



HAL
open science

Use of ecotoxicity test and ecoscores to improve the management of polluted soils: case of a secondary lead smelter plant

Yann Foucault, Marie-José Durand, Karine Tack, Eva Schreck, Florence Geret, Thibaut Lévèque, Philippe Pradère, Sylvaine Goix, Camille Dumat

► To cite this version:

Yann Foucault, Marie-José Durand, Karine Tack, Eva Schreck, Florence Geret, et al.. Use of ecotoxicity test and ecoscores to improve the management of polluted soils: case of a secondary lead smelter plant. *Journal of Hazardous Materials*, 2013, Vol. 246-247, pp. 291-299. 10.1016/j.jhazmat.2012.12.042 . hal-00815325

HAL Id: hal-00815325

<https://hal.science/hal-00815325v1>

Submitted on 18 Apr 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author-deposited version published in: <http://oatao.univ-toulouse.fr/>
Eprints ID: 8490

To link to this article: DOI: 10.1016/j.jhazmat.2012.12.042
URL: <http://dx.doi.org/10.1016/j.jhazmat.2012.12.042>

To cite this version: Foucault, Yann and Durand, Marie-José and Tack, Karine and Schreck, Eva and Geret, Florence and Leveque, Thibaut and Pradère, Philippe and Goix, Sylvaine and Dumat, Camille *Use of ecotoxicity test and ecoscores to improve the management of polluted soils: case of a secondary lead smelter plant.* (2013) Journal of Hazardous Materials, Vol. 246-247 . pp. 291-299. ISSN 0304-3894

Any correspondence concerning this service should be sent to the repository administrator: staff-oatao@listes-diff.inp-toulouse.fr

Use of ecotoxicity test and ecoscores to improve the management of polluted soils: case of a secondary lead smelter plant

Yann Foucault^{a,b,c}, Marie-José Durand^d, Karine Tack^e, Eva Schreck^f, Florence Geret^g, Thibaut Leveque^{a,b}, Philippe Pradere^c, Sylvaine Goix^{a,b}, Camille Dumat^{a,b,*}

^a Université de Toulouse, INP-ENSAT, Avenue de l'Agrobiopôle, 31326 Castanet-Tolosan, France

^b UMR 5245 CNRS-INP-UPS, EcoLab (Laboratoire d'Ecologie Fonctionnelle), Avenue de l'Agrobiopôle, BP 32607, 31326 Castanet-Tolosan, France

^c STCM, Société de Traitements Chimiques des Métaux, 30 Avenue Fondeyre, 31200 Toulouse, France

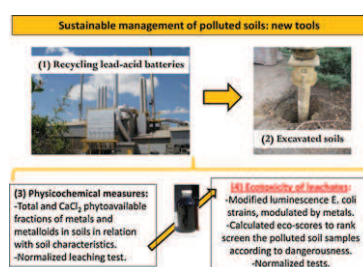
^d Université de Nantes, UMR 6144 CNRS GEPEA, Département Génie Biologique, 18 Boulevard G. Defferre, 85000 La Roche sur Yon, France

^e INERIS, Parc Technologique Alata, BP 2, 60550 Verneuil-en-Halatte, France

^f UMR 5563 CNRS/UPS/IRD/CNES GET, 14 Avenue Edouard Belin, 31400 Toulouse, France

^g UMR CNRS 5602, Laboratoire GEODE, PRES Université de Toulouse, Centre Universitaire Jean-François Champollion, Place de Verdun, 81012 Albi Cedex 9, France

GRAPHICAL ABSTRACT



ABSTRACT

With the rise of sustainable development, rehabilitation of brownfield sites located in urban areas has become a major concern. Management of contaminated soils in relation with environmental and sanitary risk concerns is therefore a strong aim needing the development of both useful tools for risk assessment and sustainable remediation techniques. For soils polluted by metals and metalloids (MTE), the criteria for landfilling are currently not based on ecotoxicological tests but on total MTE concentrations and leaching tests. In this study, the ecotoxicity of leachates from MTE polluted soils sampled from an industrial site recycling lead-acid batteries were evaluated by using both modified *Escherichia coli* strains with luminescence modulated by metals and normalized *Daphnia magna* and *Alivibrio fischeri* bioassays. The results were clearly related to the type of microorganisms (crustacean, different strains of bacteria) whose sensitivity varied. Ecotoxicity was also different according to sample location on the site, total concentrations and physico-chemical properties of each soil. For comparison, standard leaching tests were also performed. Potentially phytoavailable fraction of MTE in soils and physico-chemical measures were finally performed in order to highlight the mechanisms. The results demonstrated that the use of a panel of microorganisms is suitable for hazard classification of polluted soils. In addition, calculated eco-scores permit to rank the polluted soils according to their potentially of dangerousness. Influence of soil and MTE characteristics on MTE mobility and ecotoxicity was also highlighted.

Keywords:
Sustainable management of polluted soils
Metal trace elements
Ecotoxicity
Landfilling
Leaching

* Corresponding author at: EcoLab (Laboratoire Ecologie Fonctionnelle et Environnement), INP-ENSAT, Avenue de l'Agrobiopôle, BP 32607, Auzeville Tolosane, 31326 Castanet-Tolosan, France. Tel.: +33 5 34 32 39 03; fax: +33 5 34 32 39 01.
E-mail address: camille.dumat@ensat.fr (C. Dumat).

1. Introduction

Originally located on the outskirts of cities, numerous industrial sites, sometimes abandoned, are now in urban areas and are therefore likely to have environmental and health risks to surrounding populations [1,2]. Currently, rehabilitation of the sites frequently entails excavation of polluted soils [3]. Excavated soils can thus follow two different ways: landfilling, expensive and energy intensive, or reuse/recycling, integrated to sustainable development. The choice of a specific track mainly depends on total and leachable concentrations of the pollutant in the soil [2]. Among the numerous pollutants observed in urban and peri-urban areas, trace metals are often present in soils [4]; atmosphere emissions by smelters being one of the main anthropogenic source [5,6]. MTE speciation and compartmentalization in soils can modify their impact on living organisms [5]. Now, numerous publications concluded that these two parameters are strongly influenced by soil organic matter (OM) content, pH and texture [7–9]. According to Matejczyk et al. [10], chemical weathering of soil minerals favours MTE solubilization and leachates production. Then, these leachates can pollute surrounding soils and waters. According to the council directive n°1999/31/CE, leaching tests with chemical analysis are therefore currently used for the assessment of environmental hazards of polluted soils. But, landfilling is often inevitable for strongly polluted soils, with high “hazard level” (assessed by leached and total MTE concentrations). Moreover, according to Foucault et al. [1], professionals consider the threshold set as too restrictive and they regret that excavated soils are almost always managed as waste.

In addition to the measure of total and leached MTE concentrations, it appears therefore that knowledge of MTE availability [11] and ecotoxicity may carry useful information [12–14] to improve environmental risk assessment [10]. Actually, the accurate estimation of metal phytoavailability in polluted soils and solid wastes, using single chemical extraction [15] carry interesting data to perform pertinent risk assessment and remediation efforts [16,17]. Soil quality integrates both physicochemical and biological characteristics [18]. Moreover, according to Plaza et al. [14] microorganisms play important roles in numerous soil functions. Soils are often polluted with a large variety of compounds leading to possible interactions [19], thus as reviewed by Kim and Owens [20] study of leachates ecotoxicity provides a direct functional characterization of various pollutant mixtures. But, only few studies concern the use of ecotoxicological tests to monitor contamination and bioremediation efficiency of polluted soils [21] and new tests are required by industrial sites managers to assess environmental risks. Among them, microbial bioassays offer quick, cheap and easy ecotoxicity (toxicity and mutagenicity) and bioavailability measurements on bacteria [22,23]. However, in many cases, microbial bioassays cannot be directly used for the identification and quantification of compounds due to the lack of specificity of the engineered microorganisms [24] and further studies are needed to improve these biotests.

The aim of this study was therefore to assess the ecotoxicity of leachates for landfilling of MTE contaminated soils by various complementary biotests, in addition to usual physicochemical measures. More precisely, the following two scientific objectives were aimed: (1) what is the pertinence of ecotoxicity tests to assess a more realistic human exposition to contaminated soil leachates? (2) What is the influence of soil physicochemical parameters on MTE mobility and leachates ecotoxicity? Several studies use specific bacteria strain to sense the presence of metals in soils [25–28], nevertheless, the development of statistical model to understand the link between chemical concentration of compound and bacteria sensors is still on-going work [23]. So, the originality of this study was to combine the use of new bacterial strains never tested in a context of the remediation of an industrial polluted site

and calculation of eco-scores which facilitates the comparisons between different soils.

2. Materials and methods

2.1. Soil sampling and preparation

According to Wong et al. [29], the most relevant soil layer to study the environmental and sanitary impacts of MTE in urban areas is between 0 and 25 cm. Ten top soil samples (Fig. 1) were therefore collected in the courtyard of the Chemical Metal Treatments Society (STCM), a secondary lead smelter which currently recycles batteries located in the urban area of Toulouse (43°38'12"N, 01°25'34"E). This plant was chosen because of its activity and urban location, and many data are already available [1,4–6,30]. These data allowed defining different areas in terms of environmental and sanitary risks that can vary according to past and present activities. Moreover, previous studies of the particles released in the atmosphere by Uzu et al. [5] and Schreck et al. [4] revealed the presence of several MTE (Pb, As, Cu, Cd, Zn and Sb) and gave information on the main lead speciation: PbS, PbSO₄, PbO–PbSO₄, α-PbO and Pb (by order of abundance). All soil sampling points are presented in Fig. 1; they were dried, sieved under 2 mm and treated in triplicate.

2.2. Physico-chemical analysis

pH, organic matter and limestone contents, cation exchange capacity (CEC Metson) and texture, were determined for all soil samples, respectively, according to the norms ISO 10390 [31], ISO 10694 [32], ISO 10693 [33], NF X31-310 [34] and ISO 11277 [35]. Pb, As, Cu, Cd, Zn and Sb total concentrations were measured by ICP-OES (IRIS Intrepid II XXDL) after mineralization in *aqua regia* according to ISO 11466 [36] (HNO₃ 65%, HCl 37%, ratio 1:3 (v/v)). The detection limits of Pb, Cd, Sb, As, Cu and Zn were 0.3, 0.2, 0.2, 0.2, 1.3 and 2.2 μg L⁻¹, respectively, whereas the limits of quantification were about 0.4, 0.3, 0.4, 0.3, 2 and 3 μg L⁻¹, respectively. The accuracy of measurements was checked using a certified reference material 141R (BCR, Brussels). The concentrations found were within 95–102% of the certified values for all measured elements.

2.3. Leaching tests

Normalized leaching test [37] was applied to all soil samples. This procedure consisted of a single extraction with deionised water, using a solid-to-liquid ratio of 1/10. 10 g of soil (granulometry at least < 4 mm according to the norm) was mixed with 100 mL deionised water during 24 h with end-over-end agitation at 5 rpm. After centrifugation at 3000 × g during 15 min, the leachates were filtered with cellulose 0.45 μm (Millipore®) filters. 10 mL of each leachates were then acidified with HNO₃ 65% prior to analysis by ICP-OES (IRIS Intrepid II XXDL, analytical errors < 5%). The other part of leachates was not acidified so as not to disturb microorganisms used for further ecotoxicological tests.

2.4. MTE phytoavailability estimate

Potentially phytoavailable MTE concentrations were estimated by CaCl₂ extractions according to Uzu et al. [5]. In 25 mL polypropylene centrifugation tubes, 10 mL of 10⁻² M CaCl₂ were added to 1.0 g of soil. The liquid to solid ratio of 10 is high enough to avoid samples heterogeneities [38]. After agitation end-over-end during 2 h at 5 rpm at 20 °C, samples were then centrifuged during 30 min at 10,000 × g. Supernatant was sieved through a 0.22 μm mesh and acidified at 2% with HNO₃ (15 N, suprapur 99.9%). MTE concentrations were finally measured by ICP-OES (IRIS Intrepid II XXDL,

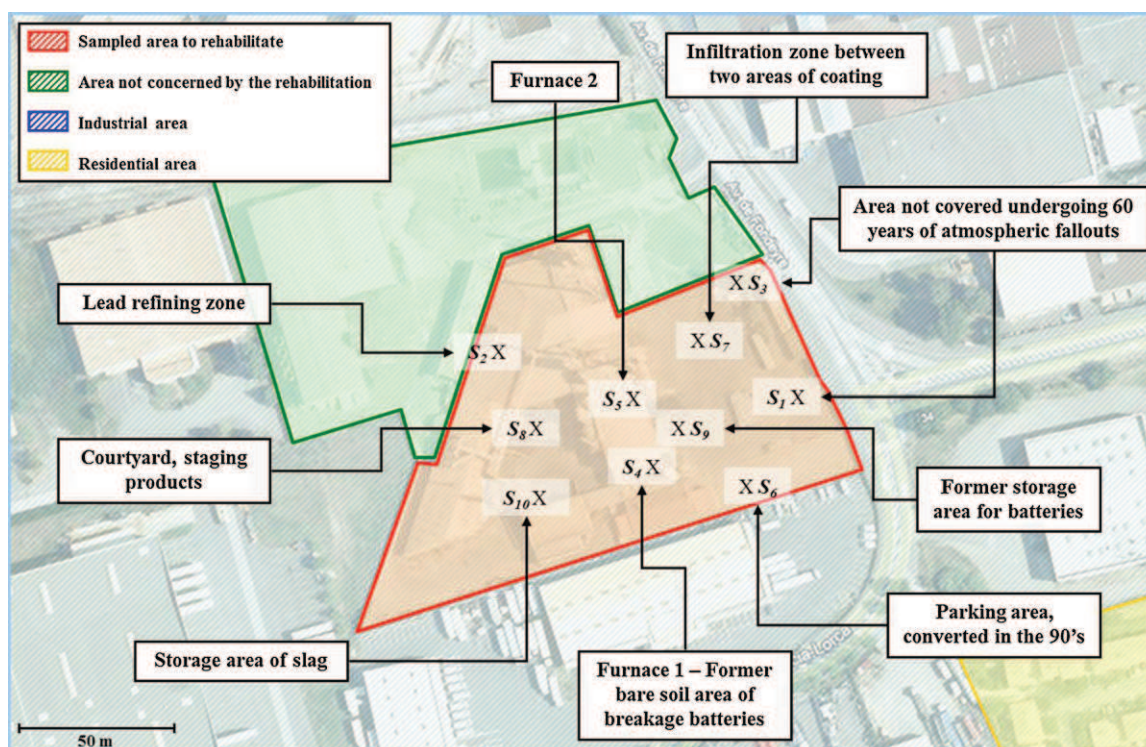


Fig. 1. Situation of the industrial study site, location and characteristics of the 10 sampling points.

analytical errors <5%). A house reference soil was used to quality control of CaCl_2 extraction: this soil described by Schreck et al. [4] has the advantage of presenting the same type of contamination that the soils studied in this work. Actually, it is a soil historically polluted by battery recycling emissions ($[\text{Pb}] = 1650 \pm 20 \text{ mg} \cdot \text{kg}^{-1}$). Using that reference soil, the detection limits of Pb, Cd, Sb, As, Cu and Zn were 0.3, 0.2, 0.2, 0.2, 1.3 and $2.2 \mu\text{g} \cdot \text{L}^{-1}$, respectively, whereas the limits of quantification were about 0.4, 0.3, 0.4, 0.3, 2 and $3 \mu\text{g} \cdot \text{L}^{-1}$, respectively. The concentrations found were within 95–102% of the reference values for all measured elements.

2.5. Ecotoxicity assessment

2.5.1. *Daphnia magna*

A first acute toxicity of leachates was performed on the water flea *D. magna* (Origin, less than 24 h old) according to [39]. Four replicates were tested for each soil solution and five neonates were used in each replicate, with 10 mL of test solution. Organisms were fed 2 h before but not during the experiment. A parafilm strip was then put on the multiwell plate placed in the incubator at 20°C in darkness. The mobility of *D. magna* was recorded after 24 h and 48 h, and inhibition rate was calculated.

2.5.2. Microtox®

First used to assess acute ecotoxicity of metals in aquatic media [40] and normalized since 2007 [41], solid-phase Microtox® test is now currently used to evaluate the toxicity of contaminated soils or sediments [4,42,43]. The Microtox test measures the decrease in light emitted by the bioluminescent bacteria *Vibrio fischeri* after 5, 15 and 30 min of exposure [43]. In view of evaluating toxicity of collected soils, the Microtox 81.9% Basic Test with the instrument MICROTOX M 500 purchased from R-Biopharm (France) was used. 100 μL of revitalized bacteria (Lot 10J1010A) were added to each Microtox® tube, gently mixed with a pipette. 900 μL of each leachate was transferred into the glass cuvette in the Microtox® analyzer and allowed to equilibrate for 5 min before reading [44].

Light emission was recorded and the output data analyzed using Microtox® Omni software Version 1.18 [45]. All samples were tested in triplicate. Bacteria validity and the set-up of the measurement procedures were verified by reference toxin ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) according to the ISO 11348 regulation specifications [41].

2.5.3. Induction of bioluminescent bacteria

Many bacterial sensors are dedicated to the specific detection of pollutants or pollutant family. These used bio-elements are bacterial strains (*Escherichia coli*) genetically modified. In all cases, reporter genes *lux CDABE* are cloned downstream of a promoter allowing to highlight the specific or semi-specific presence for certain compounds in a sample [46,47]. In the case of metal detection, the promoters used are mostly involved in the mechanisms of bacterial resistance to heavy metals [48–50]. A set of five bioluminescent bacteria namely *E. coli* Taclux, *E. coli* Zntlux, *E. coli* Arslux, *E. coli* Coplux and *E. coli* Merlux was used in this study (Table 1). Bacterial growth and lyophilization were realized according to Jouanneau et al. [24]. At the beginning of the bioassay, the lyophilized bacteria were reconstituted with 100 μL per well of distilled water for 30 min at $+30^\circ\text{C}$. 25 μL of leachate was added to each well, and afterward the microplate was incubated for 60 min at $+30^\circ\text{C}$. Monitoring of bioluminescence was recorded using a microplate luminometer (Microumat Plus Lb96V). The results were expressed by the logarithm of the induction ratio or the inhibition rate for the inducible strains and the constitutive strain, respectively. The induction ratio (IR) was calculated as follows:

- $\text{IR} = (\text{RLU} \cdot \text{s}^{-1})_{i\text{-IR}} / (\text{RLU} \cdot \text{s}^{-1})_{0\text{-IR}}$. $(\text{RLU} \cdot \text{s}^{-1})_{i\text{-IR}}$ is the bioluminescence after induction with a sample, and $(\text{RLU} \cdot \text{s}^{-1})_{0\text{-IR}}$ is the background luminescence. The inhibition rate (InR) was calculated as follows:

- $\text{InR} = 1 - (\text{RLU} \cdot \text{s}^{-1})_{i\text{-InR}} / (\text{RLU} \cdot \text{s}^{-1})_{0\text{-InR}}$. $(\text{RLU} \cdot \text{s}^{-1})_{i\text{-InR}}$ is the bioluminescence after exposure with a sample, and $(\text{RLU} \cdot \text{s}^{-1})_{0\text{-InR}}$ is the background luminescence.

Table 1
Observed MTE and detection limits for the different *E. coli* strains [24].

MTE	Detection limit of bacterial strains (standard deviation) (μM)			
	Zntlux	Arslux	Merlux	Coplux
Cd	0.0045 (0.0003)	5.9 (2.3)	0.011 (0.002)	-
Hg	0.01 (0.005)	-	1.2×10^{-7} (1×10^{-7})	-
As ^(III)	28.52 (7.1)	0.256 (0.0014)	15.6 (4.3)	-
Cu	16.92 (2.9)	-	-	90.5 (11.7)
Pb	2.2 (0.6)	4.16 (0.8)	-	-
Sn	12.95 (4.24)	-	-	-
As ^(V)	9.32 (1.24)	0.3 (0.06)	12.65 (5.4)	-
Zn	1.7 (0.62)	-	2.3 (0.14)	-
Ni	4.4 (1.6)	-	-	-
Co	0.22 (0.014)	-	-	-
Cr ^(VI)	597.2 (121.3)	-	-	-
Ag	-	-	-	2.75 (0.11)
Fe	4.34 (0.48)	-	16.1 (7.6)	-
Mn	-	-	-	-

Decision trees were designed from the learning set of bacterial bioluminescence data using the software "Metalsoft". They are specific to only one compound and organized in several binary branches. Each branch splits data according to the values of only one variable: induction ration or inhibition rate obtained from one strain. The process continues until the target value is obtained [24].

2.6. Statistical analysis

All tests were performed in triplicate and results are presented as mean \pm SD (standard deviation). The statistical significance of values was checked using an analysis of variance (ANOVA) using the Statistica 9.0 package software. Each MTE-extracted concentration (both by water and CaCl_2 procedures) was compared to respective total concentration. Significant differences (p -value < 0.05) were measured by the LSD Fisher test.

3. Results

3.1. Physico-chemical characteristics

Soil properties are reported in Table 2. These physicochemical characteristics significantly differ in function of sample origin: it means localization on the industrial site in relation with process. pH value varied between 6.9 and 9.2, CEC value varies between 2.6 and 10.5 $\text{cmol}(+)\text{kg}^{-1}$ and amounts of soil organic matter and carbonates (CaCO_3) were highly variable, respectively, from 0.9 to 46.7 g kg^{-1} and from 0 to 15.0 g kg^{-1} . MTE concentrations in polluted soil samples (Table 3) were also very heterogeneous: maximum lead concentration is 42,400 mg Pb kg^{-1} and other elements are also present at high levels (up to 2095 mg Sb kg^{-1} , 288 mg As kg^{-1} , 286 mg Cu kg^{-1} , 294 mg Zn kg^{-1} and 80.9 mg Cd kg^{-1}). All these concentrations were clearly above the national geochemical background as shown in Table 3.

3.2. Chemical extractions

MTE (Pb, As, Cu, Cd, Zn and Sb) concentrations were measured for all the solutions obtained by performing the three extractions (*aqua regia*, water and CaCl_2) on the studied polluted soil samples (Tables 3–5; Figs. 2 and 3). Leached MTE amounts in water and corresponding ratios (in comparison with *aqua regia* extraction, considered as "total") were significantly depending on element nature (Table 4a and Fig. 2). The highest extracted concentrations were recorded for lead, antimony and zinc (MTE with high total concentrations), respectively, 152.5, 158 and 9 mg kg^{-1} , i.e. 8.7%, 7.3% and 7.9%. In comparison, copper (at equivalent content) was significantly less extracted than zinc. Although quantitatively

low extracted ($\leq 5.4 \text{ mg Cd kg}^{-1}$), Cd was proportionally one of the most water-soluble element (up to 15.9% for S_6). Arsenic was the less extracted MTE with a maximum concentration reached of 2.2 mg As kg^{-1} (7.5% of the total for S_4).

CaCl_2 extractions results (Table 4b and Fig. 3) showed several contrasted behaviours depending both on chemical element and soil properties. The highest lead quantities extracted by CaCl_2 were observed for S_1 , S_3 and S_7 (up to 178.5 mg kg^{-1}). However, for S_4 sample with high total lead concentration, the extracted fraction is low (1.3 mg kg^{-1}). Conversely, antimony extracted concentration reached 306.6 mg Sb kg^{-1} . Other MTE showed a low extractability (in terms of quantity and ratio) whatever the sample, except

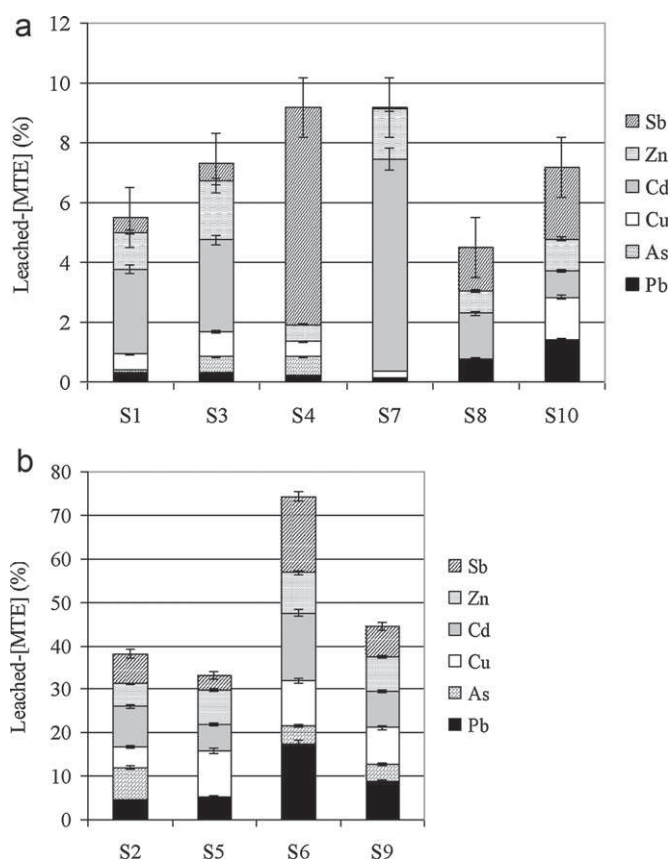


Fig. 2. Ratios (%) between MTE leached according to the EN 12457-2 procedure and *aqua regia* extraction, for the 10 soil samples: (a): sum of the ratios of leached-concentrations (in %) $< 10\%$ and (b) sum of the ratios of leached-concentrations (in %) $> 30\%$.

Table 2
Main physicochemical characteristics of the ten soil samples.

Sample	pH water	Organic matter (g kg ⁻¹)	CEC (cmol(+) kg ⁻¹)	Limestone CaCO ₃ (g kg ⁻¹)	Granulometry (%)				
					Clay	Thin silt	Rough silt	Thin sand	Rough sand
S ₁	7.0	31.4	8.9	8.0	13.3	18.3	14.2	15.7	38.5
S ₂	8.7	9.6	3.7	7.0	3.6	10.7	10.9	19.7	55.1
S ₃	6.7	14.0	6.0	0.0	9.2	13.7	12.6	19.7	44.8
S ₄	8.7	12.3	8.9	15.0	7.8	12.1	11.4	21.3	47.3
S ₅	9.2	0.9	2.6	7.0	2.7	4.3	4.8	2.9	85.3
S ₆	9.0	1.6	3.3	4.0	4.3	8.1	6.7	15.5	65.4
S ₇	6.9	46.7	10.5	0.0	6.7	9.4	8.7	21.6	53.5
S ₈	7.5	3.4	3.3	4.0	3.3	5.9	4.1	8.6	78.0
S ₉	8.5	6.0	6.9	4.0	10.4	17.3	11.2	19.5	41.6
S ₁₀	8.9	1.3	3.3	8.0	1.5	5.6	4.4	7.3	81.2

Table 3
Aqua regia MTE concentrations for the 10 soil samples (mineralization according to [29]).

MTE concentrations (mg kg ⁻¹)	NGB ^a	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀
Pb	9–50	39,800	1425	42,400	37,250	1020	297	35,700	1445	1750	1065
As	1–25	288	28.7	51.5	52.5	5.8	9.3	34.3	8.65	13.3	10.2
Cu	2–20	286	14.7	143.5	116	13.6	16.1	249	19.4	59.5	60.5
Cd	0.05–0.45	18.4	2.24	34.3	4.15	3.39	0.69	80.9	3.39	4.7	11.3
Zn	10–100	294	37.1	216	218	42.8	41.9	545	55	116	94
Sb	0.2–10	2095	53.5	1555	2175	23.5	13.1	1955	15.9	44.5	9.15

^a NGB: Natural Geochemical Background in France.

Table 4
Leached and phyto-available MTE concentrations (mg kg⁻¹) in the ten polluted soil samples.

Leached concentrations (mg kg ⁻¹)	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
(a) Leached TE concentrations (mg kg ⁻¹)											
Pb	127.8	63.0	126.2	85.6	53.4	51.8	51.7	11.1	152.5	14.9	
As	0.29	2.17	0.28	0.33	nd ^a	0.38	nd	nd	0.54	nd	
Cu	1.50	0.71	1.20	0.57	1.44	1.67	0.52	nd	5.04	0.87	
Cd	0.52	0.21	1.06	nd	0.21	0.11	5.75	0.05	0.39	0.10	
Zn	3.71	1.90	4.19	1.26	3.32	3.86	9.04	0.41	9.13	1.00	
Sb	10.0	3.69	9.63	158.2	0.81	2.30	1.64	0.23	3.13	0.22	
CaCl ₂ -extracted concentrations (mg kg ⁻¹)											
(b) Phyto-available MTE concentrations assessed by the CaCl ₂ procedure											
Pb	178.5	0.71	162.9	1.30	0.52	0.60	89.4	14.7	0.31	3.64	
As	nd	nd	nd	0.17	nd	nd	nd	nd	0.04	0.01	
Cu	0.43	0.20	0.37	0.38	0.19	0.18	0.32	0.21	0.29	0.64	
Cd	3.16	0.20	4.16	0.22	0.31	0.22	20.1	0.68	0.22	0.24	
Zn	2.77	nd	3.51	0.06	0.20	nd	25.6	0.44	0.08	0.10	
Sb	1.54	0.48	1.56	306.6	0.16	0.28	0.77	0.08	0.35	0.11	

^a nd: not detected.

for S₇ which registered pronounced pools of Cd and Zn associated with high total concentrations. Fig. 3a and b shows the fraction of the extracted element in relation to total concentration. Cadmium appeared as the most potentially phytoavailable element. Sb and Zn also represented high extracted fractions, respectively, for S₄ and S₇. Moreover, compared to the *aqua regia* fraction, the CaCl₂ fraction remained lower, except for the most potentially phytoavailable Cd element (2–32%).

3.3. Ecotoxicity tests

Ecotoxicity of leachates measured by the inhibition of *D. magna* mobility was highly variable (Table 5). Whatever the sample tested, the inhibition of daphnia mobility increased between 24 h and 48 h, except for S₃ whose inhibition was near 100% after only 24 h. Ecotoxicity was also maximal (i.e. 100%) for S₁, S₂, S₇ and S₈ after 48 h; while the lower inhibition was observed for S₅ (15%). Ecotoxicity

Table 5
Results of the *Daphnia magna* and Microtox[®] tests for the 10 leachates.

Ecotoxicity test	Sample										
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
<i>D. magna</i> ^a	24 h	90	35	100	40	10	25	80	65	45	5
	48 h	100	100	100	65	15	80	100	100	65	70
Microtox [®] ^b	5 min	29	nt ^c	nt	nt	nt	nt	63	nt	nt	nt
	15 min	78.5	nt	nt	12	nt	nt	92	nt	nt	nt
	30 min	93	nt	34	34	nt	nt	96	nt	nt	nt

^a Inhibition of mobility (%).

^b Inhibition of bioluminescence (%).

^c nt: not toxic.

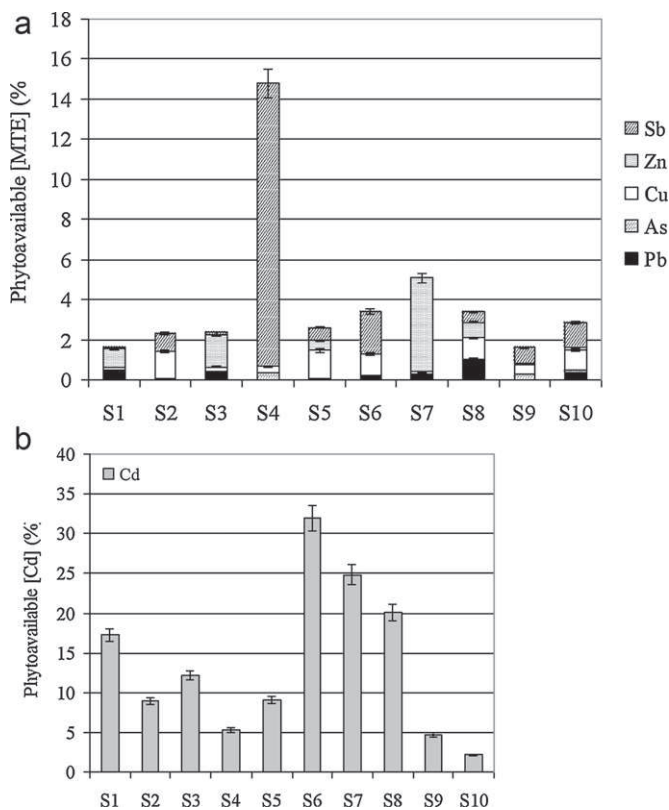


Fig. 3. Ratios (%) between MTE extracted by CaCl_2 and *aqua regia*, for the 10 soil samples. (a) Pb, Sb, As, Zn and Cu and (b) Cd.

was not simply dependant of MTE concentration: (i) the most MTE-enriched leachates were not always the more toxicant; (ii) leachate of S_8 had low MTE concentrations while the inhibition of daphnia mobility was 100%.

The mean $\text{EC}_{50-30 \text{ min}}$ value obtained for zinc sulphate heptahydrate (expressed as Zn^{2+}) was 2.38 mg L^{-1} , allowing concluding that the invertebrates lot fulfilled the validation specifications according to [34]. Microtox[®] test results (Table 5) showed an increase in the number of toxic samples with the contact time: inhibition of bioluminescence was detected in two samples at the beginning of the experiment and for four of them at the end (S_1 and S_7 to 5 min; S_1 , S_4 and S_7 to 15 min; S_1 , S_7 , S_4 and S_3 at 30 min). The measured ecotoxicity also increased over time and was above 90% for S_1 and S_7 after 30 min of contact. The bacteria were most affected by S_7 with an inhibition of the luminescence of 63% (5 min). Unlike the test on *Daphnia*, S_2 , S_6 , S_8 , S_9 and S_{10} showed no toxicity, as S_5 in both bioassays. As described above, S_1 , S_3 , S_4 and S_7 are among the most contaminated leachates ($[\text{Pb}] > 80 \text{ mg kg}^{-1}$; $[\text{Sb}] > 10 \text{ mg kg}^{-1}$ (except S_7)) (Table 4a).

The sensitivity and specificity of inducible bacteria were measured after 60 min in contact with leachates. None of the sample induced the luminescence of Coplux strain. Only two samples, S_1 and S_7 , showed a slight toxicity as demonstrated by the inhibition of luminescence of the constitutive strain pBtaclux (Table 6a).

These samples also induced the luminescence of Zntlux, Arslux and Merlux strains. For S_1 and S_7 the maximum IR was recorded for Arslux (IR=99.6) and Merlux (IR=138.1), respectively. S_3 increased moderately the luminescence of Merlux (IR=12.9) and Zntlux (IR=4.0) while S_2 induced only Arslux (IR=324.9). The analysis with decision trees was then used to determine the elements potentially responsible for ecotoxicity of S_1 , S_2 , S_3 and S_7 . Crosses between results suggested the presence of arsenic in these four leachates (up to 10^{-5} M , i.e. more than by chemical

analysis), cadmium for S_1 , S_3 and S_7 , biologically at levels lower than those measured chemically (Table 6b). Analysis of S_7 also showed the presence of copper and mercury (from 10^{-4} to 10^{-5} M and 10^{-9} to 10^{-5} M). According to the previous tests, these results also concluded to the ecotoxicity of S_1 and S_7 , and, to a minor extent, the ecotoxicity of S_3 and S_4 .

4. Discussion

4.1. Mobility and phytoavailability of MTE

In this study, soil pH were basic or close to neutral conditions and leaching procedure slightly reduced the pH by water addition. Conversely, CaCl_2 is already known to not modify soil pH and give results closer from field reality [15]. Thus, hazard proposed classification of polluted soils differs between water leaching and CaCl_2 procedures. Several studies already showed that MTE extractability is strongly influenced by the nature of the extracting agent, which can control element mobility [15,16]. Moreover, according to Dumat et al. [51] or Ferrari et al. [52], solid-liquid MTE transfers during chemical extractions are complex reactions involving numerous factors that can influence MTE speciation and release. Contact times chosen for chemical extractions were 24 h for water and only 2 h for CaCl_2 in accordance with the commonly used protocols: these two procedures carry complementary information but the results are not directly comparable.

All MTE were extracted in substantially equal proportions with water (from 0 to 18%); MTE concentrations in CaCl_2 extracts and corresponding ratios, varied in the range of those reported in the literature [5,58,61], and Cd was the most available element (up to 32%). Extracted concentrations were strongly correlated to total concentrations: $R^2 = 0.92$ and 0.95 ($p < 0.0001$), respectively, for water and CaCl_2 . At the reverse side, for all the other elements no relevant correlation was found between total and extracted fractions. In agreement with previous publications [5,15], these results highlighted the influence of soil properties and MTE nature on its behaviour. Differences observed in function of MTE nature can be explained by different OM or CaCO_3 soil contents, CEC or soil pH. In soils, cadmium is generally easy to dissolve which explain its relatively high extractability [53]. High correlation factors were observed between exchangeable Cu and Zn fractions and soil organic matter amount: $R^2 = 0.82$ for Cu ($p < 0.05$) and $R^2 = 0.91$ for Zn ($p < 0.001$). These elements were thus less mobile because of their affinity for this soil fraction [11,54]. Concerning lead behaviour, no relationship was found between extracted and total concentrations, and the influence of even one soil parameter was difficult to highlight. Nevertheless, low extraction ratios compared to the most concentrated samples (S_1 , S_3 , S_4 and S_7) can be explained by stronger bounds on soil phases as mineral fraction [29]. Finally, sorption of metalloids as As and Sb, is mainly controlled by mineral phases [55]. The high Sb amount extracted from S_4 could be explained not only by a higher total concentration but also by the highest CaCO_3 content [56]. Sb could be solubilized under the influence of soil bio-physico-chemical parameters controlling its sorption [57–59]. pH and CEC were already described as influent parameters of element extractability [60], retention and mobility in soils [11]. Thus, according to the origin of soil sampling, differences in soil parameters were observed (Fig. 1): in the industrial site, areas not covered or infiltration zones were the most impacted by MTE. Their organic matter content and CEC were also the higher, thus confirming their role in sorption/desorption mechanisms. The choice of the extractant is thereby an important step to be relevant in risk assessment and to avoid an under- or over-estimation of phytotoxicity. Finally, the data obtained by chemical tests are difficult to interpret because of the many parameters interact. The realization of ecotoxicity tests to measure the impact of

Table 6
Ecotoxicity results of bioluminescence emitted by the bacterial strains.

Sample	Bacterial strain							
	ZntLux ^a	Arslux ^a	Merlux ^a	pBtaclux ^b				
(a) Induction factor and inhibition rate calculated from the bioluminescence								
S ₁	4.8	99.6	9.9	6.5				
S ₂	0.9	324.9	0.8	–				
S ₃	4.0	1.2	12.9	–				
S ₄	1.4	0.8	0.9	–				
S ₅	0.7	0.9	1.0	–				
S ₆	0.6	0.8	0.9	–				
S ₇	5.4	79.8	138.1	18.4				
S ₈	1.2	0.9	0.9	–				
S ₉	1.3	2.1	0.8	–				
S ₁₀	1.2	1.2	0.8	–				
Sample	Chemical analysis				Biological analysis (prediction with decision trees)			
	Cd	As	Cu	Hg	Cd	As	Cu	Hg
(b) Comparison between MTE leached water-soluble concentrations and range “biologically” detected by the bacterial strains (unit: M)								
S ₁	4.5 × 10 ⁻⁷	3.7 × 10 ⁻⁷	2.3 × 10 ⁻⁶	–	10 ⁻⁸ to 10 ⁻⁷	10 ⁻⁶ to 10 ⁻⁵	–	–
S ₂	1.8 × 10 ⁻⁷	29.7 × 10 ⁻⁷	1.1 × 10 ⁻⁶	–	–	10 ⁻⁶ to 10 ⁻⁵	–	–
S ₃	9.1 × 10 ⁻⁷	3.6 × 10 ⁻⁷	1.8 × 10 ⁻⁶	–	10 ⁻⁸ to 10 ⁻⁷	< 10 ⁻⁶	–	–
S ₇	48.9 × 10 ⁻⁷	3.3 × 10 ⁻⁷	0.8 × 10 ⁻⁶	–	10 ⁻⁸ to 10 ⁻⁷	10 ⁻⁶ to 10 ⁻⁵	10 ⁻⁴ to 10 ⁻⁵	10 ⁻⁹ to 10 ⁻⁵

^a IF: induction factor.
^b Inhibition rate (%).

pollution on ecosystems seems therefore particularly appropriate in this type of study.

4.2. Relevance of ecotoxicity tests for risk assessment posed by landfilling

The *D. magna* ecotoxicity test was more sensitive to MTE impact than the Microtox[®] test. But, unlike tests on the different bacterial strains, they do not both provide information on MTE quantification. Ecotoxicity differences were measured for some samples, especially S₂ and S₈. These differences can be firstly explained by water flea sensitivity. Detection capabilities of the ecotoxicity of the leachate are actually dependent on the test used [62,63] and it has been already shown that *V. fischeri* was generally less sensitive than *D. magna* [10,64]. Instead of these tests, experiments by using

bacterial strains allowed to determine and quantify the element which was potentially bioavailable and/or toxic for bacteria [50]. Then, response in ecotoxicity tests was not always directly correlated with total or water-soluble concentrations [65]. These results are in agreement with data previously obtained by Plaza et al. [14] concerning the influence of pH and CEC on MTE behaviour in soils.

Results of this study have shown that this new bioassay enables the screening of samples in terms of environmental risk during remediation process [24]. However, the drawback of the lack of specificity of one strain and the effect of a mixture of MTE (synergistic or antagonistic effects) could be overcome by using a panel of bacterial strains coupled with a predictive model [24]. Due to the lack of specific bacteria for lead, the introduction of other strains induced by lead like *Rastonia Metallidurans* AE 1433 [66] could improve the interpretation of the data.

Table 7
Hazard classification, adapted from [10,60].

Sample	Score						Class ^a	MCW ^b	W ^c	PW ^d
	<i>D. magna</i>	Microtox	Zntlux	Arslux	Merlux	pBtaclux				
S ₁	4	3	1	3	2	2	V	4	2.5	62.5
S ₂	4	0	0	4	0	0	V	4	1.3	33.3
S ₃	4	1	1	1	2	0	V	4	1.5	37.5
S ₄	2	1	1	0	0	0	III	2	0.7	33.3
S ₅	0	0	0	0	1	0	II	1	0.2	16.7
S ₆	3	0	0	0	0	0	IV	3	0.5	16.7
S ₇	4	3	2	3	4	2	V	4	3.0	75.0
S ₈	4	0	1	0	0	0	V	4	0.8	20.8
S ₉	2	0	1	1	0	0	III	2	0.7	33.3
S ₁₀	2	0	1	1	0	0	III	2	0.7	33.3

^a

Class	MCW	
I No acute toxicity	PE < 20% IR/lnR < 1	0
II Slight acute toxicity	20% ≤ PE < 50% 1 ≤ IR/lnR < 5	1
III Acute toxicity	50% ≤ PE < 75% 5 ≤ IR/lnR < 50	2
IV High acute toxicity	75% ≤ PE < 100% 50 ≤ IR/lnR < 100	3
V Very high acute toxicity	PE ≥ 100% IR/lnR ≥ 100	4

^b MCW: maximum class weight score.
^c W: class weight score.
^d PW: class weight score in percent.

1.1. Hazard classification according to ecotoxicity

According to Persoone et al. [67] and Matejczyk et al. [10], the samples were ranked into one of five classes on the basis of the percentage effect (PE) found in *Daphnia* and Microtox® tests. Ranking was based on induction/inhibition rates for bacterial strains. A weight score was calculated for each hazard class to indicate the quantitative importance (weight) of the ecotoxicity in that class. The weight score was expressed as percentage.

$$\text{Class weight score} = \frac{\sum \text{all test scores}}{\text{number of tests performed} (= 6)} \quad (1)$$

$$\text{Class weight score (\%)} = \frac{\text{class weight score}}{\text{maximum class weight score}} \times 100 \quad (2)$$

That classification system aimed at the integration of ecotoxicity data obtained in a battery of bioassays as describe by Lors et al. [68]. The classification system is based on two values: a ranking in five acute toxicity classes and a weight score for each toxicity class. The classification of the samples tested in the investigation is reported in Table 7. Samples were classified as slightly and highly toxic in 10%, toxic in 30%, and very highly toxic in 50%. The percentage of class weight class was above 5% for only S₇ and S₁ (75% and 62.5%, respectively). These samples were definitely considered as the most hazardous and acutely toxic to the microfauna. The final classification of ecotoxicity risks was S₇ > S₁ > S₃ > S₂ = S₄ = S₉ = S₁₀ > S₈ > S₅ = S₆. Although the toxicity of some samples (S₂ and S₈, for instance) could be different depending on the test used, the ranking based on total concentrations and leachable contents of MTE was almost the same; samples S₁, S₃, S₄ and S₇ presenting the greatest risks while the less contaminated areas were generally the less hazardous. However, as our results demonstrated that only a small fraction of total MTE soil concentrations can be solubilized and phytoavailable; ecotoxicity measures complete therefore efficiently standard performed tests for a realistic risks assessment of MTE-contaminated soils accordingly to [10,14]. Moreover, the use of eco-scores improves the comparisons between bioassays and suggests the use of a restricted battery of tests to perform a cost-effective risk assessment of MTE-contaminated soils.

2. Conclusion

Biotests and eco-scores improve standard tests performed to assess risk on ecosystems induced by polluted soils. In particular, modified bacteria strains sensitive to metals are useful tools highlighting the presence of different MTE and the influence of soil parameters that lead to synergistic or antagonistic effects. Moreover, eco-scores calculation allows an easy and cheap screening of a large number of polluted soil samples and suggests a restricted battery of bioassays to perform a cost-effective risk assessment.

Nevertheless, further researches are required to develop a panel of bacterial strains specific of each MTE. Moreover, the chemical characterization of leachates could provide additional relevant insight with the use of modelling software for determination of MTE speciation and thus to evaluate the predominant parameter influencing soil availability and ecotoxicity. Finally, the procedure developed in the present work could be used in the context of bioremediation techniques like phytoremediation performed on polluted soils, to assessment their efficiency and favour their use in a context of sustainable development.

Acknowledgment

ANRT (National Agency for Research and Technology) and STCM (Metal Chemical Treatments Society) are acknowledged for their financial support and technical help.

References

- [1] Y. Foucault, E. Schreck, T. Levêque, P. Pradère, C. Dumat, Toward rational management of excavated soil contaminated by metal trace elements, *Environ. Risk Health* 11 (2012) 61–66.
- [2] P.A.W. Van Hees, K. Elgh-Dalgren, M. Engwall, T. Von Kronhelm, Re-cycling of remediated soil in Sweden: an environmental advantage? *Resour. Conserv. Recy.* 52 (12) (2008) 1349–1361.
- [3] S. Colombano, A. Saada, V. Guerin, P. Bataillard, G. Bellenfant, S. Beranger, D. Hube, C. Blanc, C. Zornig, I. Girardeau, Quelles techniques pour quels traitements – analyse coûts-bénéfices, BRGM-RP-58609-FR, 2010.
- [4] E. Schreck, Y. Foucault, F. Geret, P. Pradère, C. Dumat, Influence of soil ageing on bioavailability and ecotoxicity of lead carried by process waste metallic ultrafine particles, *Chemosphere* 85 (10) (2011) 1555–1562.
- [5] G. Uzu, S. Sobanska, P. Pradère, C. Dumat, Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation, *Environ. Pollut.* 157 (4) (2009) 1178–1185.
- [6] X.S. Luo, S. Yu, X.D. Li, Distribution, availability, and sources of trace metals in different particle size fractions of urban soils in Hong Kong: implications for assessing the risk to human health, *Environ. Pollut.* 159 (2011) 1317–1326.
- [7] C. Fritsch, P. Giraudoux, M. Coeurdassier, F. Douay, F. Raoul, C. Pruvot, C. Waterlot, A. de Vaulfleur, R. Scheffler, Spatial distribution of metals in smelter-impacted soils of woody habitats: influence of landscape and soil properties, and risk for wildlife, *Chemosphere* 81 (2010) 141–155.
- [8] F. Vega, M. Andrade, E. Covelo, Influence of soil properties on the sorption and retention of cadmium, copper and lead, separately and together, by 20 soil horizons: comparison of linear regression and tree regression analyses, *J. Hazard. Mater.* 174 (1–3) (2010) 522–533.
- [9] M. Sammut, Y. Noack, J. Rose, J. Hazemann, O. Proux, M. Depoux Ziebel, E. Fiani, Speciation of Cd and Pb in dust emitted from sinter plant, *Chemosphere* 78 (4) (2010) 445–450.
- [10] M. Matejczyk, G.A. Plaza, G. Nałęcz-Jawecki, K. Ulfig, A. Markowska-Szczupak, Estimation of the environmental risk posed by landfills using chemical, microbiological and ecotoxicological testing of leachates, *Chemosphere* 82 (2011) 1017–1023.
- [11] M.A. Kashem, B.R. Singh, T. Kondo, S.M. Imamul Huq, S. Kawai, Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils, *Int. J. Environ. Sci. Technol.* 4 (2) (2007) 169–176.
- [12] CEN 14735, Characterization of Waste. Preparation of Waste Samples for Ecotoxicity Tests, European Committee for Standardization, Brussels, 2006, 47 pp.
- [13] I. Buj, J. Torras, M. Rovira, J. de Pablo, Leaching behaviour of magnesium phosphate cements containing high quantities of heavy metals, *J. Hazard. Mater.* 175 (1–3) (2010) 789–794.
- [14] G.A. Plaza, G. Nałęcz-Jawecki, O. Pinyakong, P. Illmer, R. Margesin, Ecotoxicological and microbiological characterization of soils from heavy-metal and hydrocarbon-contaminated sites, *Environ. Monit. Assess.* 163 (2010) 477–488.
- [15] E. Meers, R. Samson, F.M.G. Tack, A. Ruttens, M. Vandegheuchte, J. Vangronsveld, M.G. Verloo, Phytoavailability assessment of heavy metals in soils by single extractions and accumulation by *Phaseolus vulgaris*, *Environ. Exp. Bot.* 60 (3) (2007) 385–396.
- [16] N.W. Menzies, M.J. Donn, P.M. Kopitke, Evaluation of extractants for estimation of the phytoavailable trace metals in soils, *Environ. Pollut.* 145 (1) (2007) 121–130.
- [17] M. Arshad, J. Silvestre, E. Pinelli, J. Kallerhoff, M. Kaemmerer, A. Tarigo, M. Shahid, M. Guiesse, P. Pradère, C. Dumat, A field study of lead phytoextraction by various scented *Pelargonium* cultivars, *Chemosphere* 71 (11) (2008) 2187–2192.
- [18] J. Römbke, A.M. Breure, C. Mulder, M. Rutgers, Legislation and ecological quality assessment of soil: implementation of ecological indication systems in Europe, *Ecotoxicol. Environ. Saf.* 62 (2005) 201–210.
- [19] S.A. Mansour, M.F. Gad, Risk assessment of pesticides and heavy metals contaminants in vegetables: a novel bioassay method using *Daphnia magna* Straus, *Food Chem. Toxicol.* 48 (1) (2010) 377–389.
- [20] K.R. Kim, G. Owens, Potential for enhanced phytoremediation of landfills using biosolids – a review, *J. Environ. Manage.* 91 (2010) 791–797.
- [21] L. Leitgib, J. Kalman, K.K. Gruiz, Comparison of bioassays by testing whole soil and their water extract from contaminated sites, *Chemosphere* 66 (3) (2007) 428–434.
- [22] M.J. Durand, G. Thouand, T. Dancheva-Ivanova, P. Vachon, M. DuBow, Specific detection of organotin compounds with a recombinant luminescent bacteria, *Chemosphere* 52 (1) (2003) 103–111.
- [23] T. Elad, E. Benovich, S. Magrisso, S. Belkin, Toxicant identification by a luminescent bacterial bioreporter panel: application of pattern classification algorithms, *Environ. Sci. Technol.* 42 (22) (2008) 8486–8491.
- [24] S. Jouanneau, M.J. Durand, P. Courcoux, T. Blusseau, G. Thouand, Improvement of the identification of four heavy metals in environmental samples by using predictive decision tree models coupled with a set of five bioluminescent Bacteria, *Environ. Sci. Technol.* 45 (7) (2011) 2925–2931.

- [25] J.M. Ritchie, M. Cresser, J. Cotter-Howells, Toxicological response of a bioluminescent microbial assay to Zn, Pb and Cu in an artificial soil solution: relationship with total metal concentrations and free ions activities, *Environ. Pollut.* 114 (2001) 129–156.
- [26] A. Ivask, M. François, A. Kahru, H.C. Dubourguier, M. Virta, F. Douay, Recombinant luminescent bacterial sensors for the measurement of bioavailability of cadmium and lead in soils polluted by metal smelters, *Chemosphere* 55 (2004) 147–156.
- [27] P. Peltola, A. Ivask, M. Aström, M. Virta, Lead and Cu in contaminated urban soils: extraction with chemical reagents and bioluminescent bacteria and yeast, *Sci. Total Environ.* 350 (1–3) (2005) 194–203.
- [28] T. Xu, D.M. Close, G.S. Saylor, S. Ripp, Genetically modified whole-cell bioreporters for environmental assessment, *Ecol. Indic.* (2012), <http://dx.doi.org/10.1016/j.ecolind.2012.01.020>.
- [29] C.S.C. Wong, X. Li, I. Thornton, Urban environmental geochemistry of trace metals, *Environ. Pollut.* 142 (2006) 1–16.
- [30] G. Uzu, S. Sobanska, G. Sarret, J.J. Sauvain, P. Pradère, C. Dumat, Characterization of lead-recycling facility emissions at various workplaces: major insights for sanitary risk assessment, *J. Hazard. Mater.* 186 (2–3) (2011) 1018–1027.
- [31] ISO 10390, Soil Quality – Determination of pH, 2005.
- [32] ISO 10694, Soil Quality – Determination of Organic and Total Carbon After Dry Combustion (Elementary Analysis), 1995.
- [33] ISO 10693, Soil Quality – Determination of Carbonate Content – Volumetric Method, 1995.
- [34] NF X31-130, Qualité des sols – Méthodes chimiques – détermination de la capacité d'échange cationique (CEC) et des cations extractibles, 1999.
- [35] ISO 11277, Soil Quality – Determination of Particle Size Distribution in Mineral Soil Material – Method by Sieving and Sedimentation, 2009.
- [36] ISO 11466, Soil Quality – Extraction of Trace Elements Soluble in *aqua regia*, 1995.
- [37] CEN 12457-2, Characterisation of Waste. Leaching. Compliance Test for Leaching of Granular Waste Materials and Sludge. Part 2. One Stage Batch Test at a Liquid-to-Solid Ratio of 10l/kg for Materials with Particle Size Below 4 mm (Without or with Size Reduction), 2002.
- [38] V.J.G. Houba, T.M. Lexmond, I. Novozamsky, J.J. Van Der Lee, State of the art and future developments in soil analysis for bioavailability assessment, *Sci. Total Environ.* 178 (1–3) (1996) 21–28.
- [39] ISO 6341, Water Quality – Determination of the Inhibition of the Mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute Toxicity Test, 1996.
- [40] D. De Zwart, W. Slooff, The Microtox as an alternative assay in the acute toxicity assessment of water pollutants, *Aquat. Toxicol.* 4 (1983) 129–138.
- [41] ISO 11348-2, Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of *Vibrio fischeri* (Luminescent Bacteria Test), Part 2. Method Using Liquid-Dried Bacteria, 2007.
- [42] R. Doshi, W. Braidá, C. Christodoulatos, M. Wazne, G. O'Connor, Nanoaluminium: transport through sand columns and environmental effects on plant and soil communities, *Environ. Res.* 106 (3) (2008) 296–303.
- [43] R. Barrena, E. Casals, J. Colón, X. Font, A. Sánchez, V. Puentes, Evaluation of the ecotoxicity of model nanoparticles, *Chemosphere* 75 (7) (2009) 850–857.
- [44] Y. Mamindy-Pajany, F. Geret, M. Roméo, C. Hurel, N. Marmier, Ex situ remediation of contaminated sediments using mineral additives: assessment of pollutant bioavailability with the Microtox solid phase test, *Chemosphere* 86 (11) (2012) 1112–1116.
- [45] Azur Environmental, Microtox Acute Toxicity Test Guide, Users Manual, Carlsbad, USA, 1998.
- [46] S.F. D'Souza, Microbial biosensors, *Biosens. Bioelectron.* 16 (6) (2001) 337–353.
- [47] Y. Lei, W. Chen, A. Mulchandani, Microbial biosensors, *Anal. Chim. Acta* 568 (1–2) (2006) 200–210.
- [48] N.L. Brown, Y.C. Shih, C. Leang, K.J. Glendinning, J.L. Hobman, J.R. Wilson, Mercury transport and resistance, *Biochem. Soc. Trans.* 30 (4) (2002) 715–718.
- [49] N. Verma, M. Singh, Biosensors for heavy metals, *Biometals* 18 (2) (2005) 121–129.
- [50] T. Charrier, M.J. Durand, M. Affi, S. Jouanneau, H. Gezekel, G. Thouand, Bacterial bioluminescent biosensor characterisation for on-line monitoring of heavy metals pollutions in waste water treatment plant effluents, in: P.A. Serra (Ed.), *Biosensors*, INTECH., 2010, pp. 179–206.
- [51] C. Dumat, K. Quenea, A. Bermond, S. Toinen, M. Benedetti, Study of the trace metal ion influence on the turnover of soil organic matter in cultivated contaminated soils, *Environ. Pollut.* 142 (3) (2006) 521–529.
- [52] B.J.D. Ferrari, J.F. Masfarau, A. Maul, J.F. Féraud, Predicting uncertainty in the ecotoxicological assessment of solid waste leachates, *Environ. Sci. Technol.* 40 (22) (2006) 7012–7017.
- [53] L. Beesley, E. Moreno-Jiménez Clemente, R.N. Lepp, N. Dickinson, Mobility of arsenic, cadmium and zinc in a multi-element contaminated soil profile assessed by in-situ soil pore water sampling, column leaching and sequential extraction, *Environ. Pollution* 158 (1) (2010) 155–160.
- [54] P.F.A.M. Romkens, H.Y. Guo, C.L. Chu, T.S. Liu, C.F. Chiang, G.F. Koopmans, Prediction of cadmium uptake by brown rice and derivation of soil-plant transfer models to improve soil protection guidelines, *Environ. Pollut.* 157 (8–9) (2009) 2435–2444.
- [55] S.C. Wilson, P.V. Lockwood, P.M. Ashley, M. Tighe, The chemistry and behavior of antimony in the soil environment with comparison to arsenic: a critical review, *Environ. Pollut.* 158 (5) (2010) 1169–1181.
- [56] S. Denys, K. Tack, J. Caboche, P. Delalain, Bioaccessibility, solid phase distribution, and speciation of Sb in soils and digestive fluids, *Chemosphere* 74 (2008) 711–716.
- [57] B. Collin, E. Doelsch, Impact of high natural soilborne heavy metal concentration on the mobility and phytoavailability of these elements for sugarcane, *Geoderma* 159 (3–4) (2010) 452–458.
- [58] S.M. Rodrigues, B. Henriques, E. Ferreira da Silva, M.E. Pereira, A.C. Duarte, J.E. Groenenberg, P.F.A.M. Römkens, Evaluation of an approach for the characterization of reactive and available pools of 20 potentially toxic elements in soils. Part II. Solid–solution partition relationships and ion activity in soil solutions, *Chemosphere* 81 (11) (2010) 1560–1570.
- [59] X.S. Luo, S. Yu, X.D. Li, The mobility, bioavailability, and human bioaccessibility of trace metals in urban soils of Hong Kong, *Appl. Geochem.* 27 (5) (2012) 995–1004.
- [60] J.S. Rieuwerts, M.R. Ashmore, M.E. Farago, I. Thornton, The influence of soil characteristics on the extractability of Cd, Pb and Zn in upland and moorland soils, *Sci. Total Environ.* 266 (2–3) (2006) 864–875.
- [61] A. Lopareva-Pohu, A. Verdin, G. Garçon, A. Lounès-Hadj Sahraoui, B. Pourrut, D. Debiane, C. Waterlot, F. Laruelle, G. Bidar, F. Douay, P. Shirali, Influence of fly ash aided phytostabilisation of Pb, Cd and Zn highly contaminated soils on Lolium perenne and Trifolium repens metal transfer and physiological stress, *Environ. Pollut.* 159 (6) (2011) 1721–1729.
- [62] M. Isidori, M. Lavorgna, A. Nardelli, A. Parrella, Toxicity identification evaluation of leachates from municipal waste landfills: a multispecies approach, *Chemosphere* 52 (1) (2003) 85–94.
- [63] L. Manusadzianas, K. Sadauskas, R. Vitkus, Comparative study of indices used in toxicity evaluation of effluents, *Desalination* 250 (1) (2010) 383–389.
- [64] R. Boluda, L. Roca-Pérez, L. Marimón, Soil plate bioassay: an effective method to determine ecotoxicological risks, *Chemosphere* 84 (1) (2011) 1–8.
- [65] E.E.V. Chapman, G. Dave, J.D. Murimboh, Ecotoxicological risk assessment of undisturbed metal contaminated soil at two remote lighthouse sites, *Ecotoxicol. Environ. Saf.* 73 (2010) 961–969.
- [66] P. Corbisier, E. Thiry, L. Diels, Bacterial biosensors for toxicity assessment of solid waste, *Environ. Toxicol. Water Qual.* 11 (1996) 171–177.
- [67] G. Persoone, B. Marsalek, I. Blinova, A. Torokne, D. Zarina, L. Manusadzianas, G. Nalecz-Jawecki, L. Tofan, N. Stepanova, L. Tothova, B. Kolar, A practical and user-friendly toxicity classification system with microbioassays for natural waters and wastewaters, *Environ. Toxicol.* 18 (6) (2003) 395–402.
- [68] C. Lors, J.F. Ponge, M. Martínez Aldaya, D. Damidot, Comparison of solid and liquid-phase bioassays using ecoscores to assess contaminated soils, *Environ. Pollut.* 159 (2011) 2974–2981.