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ECOTOXICITY TEST AND ECOSCORES TO IMPROVE POLLUTED SOILS MANAGEMENT: CASE OF A SECONDARY LEAD SMELTER PLANT.

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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, 8, 9]. According to Matejczyk et al. [10], chemical weathering of soil minerals favours MTE solubilisation 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, 13, 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? 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

Ten top soil samples were 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. This plant was chosen because of its activity and urban location, and many data are already available [1,4,5]. These data allowed defining different areas in terms of environmental and sanitary risks that can vary according to past and present activities. Samples were dried, sieved under 2 mm and treated in triplicate. pH, organic matter and limestone contents, cation exchange capacity and texture, were determined for all soil samples. Pb, As, Cu, Cd, Zn and Sb total concentrations were measured by ICP-OES after mineralization in *aqua regia*.

Normalized leaching test 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 < 4 mm according to the norm) was mixed with 100 mL deionised water during 24h with end-over-end agitation at 5 rpm. After centrifugation at 3,000 g during 15 min, the leachates were filtered with cellulose 0.45 μm

(Millipore®) filters. 10 mL of each leachates were then acidified with HNO_3 65% prior to analysis by ICP-OES. The other part of leachates was not acidified so as not to disturb microorganisms used for further ecotoxicological tests.

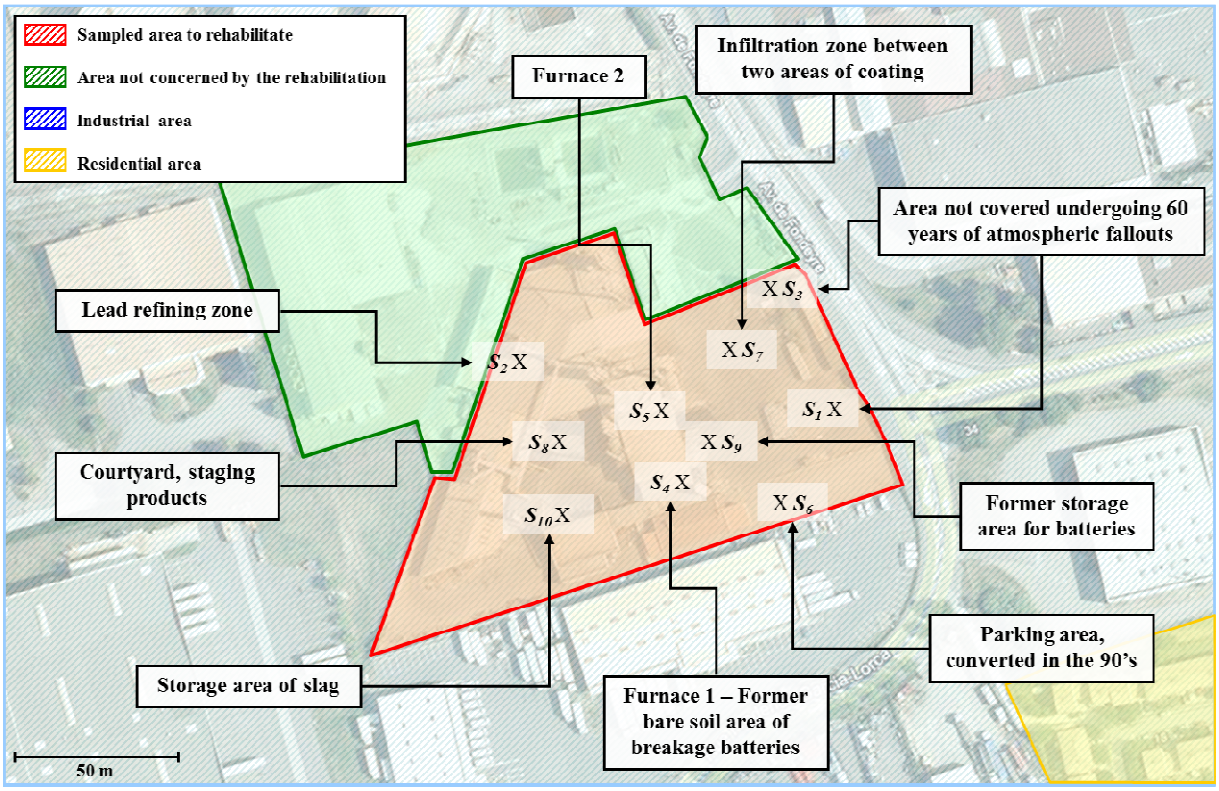

Figure 1 : Situation of the industrial study site, location and characteristics of the 10 sampling points.

Potentially phytoavailable MTE concentrations were estimated by $CaCl_2$ extractions according to Uzu et al. [5]. In 25 mL polypropylene centrifugation tubes, 10 mL of 10^{-2} M $CaCl_2$ were added to 1.0 g of soil. The liquid to solid ratio of 10 is high enough to avoid samples heterogeneities [25]. After agitation end-over-end during two hours 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 HNO_3 (15 N, suprapur 99.9%). MTE concentrations were finally measured by ICP-OES (IRIS Intrepid II XXDL, analytical errors < 5%).

Acute toxicity tests of leachates were performed on the water flea *Daphnia magna* according to ISO 6341 and with the Microtox® according to the ISO 11348 regulation specifications. Moreover, new bacterial strains which are dedicated to the specific detection of pollutants or pollutant family were used. Thus, 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. Monitoring of bioluminescence was recorded and the results were expressed by the logarithm of the induction ratio or the inhibition rate for the inducible strains and the constitutive strain respectively. Decision trees were designed from the learning set of bacterial bioluminescence data using the software "Metalsoft".

3. Results

Physico-chemical characteristics significantly differ in function of sample origin, i.e. their localization on the industrial site in relation with process. pH value varied between 6.9 to 9.2, CEC value varies between 2.6 to 10.5 cmol(+) 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 were also very heterogeneous: maximum lead concentration is 42,400 mgPb.kg^{-1} and other elements are

also present at high levels (up to 2095 mgSb kg^{-1}, 288 mgAs kg^{-1}, 286 mgCu kg^{-1}, 294 mgZn kg^{-1} and 80.9 mgCd kg^{-1}).

MTE (mg kg^{-1})	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
Pb	39800	1425	42400	37250	1020	297	35700	1445	1750	1065
As	288	28.7	51.5	52.5	5.8	9.3	34.3	8.65	13.3	10.2
Cu	286	14.7	143.5	116	13.6	16.1	249	19.4	59.5	60.5
Cd	18.4	2.24	34.3	4.15	3.39	0.69	80.9	3.39	4.7	11.3
Zn	294	37.1	216	218	42.8	41.9	545	55	116	94
Sb	2095	53.5	1555	2175	23.5	13.1	1955	15.9	44.5	9.15

Table 1: *Aqua regia* MTE concentrations for the 10 soil samples

Leached MTE amounts in water and corresponding ratios (in comparison with *aqua regia* extraction, considered as "total") were significantly depending on element nature (Table 2a). 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 (≤ 5.4 mgCd 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 mgAs kg^{-1} (7.5 % of the total for S_4).

$CaCl_2$ extractions results (Table 2b) 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 mgSb kg^{-1}. Other MTE showed a low extractability (in terms of quantity and ratio) whatever the sample, except for S_7 which registered pronounced pools of Cd and Zn associated with high total concentrations. Figures 3-a and 3-b show 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 S_4 and S_7. Moreover, compared to the *aqua regia* fraction, the $CaCl_2$ fraction remained lower, except for the most potentially phytoavailable Cd element (2–32 %).

	Leaching procedure	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Pb	*mg kg^{-1}*	127,8	63,04	126,2	85,62	53,44	51,79	51,66	11,13	152,5	14,86
	% of the total	0,32	4,42	0,30	0,23	5,24	17,44	0,14	0,77	8,72	1,40
As	*mg kg^{-1}*	0,29	2,17	0,28	0,33	nd	0,38	nd	nd	0,54	nd
	% of the total	0,10	7,55	0,55	0,62	-	4,13	-	-	4,07	-
Cu	*mg kg^{-1}*	1,50	0,71	1,20	0,57	1,44	1,67	0,52	nd	5,04	0,87
	% of the total	0,52	4,83	0,83	0,49	10,61	10,40	0,21	-	8,47	1,44
Cd	*mg kg^{-1}*	0,52	0,21	1,06	nd	0,21	0,11	5,75	0,05	0,39	0,10
	% of the total	2,81	9,28	3,08	-	6,16	15,60	7,10	1,53	8,28	0,89
Zn	*mg kg^{-1}*	3,71	1,90	4,19	1,26	3,32	3,86	9,04	0,41	9,13	1,00
	% of the total	1,26	5,13	1,94	0,58	7,77	9,22	1,66	0,75	7,87	1,06
Sb	*mg kg^{-1}*	10,01	3,69	9,63	158,2	0,81	2,30	1,64	0,23	3,13	0,22
	% of the total	0,48	6,90	0,62	7,28	3,43	17,53	0,08	1,47	7,03	2,40

Table 2a: Leached MTE in mg kg^{-1} and % of the total concentration

Phytoavailability assessment		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Pb	*mg kg^{-1}*	178,5	0,71	162,9	1,30	0,52	0,60	89,38	14,69	0,31	3,64
	% of the total	0,45	0,05	0,38	0,00	0,05	0,20	0,25	1,02	0,02	0,34
As	*mg kg^{-1}*	nd	nd	nd	0,17	nd	nd	nd	nd	0,04	0,01
	% of the total	-	-	-	0,32	-	-	-	-	0,27	0,11
Cu	*mg kg^{-1}*	0,43	0,202	0,37	0,38	0,19	0,18	0,32	0,21	0,29	0,64
	% of the total	0,15	1,374	0,26	0,33	1,41	1,10	0,13	1,06	0,48	1,06
Cd	*mg kg^{-1}*	3,16	0,20	4,16	0,22	0,31	0,22	20,08	0,68	0,22	0,24
	% of the total	17,22	9,02	12,14	5,21	9,22	31,91	24,82	20,00	4,68	2,15
Zn	*mg kg^{-1}*	2,77	nd	3,51	0,06	0,20	nd	25,57	0,44	0,08	0,10
	% of the total	0,94	-	1,62	0,03	0,48	-	4,69	0,79	0,07	0,11
Sb	*mg kg^{-1}*	1,54	0,478	1,56	306,6	0,16	0,28	0,77	0,08	0,35	0,11
	% of the total	0,07	0,894	0,10	14,10	0,66	2,09	0,04	0,50	0,78	1,20

Table 2b: Extracted MTE with CaCl2 in mg kg^{-1} and % of the total concentration

Ecotoxicity of leachates measured by the inhibition of *Daphnia magna* mobility was highly variable (Table 3). Whatever the sample tested, the inhibition of daphnia mobility increased between 24 h and 48 h, except for S_3 whose inhibition was near 100% after only 24 h. Ecotoxicity was also maximal (i.e. 100%) for S_1, S_2, S_7 and S_8 after 48 h; while the lower inhibition was observed for S_5 (15 %). Ecotoxicity 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 EC_{50}-30 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. Microtox® test results (Table 3) 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 ([Pb] > 80 mg kg^{-1}; [Sb] > 10 mg kg^{-1} (except S_7)) (Table 2a).

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 4).

Ecotoxicity test		Sample									
		S_1	***S_2***	***S_3***	***S_4***	***S_5***	***S_6***	***S_7***	***S_8***	***S_9***	***S_{10}***
D. magna [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**	*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 (%).
*nt: not toxic

Table 3: Results of the *Daphnia magna* and Microtox® tests for the 10 leachates

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 6-b). 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.

Sample	Bacterial strain			
	ZntLux [a]	***Arslux*** [a]	***Merlux*** [a]	***pBtaclux*** [b]
S1	4.8	99.6	9.9	6.5
S2	0.9	324.9	0.8	-
S3	4.0	1.2	12.9	-
S4	1.4	0.8	0.9	-
S5	0.7	0.9	1.0	-
S6	0.6	0.8	0.9	-
S7	5.4	79.8	138.1	18.4
S8	1,2	0.9	0.9	-
S9	1,3	2.1	0.8	-
S10	1.2	1.2	0.8	-

[a] IF: Induction Factor.
[b] Inhibition rate (%).

Table 4: Ecotoxicity results of bioluminescence emitted by the bacterial strains

4. Discussion

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. [27] or Ferrari et al. [28], 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 24h for water and only 2h 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 [5,34,37], and Cd was the most available element (up to 32 %). Extracted concentrations were strongly correlated to total concentrations. 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 [29]. High correlation factors were observed between exchangeable Cu and Zn fractions and soil organic matter amount. These elements were thus less mobile because of their affinity for this soil fraction [11,30]. 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 [31]. The high Sb amount extracted from S4 could be explained not only by a higher total concentration but also by the highest $CaCO_3$ content [32]. Sb could be solubilised under the influence of

soil bio-physico-chemical parameters controlling its sorption [33,34,35]. pH and CEC were already described as influent parameters of element extractability [36], retention and mobility in soils [11]. Thus, according to the origin of soil sampling, differences in soil parameters were observed (Figure 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 pollution on ecosystems seems therefore particularly appropriate in this type of study.

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 S_2 and S_8. 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 [38,39] and it has been already shown that *V. fischeri* was generally less sensitive than *D. magna* [10,40]. 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 [26]. Then, response in ecotoxicity tests was not always directly correlated with total or water-soluble concentrations [41]. 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 [42] could improve the interpretation of the data.

According to Persoone et al. [43] 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.

(1) Class weight score = ($\sum$ all test scores) / number of tests performed (= 6)

(2) Class weight score (%) = (class weight score) / maximum class weight score × 100

That classification system aimed at the integration of ecotoxicity data obtained in a battery of bioassays as describe by Lors et al. [44]. 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 5. 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 S_7 and S_1 (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 $S_7 > S_1 > S_3 > S_2 = S_4 = S_9 = S_{10} > S_8 > S_5 = S_6$. Although the toxicity of some samples (S_2 and S_8, 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 S_1, S_3, S_4 and S_7 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 solubilised 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.

Sample	Score						Class[a]	MCW[b]	W[c]	PW[d]
	D. magna	*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 / InR < 1	0
II	slight acute toxicity	20 % ≤ PE < 50 %	1 ≤ IR / InR < 5	1
III	acute toxicity	50 % ≤ PE < 75 %	5 ≤ IR / InR < 50	2
IV	high acute toxicity	75 % ≤ PE < 100 %	50 ≤ IR / InR < 100	3
V	very high acute toxicity	PE ≥ 100 %	IR / InR ≥ 100	4

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

Table 5: Ecoscores calculation and hazard classification

5. 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 characterisation 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.

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