pilot contained a layer of soil under the waste deposit: mass of soil and mass of waste were accurately defined); no transfer on soil.

^c MSWI, municipal solid waste incineration.

^d APC, air pollution control.

3.2. Materials and methods

3.2.1. Impact study on plants and microorganisms

3.2.1.1. Laboratory experiments. Plant assays: effects on *Agrostis vulgaris*. Effect of percolates or leachates were assessed on the germination rate of *A. vulgaris* and on a standard species for ecotoxicology studies. Four replicates of 50 seeds were incubated in 2 mm sieved soil moistened with serial dilutions of the studied percolates/leachates and on blank soil moistened with pure water.

Effect on biomass was followed by measuring at 30 and 50 days of cultivation on fresh soil contaminated by percolates/leachates the weight of aerial parts and root system separately. Oven dry weight (o.d.w. at 100 °C, 48 h) and fresh weight (f.w.) per plant were determined, the difference giving the water content of plants.

Microbial assays. Studies on soil bacteria were conducted on fresh soil columns added with 60 ml of percolate/leachate and left to drain at constant temperature and moisture for 30 days. At each sampling time three columns were removed and the soil collected to further analysis. Amongst the package of techniques which were assayed, the general analysis only is described, more specific studies will be developed elsewhere.

1. Enumeration of bacteria: direct enumeration of bacterial cells was done by Acridine Orange staining and fluorescent microscopy on serial dilution of 1 g fresh samples. Cultivable heterotrophic bacteria (CHB) were counted as colony forming units (CFU) on diluted nutrient agar (Trypto Soja Agar, Difco) plates inoculated with 100 µl of the same serial dilution and incubated for 5 days at 27° C in a constant temperature room in darkness.
2. DNA extraction and genetic diversity of soil bacteria: DNA was extracted and purified according to Ranjard et al. [8] on fresh soil samples (1 g equivalent dry weight), the Ribosomal Intergene Spacer region amplified by PCR [9] with bacterial specific and universal fluorescent primers, and the different components separated by electrophoresis, revealed and quantified using the labelled primers (RISA). Due to the complexity of the resulting profiles, a statistical analysis was performed by principal components analysis (PCA). This method provided an ordination of bacterial pools that were plotted in two dimensions based on scores on the first two principal components.

3.2.1.2. Field experiments (plant and microbial assays). Alfisol soil type was chosen for it is a common soil type in western Europe. As mentioned above, the experimental site was located at Vernon in Normandy (France). Soil blocks of half a cubic meter were carefully

transferred, with their plant cover, on a geomembrane to establish a plot of 100 m² on 0.50 m depth. The resulting soil conditions were as similar as possible to undisturbed close land but underground was protected against depth percolation of the pollutants. A slight slope led the drainage water to be collected in a container. To achieve the stabilization of the soil, the experimental plot was set up 1 year before the addition of pollutants. For laboratory use, undisturbed soil columns (6 cm in diameter, 10 cm high) were cored at the top of the soil after the vegetal layer has been cut. Since it was dominant amongst the vegetal species on the site, *Agrostis vulgaris*, a metallotolerant grass, was selected to assess the effect on prairie species.

Percolates/leachates were added at early spring on standard surfaces of 10 m², saving surfaces for unamended control soil under planted *Agrostis* (10 m²) or under indigenous grass cover (10 m²). Fresh soil samples were cored at 8, 16, 30, 60 and 90 days after pollution and brought to the laboratory for microbial analysis without drying. Plant species were enumerated 180 days after pollution. Heavy metal content of collected plants was analyzed on aerial and root parts, after ashing, by ionization plasma mass spectrometry (ICPMS) of the dissolved ash.

3.2.2. Impact study on soil fauna

3.2.2.1. Laboratory experiments. Laboratory experiments were performed on two earthworm species (*Lumbricus terrestris* and *Allolobophora caliginosa*) to evaluate the toxicity of waste percolates. These species belong to different ecological category [10]. Microcosms (soil columns with earthworms) are successively sprayed with three percolates P1, P2, P3 (see Section 2.2.3) at 7-day intervals and a range of concentrations is defined according to the waste. The studied biological parameters are survival, weight evolution, maturity, fecundity and grass consumption.

3.2.2.2. Field experiments. Field study consisted of a first experiment on introduced earthworms and another one on in situ communities. The impact of percolates from bottom ash and lead slags was therefore assessed through the evolution of biological parameters in field conditions and through the variation of the in situ communities density and structure. Experiments were performed on experimental plots; they consisted of soil unit strips moved from a near grassland and put on a waterproof area [11]. Two soil strips corresponded to bottom ash and lead slags and a third one corresponded to the control (sprayed with drinking water). Before spraying, soil sampling was done to study the in-situ communities, then, soil columns with an earthworm species were placed in the resulting holes. The spraying protocol was the same to the one applied at laboratory (i.e. three successive sprayings of solution at 7-day intervals).

Nevertheless, only one concentration with a volume of 28 l/m² was used according to the waste percolate toxicity.

3.2.3. Impact study on aquatic receiving environments

3.2.3.1. Laboratory experiments. The experimental systems were microcosms in the laboratory (12 series of partitioned glass tubes where 3 l of toxic solution circulated in each unit of experimental tubes). Six species, with diverse ecological requirements for biotope, diet, etc., were used for the assays (three crustaceans including one interstitial species, one mollusc, two insects including one Trichoptera and one Ephemeroptera). The length of exposure was 10 days and three replications were performed for each concentration and for the control. Mortality and post exposition recovery were considered as toxicity parameters and LC 50–240 h was calculated.

3.2.3.2. Field experiments. Outdoor mesocosms made of four 5-m long artificial steel channels, with a 440 l capacity and partially recirculating water (turnover 1.5 in 24 h at 10 cm/s) were used for the field-like study [12]. Artificial substrates colonised by known invertebrate communities collected in an unpolluted river were used. The experimentation lasted 30 days. During this time, artificial substrates were regularly removed and analysed to study the temporal changes of their fauna. Drifting, dead organisms and emerging insects were collected at regular intervals and water temperature, dissolved oxygen, and chemical water quality were measured.

Concerning the second approach, the experimental device was representative of “average” artificial stream channels used over the last 20 years for experimental studies such as behaviour studies or disturbance effects (see reviews in Kosinski [13] and Lamberti and Steinman [14]) and the experimental procedure was quite similar to that used by Crossland and Mitchell [15] to test the effects of various heavy metals.

3.3. Results

3.3.1. Impact study on plants and microorganisms

3.3.1.1. Effects on plants. Laboratory experiments. Laboratory experiments showed a significant increase in

fresh and dry biomass of *Agrostis* grown in soil amended with leachates from solidified APC residues when biomass was strongly depressed by percolates from MSWI bottom ash which resulted in 75% of the fresh biomass of *Agrostis* in an uncontaminated situation. Percentage germination of *Agrostis* seeds was significantly but differently depressed by MSWI bottom ash percolates and remained unchanged with solidified APC residues leachates added. The stronger effect resulting from bottom ash percolate addition was obtained at a dilution of 18% in water, which caused a decreased of 25% in the germination rate of *Agrostis*, and remained constant up to the undiluted percolate.

Field experiments. Effect of percolates/leachates assayed in the field resulted in a significant decrease in the ratio of aerial versus root biomass (a/r ratio) on the pool of indigenous gramineae when *Agrostis* a/r ratio was not affected by the MSWI bottom ash percolate and strongly depressed by solidified APC residues leachate which reflected the sensible increase of root biomass when solidified APC residues leachate was added.

Accumulation in plants of selected elements brought by the percolates/leachates where Na resulting from salt-contamination and Cu from heavy metal-contamination are shown in Table 3.

3.3.1.2. Effects on microorganisms. Laboratory experiments. Numbers of bacteria either from direct counts or from CFU remained unchanged whatever the added percolate/leachate which means that the assay was not sensitive enough to reveal the effect of the type of pollution resulting from the scenarios.

We also studied the genetic structure of soil bacterial communities. Ribosomal intergenes spacer analysis (RISA) revealed that bacterial communities were slightly but significantly (Monte-Carlo test) affected by the addition of MSWI bottom ash percolate in soil columns compared to the uncontaminated soil. Increasing effect was obtained with solidified APC residues leachate (results not presented).

Field experiments. The PCA allowed us to ordinate bacterial pools. The first principal component F1

Table 3
Na and Cu content in aerial and root parts of plants grown in field experiment ($\mu\text{m/g}$ of dry matter)

	Uncontaminated soil				MSWI ^a bottom ash contaminated soil				APC ^b residues contaminated soil			
	Leaves		Roots		Leaves		Roots		Leaves		Roots	
	Na	Cu	Na	Cu	Na	Cu	Na	Cu	Na	Cu	Na	Cu
<i>Agrostis vulgaris</i> (introduced)	1.9	90	1.5	152	4.8	97	1.7	202	5.5	85	1.5	132
Indigenous grasses	1.3	100	1.6	110	2.5	145	1.8	165	1.2	85	1.5	110

^a MSWI, municipal solid waste incineration.

^b APC, air pollution control.

explained 23% of the variance in the data and the second component F2 explained 17.7% of this variance. The factorial map (Fig. 6) showed that ordination on F1 differentiates the RISA profile of the contaminated soils and uncontaminated soils, excepted for MSWI bottom ashes under *Agrostis* cover. Uncontaminated soil communities were close to the MSWI bottom ash contaminated soil even if the low number of samples (4) made unusable the Monte-Carlo test. To get a maximum of samples for statistic analysis, RISA results from planted soil and indigenous plant covered soil were mixed. This global analysis revealed a significant difference between the RISA profile of the communities in soil amended with solidified APC residues leachate and those from the uncontaminated soil. Differences between RISA results on communities under MSWI bottom ash were masked when RISA on bacteria from soil under *Agrostis* planted soil were mixed with RISA on bacteria from soil under indigenous plant cover. This difference between planted and indigenous grass soil reaction evidenced that indigenous plant cover could protect soil against the addition of percolate via aerial watering.

3.3.2. Impacts study on soil fauna

3.3.2.1. Laboratory experiments. MSWI bottom ash. In the laboratory, no modification of earthworm biological parameters has been noted with pure percolate: no

mortality, no difference of weight variation, cocoon production or litter consumption.

Lead slags. High toxicity has been observed in the laboratory. Moreover, concentrations below 5% were used. Mortality was higher for *L. terrestris* (anebic) than for *Allolobophora caliginosa* (endogeic). Significant increase of weight was observed for *L. terrestris* caused by water gain. Fecundity was also disturbed during experiment at $C \geq 2.5\%$. This effect is reversible after 1 month in healthy soil. *L. terrestris* presented a lower litter consumption at $C = 2.5\%$.

3.3.2.2. Field experiments. Bottom ash. On field, introduced earthworms presented no mortality, weight loss but without any relation with percolates, no modification of fecundity and they consumed more litter than control.

The communities observed in-situ were not significantly different in terms of abundance or diversity between:

1. soil units; and
2. “before” and “after” spraying.
3. These observations are for the whole community and for each ecological category.

Lead slags. On field, a low mortality has been observed in introduced earthworms. The weight loss

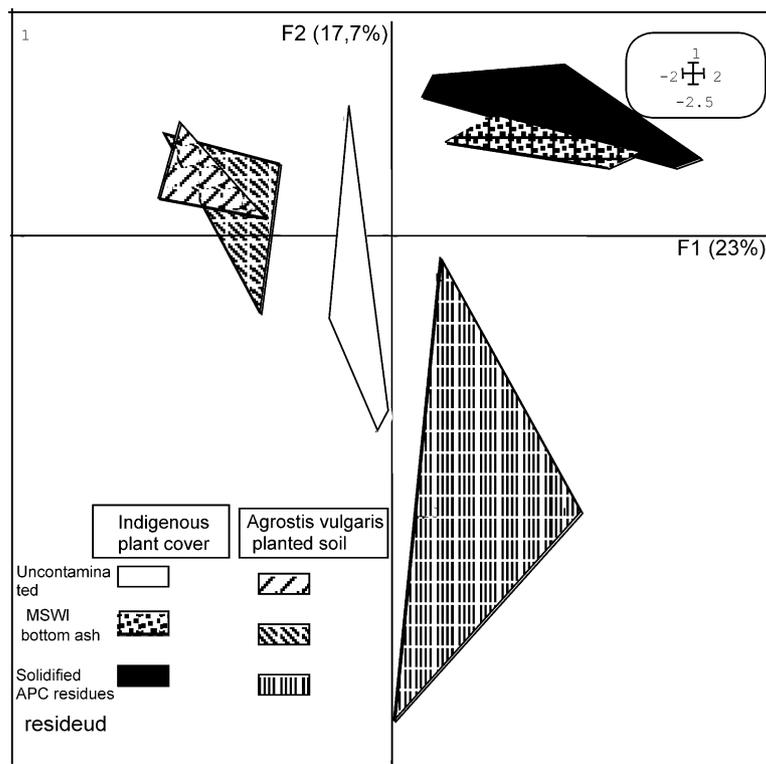


Fig. 6. PCA plot showing the position of ribosomal intergenes spacer analysis (RISA) profiles for the bacterial DNA extracted from soils contaminated by municipal solid waste incineration (MSWI) ash percolates compared to RISA obtained from uncontaminated soil.

Table 4
Laboratory experiments: impact on recipient environments (impact term)

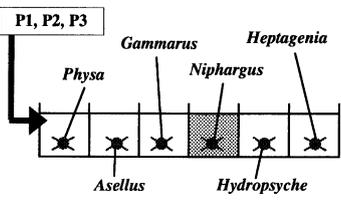
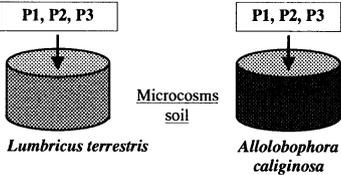
		Presentation of experiments	Results		
			Scenario 1 Percolate from MSWI bottom ash	Scenario 2 Percolate from slags (2 nd smelting of lead)	Scenario 3 Leachate from solidified APC residues
Aquatic ecosystems	Fauna	<ul style="list-style-type: none"> Study of percolate (or leachate) effects on six aquatic invertebrate species in experimental microcosms (series of partitioned glass tubes). Survey of mortality after 10 days of exposure (LC50 240h) and after 48h of post-exposition in a healthy environment. 	<ul style="list-style-type: none"> Raw bottom ash (no percolation through a soil) : <ul style="list-style-type: none"> Global LC 50 = 27.8% of percolate. Differences of sensitivity between species : <i>Gammarus</i> and <i>Heptagenia</i> LC50 = 15.5 % vs <i>Asellus</i> and <i>Hydropsyche</i> LC50 ≥ 50% of percolate. Bottom ash (percolation through a soil) : <ul style="list-style-type: none"> No significant mortality effect after 10 days of exposure with a pure percolate. Physiological effects (metabolism) during post-exposition for <i>Gammarus</i>. 	High toxicity : Global LC 50 = 0.011% of percolate All species in toxicity class 1 according to Bulich's grid (LC 50 <i>Gammarus</i> = 0.006% of percolates), i.e. the class of highest toxicity	<ul style="list-style-type: none"> A 100% concentration of leachate killed nearly 50% of <i>Gammarus</i> and nearly 20% of <i>Physa</i>. During the post exposition survey <i>Gammarus</i> and <i>Physa</i> showed physiological effects (mortality of 14% and 42% respectively of the surviving individuals)
	Fauna	<ul style="list-style-type: none"> Exposure of two earthworm species to percolate (or leachate). Study of the following parameters : <ul style="list-style-type: none"> survival, weight variation, fecundity, litter consumption (<i>L. terrestris</i>). 	For all concentrations and volumes used, percolates of bottom ash have no effect on parameters.	<ul style="list-style-type: none"> Survival : different according to the species but only with concentrations below 5% of percolates. Weight variation : increase at 2.5 %. Fecundity : decrease at 2.5 % during experiment but reversible in healthy soil. Litter consumption : decrease at 2.5%. 	<ul style="list-style-type: none"> Survival : no effect with pure leachate, Weight variation : low decrease with 100% of leachate. Fecundity : no effect observed after 1 month in healthy soil. Litter consumption : decrease with 25% and 50% ; no consumption with 100% of leachate.
	Microflora	<ul style="list-style-type: none"> Dose effect on microflora <ul style="list-style-type: none"> Enumeration of cultivable bacteria (CFU) from soil suspension in serial dilution of percolates (or leachates). Studies on intact soil core : <ul style="list-style-type: none"> Enumeration as for dose effect, Effect on structure of bacteria communities (molecular biology tools : RISA). 	<ul style="list-style-type: none"> Heterotrophic cultivable bacteria (Tryptic Soja Agar 1/10). N fixing bacteria cultivable on N free broth. Idem as above (dose effect). Direct extraction of DNA from soil, clean up, amplification by PCR and electrophoresis on polyacrylamide gel. 	<ul style="list-style-type: none"> No effect on CFU whatever the dose. No effect of the relevant amount of percolate on CFU from reference soil. Low effect of MSWI bottom ash percolate on RISA profiles. 	<ul style="list-style-type: none"> No effect on CFU whatever the dose. No effect of the relevant amount of percolate from slag on CFU from reference soil. Stronger effect of percolate from slag than percolate from bottom ash on RISA profiles.
Plants	Ecotox assay on lettuce and <i>Agrostis vulgaris</i> (germination, aerial biomass). Calculation of the a/r ratio : aerial biomass/root biomass	Assay on 50 seeds on sterile soil moistened with percolate (or leachate). <i>Agrostis</i> seeds grown on soil reference soil columns added with percolate (or leachate).	Ecotox test positive on both lettuce and <i>Agrostis</i> . Decrease of a/r ratio for indigenous and planted grasses. Strong decrease of <i>Agrostis</i> biomass.	Strong inhibition of germination caused by percolate from slag. In laboratory experiments, strong impact on <i>Agrostis</i> – Decrease of a/r ratio.	Ecotox test positive on lettuce and <i>Agrostis</i> seeds Increase fresh and dry biomass of <i>agrostis</i> . Increase of root biomass, so decrease of a/r ratio.

Table 5
Field experiments: impact on recipient environments (impact term)

First step of the program	First step of the program	Results for the Impact Term		
		Scenario 1 Percolate from MSWI bottom ash	Scenario 2 Percolate from slags (2 nd smelting of lead)	Scenario 3 Leachate from solidified APC residues
<p>Percolates and leachate production</p> <p>→ Validation of the pollutants flux model</p> <p>Granular waste Slags : 30 m³ pilot Bottom ash : 30 m³ pilot</p> <p>Monolithic waste Solidified APC residues : area : 30 m²</p> <p>Experimental prairie</p> <p>→ Analyses before percolates and leachate spraying : soil characteristics & study of soil fauna, plants and microbial communities</p> <p>100 m² x 0,5 m</p>	<p>Percolates and leachate production</p> <p>Slags and bottom ash : 2nd pilots Solidified APC residues : water</p> <p>Outdoor artificial streams : 5 m, 440 litres</p> <p>→ Supplying with percolates or leachate coming from the pilots. Dilution range from C0 to C3</p> <p>C0 C1 C2 C3</p> <p>Experimental prairie</p> <p>→ Spraying with percolates or leachate coming from the pilots.</p> <p>Note : pilots for leachate production contain a soil layer or not according to the concerned scenario</p>	<p>Aquatic fauna :</p> <ul style="list-style-type: none"> • Study of percolate effects on artificial substrates colonized by aquatic invertebrate communities in experimental artificial channels. • Survey of following parameters : abundance, richness and emergence during a 30 days exposure at the community level and at the taxa level. <p>Soil fauna :</p> <ul style="list-style-type: none"> • Introduced microcosms with <i>L. terrestris</i> ; Study of : <ul style="list-style-type: none"> - survival, - weight variation, - fecundity, - litter consumption. • Experimental plots ; effect on structure and diversity of natural communities. <p>Soil microflora :</p> <ul style="list-style-type: none"> • Cored soil columns added with percolate (or leachate) : see laboratory experiments. • Experimental plots : <ul style="list-style-type: none"> - under indigenous plant cover, - under planted <i>agrostis</i>, <p>Effect on demography and structure of bacterial communities after 3, 8, 30, 90 days.</p> <p>Plants :</p> <ul style="list-style-type: none"> • Experimental plots : see soil microflora. <ul style="list-style-type: none"> - Taxonomy and diversity of plant species at 180 days after percolate (or leachate) spraying. - Collect of plants : Biomass (aerial and roots) and bioaccumulation of salt and heavy metals. 	<ul style="list-style-type: none"> • The effects were significant : in channel C1 (10% of percolate), the abundance, richness and emergence decreased compared to control channel). • Difference of sensitivity between species : <i>Gammaridae</i> and <i>Heptageniidae</i> vs <i>Asellidae</i> and <i>Elmidae</i>. <ul style="list-style-type: none"> • Introduced microcosms with <i>L. terrestris</i> : no negative effects. • Experimental plots ; No significant difference of abundance or diversity between blocs for the whole community and for each ecological category. Same observation between before and after spraying. <p>No effect on bacterial demography. Structure of soil communities less affected by bottom ash percolate than by plant cover.</p> <p>Decrease of plant fresh biomass (loss of water). Phytotoxicity : abnormal accumulation of Pb and Cu in plants.</p>	<ul style="list-style-type: none"> • The effects were significant at C2 = 1% (abundance, richness). • No emergence (season). • Differences of sensitivity : <i>Gammaridae</i> vs <i>Asellidae</i>, <i>Elmidae</i>, <i>Hydropsychidae</i>. <ul style="list-style-type: none"> • Introduced microcosms with <i>L. terrestris</i> : negative effects on survival, fecundity and litter consumption. • Experimental plots ; No significant difference of abundance or diversity for the whole community but significant decrease of abundance for anecics and endogeics <p>- not experimented -</p> <p>- not experimented -</p> <p>Effect on soil community structure. Similar effect to bottom ash percolate on soil bacteria under indigenous cover. Strong effect under <i>Agrostis</i>.</p> <p>Positive effect on plant biomass (indigenous and planted). No effect on plant community (short term).</p>

Table 6
Laboratory versus field impact on recipient environments (impact term)

Scenario 1: percolate from MSWI bottom ash		Scenario 2: percolate from slags (second smelting of lead)		Scenario 3: leachate from solidified APC residues	
Similarities	Differences	Similarities	Differences	Similarities	Differences
<i>Aquatic environment— aquatic fauna</i>					
Constancy of taxa to be sensitive or resistant: e.g. <i>Gammarus</i> was always more sensitive than <i>Asellus</i> .	No effect at 100% considering the parameter “mortality” in the laboratory; significant biological effects at 10% in experimental streams.	Constancy of taxa to be sensitive or resistant (cf <i>Gammarus</i> vs <i>Asellus</i>). Significant toxicological effects whatever the concentration.	Differences between laboratory and experimental streams (ratio 1:100)	No field experiments	No field experiments
<i>Soil environment— soil fauna</i>					
No effect on biodemographic parameters. Individual and communities integrity was preserved at short term.	No information on field long term for <i>in-situ</i> communities.	Individual and communities integrity was affected at short term: there was mortality at low concentration of percolates. Sublethal effects (fecundity and litter consumption). Different reply according to the species.	Stronger effects at laboratory than on field. No information on field long term for <i>in-situ</i> communities	No field experiments	No field experiments
<i>Soil environment— soil microflora</i>					
No effect on demography of bacteria. Comparable effect on RISA of soil under planted <i>Agrostis</i> .	Less effect on microbial parameters in the field under indigenous cover.	No field experiments	No field experiments	No effect on bacteria number. Stronger effect on RISA in the field as in laboratory than bottom ash percolate.	Large dispersion of RISA profiles from <i>Agrostis</i> planted soil.
<i>Soil environment— Plants</i>					
Similar effect on <i>Agrostis</i> planted in the field or in-situ.	Indigenous cover protected soil against percolate watering. Bioaccumulation lower in indigenous species.	No field experiments	No field experiments	Positive effect on biomass yield was detected on field grown as well on laboratory grown <i>Agrostis</i> .	Indigenous plants (including <i>Agrostis</i>) did not react as planted <i>Agrostis</i> . Bioaccumulation was not tested on laboratory plants.

had no relation with percolates. However, there were inhibition of cocoon production and decrease of litter consumption.

For the whole in-situ community, no significant difference of abundance or diversity was observed. Nevertheless, anecic and endogeic abundances presented a significant decrease after spraying.

3.3.3. Impact study on aquatic receiving environments

3.3.3.1. Laboratory experiments. Bottom ash. With no percolation through a soil transfer, percolate from bottom ash was toxic: a 28% dilution of its percolate killed 50% of the macroinvertebrates (Table 4). The sensitivity of all taxa was not the same: for example, considering the crustaceans, the bottom ash was very toxic for *Gammarus* and *Niphargus* (a hypogean species) and toxic for *Asellus*, according to Bulich's grid of toxicity [16]. These differences of sensitivity may be explained by sensitivity to different chemicals (lead, copper, zinc). A purification-like effect was noted after percolation of the waste percolate through the substratum and decreasing concentrations of Pb, Cu, Na and K were measured. In contrast, Zn and Ca were dissolved and their concentrations increased. This lower toxicity after percolation did not necessarily mean that the product was less toxic, as it might have other effects than a simple short-term mortality. The effects on metabolism (respiration for ex.) were probably important for *Gammarus* which did not recover very well 48 h after the end of the bioassay (30% of mortality). Acute toxicity was infrequent in the natural environment where sub-acute and chronic effects were detected. Pollution affected the development or the reproduction rates of individuals, the diversity or the trophic equilibrium of communities, and modified the whole ecosystem.

Lead slags. The toxicity of the percolates from slags was lower in the experimental streams: mortality was significant for a 1% concentration, i.e. a 1:100 ratio when compared to the laboratory LC 50 (Table 6).

3.3.3.2. Field experiments. Bottom ash. Biological effects were noted for percolated bottom ash in the outdoor experimental streams since a 10% concentration: decrease of 60% in abundance, of 25% in taxonomic richness, of 80% in emergence compared to the control stream. From a taxonomic point of view, sensitivity decreased from Gammaridae (Crustacea) and Heptageniidae (Ephemeroptera) to Asellidae (Crustacea) and Elmidae (Coleoptera; Table 5).

Lead slags. In the same way as with bottom ash, Asellidae and Elmidae were the most resistant taxa during the outdoor experiments.

3.4. Synthesis

A summary presentation of the laboratory and field results is provided in Tables 4 and 5. Table 6 is a comparison of these two approaches.

4. Conclusion and discussion

4.1. Impact study on plants and microorganisms

Field experiment results were supported by the various effects observed on laboratory assays. *Agrostis vulgaris* planted on contaminated soils was deleteriously affected by the addition of MSWI bottom ash percolates. The reaction of *Agrostis vulgaris* to the addition of solidified APC residues leachate was to maximize its root system, increasing biomass and increasing the possible accumulation of heavy metals in the roots. New tools on the genetic structure of bacterial communities evidenced effects that were not detected by the classical techniques of enumeration. RISA revealed deep changes in soil bacteria communities when soil was added with solidified APC residues leachate and moderate changes in the presence of MSWI bottom ash percolate.

4.2. Impact study on soil fauna

The experiment with introduced-earthworms allowed the assessment of earthworms reactions under field conditions. So, integration of toxic data to field observations is easier.

We have extreme examples of waste percolates effects. No toxicity was observed for MSWI bottom ash percolates in the laboratory as well as in the field while lead slags percolates caused strong effects, one of which was death. Similar reactions were observed at laboratory and on field. Nevertheless, effects seem stronger in the laboratory and species have different sensitivity.

The study period of natural communities is short. Consequently, the observed effects are only due to lethal toxicity. At this stage, it appears necessary to carry out field studies over a longer period (more than 1 year after pollution) in order to include sublethal effects.

4.2.1. Impacts study on aquatic receiving environments

From a faunal point of view, similar trends (Table 6) such as the constant sensitivity or resistance of taxa were noted between the two approaches or for the percolates (e.g. *Gammarus* more sensitive than *Asellus*, resistance of Asellidae and Elmidae).

In contrast, differences in toxicity between the two approaches appeared for percolates from bottom ash and lead slags (Table 6).

1. The differences in toxicity between laboratory and outdoor experiments could be explained by:

2. the increasing complexity of the experimental device (duration of exposure, species level vs community level, size of the device) and of the environment (substratum, biofilm development, organic matter input into the artificial stream channels);
3. a greater production of organic compounds compared to the heavy metals present under ionic form in percolated bottom ash; and
4. the precipitation and the adsorption of heavy metals which became less bioavailable in lead slags.

The complementarity between the two approaches has been validated: the 1:100 toxicity ratio in the experiment with lead slags shows the risk of the separation of laboratory and field studies. The former allows to establish acceptance thresholds, whereas the latter measure the ecological effects on in situ communities and on the structure and functioning of the receiving environment, i.e. ecosystem health.

On-site studies are thus needed for a better understanding of the synergetic and antagonistic effects of the chemical compounds of the different percolates and of their fate.

Mortality does not appear to be an adequate parameter for evaluating toxicity; rate of emergence or physiological response also seem to be good descriptors. Extensive research must be carried out in this path, such as the study of species traits, to establish what are the reasons for differences of sensitivity between species, for example why *Gammarus*, a swimmer and omnivorous taxon, is more sensitive than *Asellus*, a crawler and detritivorous taxon.

The notion of ecocompatibility is close to that of impact, and it leads to decisions based on reliable tools for measuring and evaluating. This study tested of a monitoring tool (the experimental artificial streams) which made laboratory results more efficient.

4.3. Methodology for the assessment of waste ecocompatibility: utilisation limitations and improvement perspectives

The research program on Waste Ecocompatibility, partly described here (see the evaluation of the “source term” in Barna et al. [17,18], led to an operational methodology [19].

A fully descriptive guide for the methodology will be forthcoming at the beginning of 2000 and will contain the set of practical elements necessary for the implementation of such a methodology in order to generalise its utilisation.

This methodology can be used for the study of new scenarios or the characterisation of existing scenarios (deposits, waste utilisation...). It can also be used as decision-making tool for the conception of new storage centres or civil works compatible with the environment.

There are two main limits of this methodology:

1. Some evaluation tools are still not available and validated for a few scenarios. e.g. tools for evaluating impacts on aquatic biological communities living in groundwater, ponds, lakes...
2. This methodology is applicable for the medium-term behaviour assessment. Investigations on the long-term predictions are needed. e.g. to assess the long-term impact on plants, the phyto-genotoxicity is to be considered.

Acknowledgements

This study is part of a French national research program on waste ecocompatibility (duration: 3 years) funded by the Agency for Environment and Energy Management (ADEME). We acknowledge all the laboratories and research teams involved in the “Waste Ecocompatibility” program. Apart from the authors, this work is the result of the work of numerous researchers: Valérie Gaveglia (POLDEN), Pierre Moszkowicz, Rémy Gourdon (LAEPSI-INSA de Lyon), Gérard Didier, Véronique Norotte and Ibrahim Alimi (Laboratoire de Géotechnique-INSA de Lyon), Jean-François Féraud, Benoît Ferrari (CSE-Metz), Hervé Billard, Lucie Lambolez and Philippe Salmon (CERED).

References

- [1] Perrodin Y, Cavégia V, Barna R, Moszkowicz P, Gourdon R, Féraud JF, Ferrari B, Fruget JF, Plenet S, Jocteur-Monrozier L, Poly F, Texier C, Cluzeau D, Lambolez L and Billard H. Programme de recherche sur l'écocompatibilité des déchets: étude bibliographique. Report, ADEME, 1996.
- [2] Ward D, Weller R, Bateson MM. 16S rRNA sequences reveal numerous uncultured microorganisms in natural community. *Nature* 1990;345:63–5.
- [3] Linder G, Greene JC, Ratsch H, Nwosu J, Smith S, Wilborn D. Seed germination and root elongation toxicity tests in hazardous waste site evaluation: methods and applications. *Plants for Toxicity Assessment ASTM STP1091* 1990:177–87.
- [4] Cluzeau D, Lagarde R, Texier C, Fayolle L. Relevance of life-history parameters in earthworm ecotoxicology. *Ecotoxicology of Earthworms Intercep*, Hants 1992:213–6.
- [5] Texier C, Cluzeau D, Cortet J, and Gomot A. La faune du sol indicateur de la qualité des sols. *Séries données et références*. brochure 2588, ADEME, 1996.
- [6] Légize L and Nourisson M. Les micropolluants dans les divers compartiments de l'écosystème des eaux douces: utilisation des niveaux de contamination comme indicateurs de qualité. Cas des Invertébrés. In: *Proceeding of the 5èmes journées scientifiques et techniques*, Lille. Collection Recherche & Environnement 1983; volume 22, p. 55–60.
- [7] Jean G, Fruget JF. Aquatic macroinvertebrates as ecotoxicological indicators. *Verh Internat Verein Limnol* 1994;25(3): 2004–7.

- [8] Ranjard L, Poly F, Combrisson J, Richaume A, Nazaret S. A single procedure to recover DNA from the surface and inside aggregates and in various size fractions of soil suitable for PCR based assays of bacteria. *European Journal of Soil Biology* 1998; 34(2):89–97.
- [9] Normand P, Ponsonnet C, Nesme X, Neyra M, Simonet PITS analysis of procaryotes. *Molecular Ecology Manual*. Dordrechts: Kluwer, 1996.
- [10] Bouché MB. *Ecologie et systématique*. Ann Zool INRA 1972 special issue.
- [11] Texier C, Cluzeau D and Bellido A. In: Programme de recherche sur l'écocompatibilité des déchets. Report, ADEME, 1999.
- [12] Canivet V, Fruget JF and Gibert J. In: Programme de recherche sur l'écocompatibilité des déchets. Report, ADEME, 1999.
- [13] Kosinski RJ. Artificial streams in ecotoxicological research. *Aquatic ecotoxicology: fundamental concepts and methodologies*. Volume I: 297–316, CRC Press, Boca Raton, 1989.
- [14] Lamberti GA, Steinman AD. Research in artificial streams: applications, uses, and abuses. *J N Am Benthol Soc* 1993;12(4):313–84.
- [15] Crossland NO, Mitchell GC. Use of outdoor artificial streams to determine threshold toxicity concentrations for a petrochemical effluent. *Environ Toxicol Chem* 1992;11:49–59.
- [16] Bulich AA. A practical and reliable method for monitoring the toxicity of aquatic samples. *Process Biochemistry* 1982:45–7.
- [17] Barna R, Rethy Z, Imyim A, Perrodin Y, Moszkowicz P, Tiruta-Barna L. Environmental behaviour of a construction made of a mixture of hydraulic binders and air pollution control residues from Municipal Solid Waste Incineration. Part 1. Physico-chemical characterisation and modelling of the source term. *Waste Management* 2000;20:741–50.
- [18] Barna R, Rethy Z, Imyim A, Perrodin Y, Moszkowicz P, Tiruta-Barna L. Environmental behaviour of a construction made of a mixture of hydraulic binders and air pollution control residues from Municipal Solid Waste Incineration. Part 2. Simulation tests and validation of the source term modelling. *Waste Management* 2000;20:751–9.
- [19] Perrodin Y, Grelier-Volatier L, Barna R and Gobbey A. Assessment of the Ecocompatibility of waste disposal or waste use scenarios: towards the elaboration and implementation of a comprehensive methodology. In: Woolley GR, Goumans JJJM, Wainwright PJ, editors. *Proceeding of the International Conference on the Science and Engineering of recycling for Environmental Protection*. United Kingdom, Harrogate International Conference Centre, 31 May–2 June, 2000.