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"Gheorghe Asachi" Technical University of Iasi, Romania

ECOTOXICITY OF FOAMING AGENT CONDITIONED SOILS TESTED ON TWO TERRESTRIAL ORGANISMS

Emanuela Galli[1]*, Valerio Giorgio Muzzini[1], Antonio Finizio[2], Pietro Fumagalli[2], Paola Grenni[3], Anna Barra Caracciolo[3], Jasmin Rauseo[3], Luisa Patrolecco[3]

[1]Institute of Research on Terrestrial Ecosystems (IRET), National Research Council (CNR), Via Salaria km 29.300, 00015 Monterotondo (Rome), Italy
[2]Department of Earth and Environmental Sciences, University of Milano Bicocca, Milano, Italy
[3]Water Research Institute (IRSA), National Research Council (CNR), Via Salaria Km 29.300, 00015 Monterotondo (Rome), Italy

Abstract

Huge amounts of soil debris are produced during the underground excavation with Earth Pressure Balance-Tunnel Boring Machines (EPB-TBM). Soil debris may contain residual concentrations of the anionic surfactant sodium lauryl ether sulphate (SLES), the main component in some foaming agents used as excavation additives. The reuse of this debris or its discharge as waste is a critical environmental question in construction engineering. There are only few studies on ecotoxicological effects on soil debris coming from a real excavation site.
The aim of this study was to evaluate the ecotoxicity of two deep soils, with different lithological compositions, conditioned with three foaming agents. In some cases, lime was added to the soil. The soils were placed in mesocosms (1 m^3) to simulate the temporary storage of the soil debris at a construction site. At fixed times, soil sub-samples were collected and ecotoxicological tests on terrestrial organisms (*Lepidium sativum, Eisenia foetida*) and an assessment of SLES concentration were performed with soils and aqueous elutriates produced from them. Results showed that at day 28, a SLES reduction was observed in both the soil and aqueous elutriates, with various rates of decrease. The differences were due to different soil lithological compositions and foaming agent products composition. In general, the two soils were not suitable for both plant growth and earthworm reproduction, but in Soil 1 the earthworm mortality was very low, except when lime was added. Tests with soil elutriates showed that 7 days after conditioning no toxic effect was found for the organisms tested.

Key words: anionic surfactants, cress, earthworms, mechanized tunnelling

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1. Introduction

In the coming years, due to the tunnelling projects planned in Europe and worldwide, several hundreds of millions of tonnes of spoil material will be produced. Mechanized excavation has rapidly evolved, changing the approach to tunnel construction: the conventional techniques have been replaced by full face Tunnel Boring Machines (TBM) for rock and soils, which allow the simultaneous realization of excavation and the lining phases (Maidl et al, 2012; Peila et al., 2016). In tunnelling excavation, the most frequently used technology is the EPB (Earth Pressure Balanced) - TBM, which requires a conditioning of the excavated soil.

Thanks to the use of proper agents, the conditioning process makes it possible to change the properties of the soil both in the excavation chamber and along the screw conveyor by transforming it into a plastic workable paste with the necessary

* Author to whom all correspondence should be addressed: e-mail: emanuela.galli@cnr.it; Phone: +3906 90672526; Fax: +3906 90672990

mechanical properties and water content for proper tunnelling (Milligan, 2000).

Soil debris produced from tunnelling excavation can be reused as a by-product for different purposes, such as building materials or land covering; however, the presence of residual foaming agents can compromise the reuse of tons of soil debris, posing a potential environmental risk, especially for the terrestrial and aquatic ecosystems (Jackson et al., 2016). Soil debris can be classified in Italy as waste when it exceeds the chemical thresholds for organic and inorganic contaminants (Italian Decree 120, 2017), but there are currently no threshold limits for soil conditioning agents in European and Italian legislation, or complete studies on their eco-toxicological effects. Even in small concentrations, many chemicals can have a negative effect on soil chemistry; moreover, due to the quantities generated by the excavation processes (e.g. a 40 km tunnel is estimated to produce 2.5-3.0 million m^3 excavation material; Oreste and Castellano, 2012), substances that are generally considered minor contaminants can have a high environmental impact with negative effects on the survival and/or reproduction of some organisms. In Italy, the temporary storage of soil debris at a construction site is considered a normal industrial practice (Italian Decree 120, 2017), in order to promote the natural degradation (biotic and/or abiotic) of the additives used during the excavation process. The disadvantage of this practice is that it requires specific and large areas and, in some cases, there is not room for huge amounts of spoil materials to be kept for a long time. For this reason, in many excavation works, soil debris is directly disposed of as waste, with enormous economic and environmental disadvantages. Recycling debris fits into the so-called "circular economy", with the undoubted advantages of lowering project costs and, more in general, of recycling a non-renewable natural resource, that is the soil (FAO, 2015), and avoiding the unnecessary production of waste (Bellopede et al., 2011). However, this requires a responsible innovative technology and common criteria, not yet available, for assessing and managing any environmental risk from the residual mixture of chemicals occurring in the excavated material.

The foaming mixtures of many commercial products contain anionic surfactants (AS) as the main component for changing the mechanical and hydraulic behaviour of the excavated soil, changing it into a plastic paste, thus permitting soil pressure applications at the tunnel face (Vinai et al., 2008; Peila et al., 2016). Other additives, generally polymers, not always of a known chemical composition, are also used to increase the foam viscosity and to improve its thixotropic properties (Milligan, 2000). Sodium lauryl ether sulphate (SLES) is one of the most used AS in foaming agents, in concentrations ranging from 10 to 50% of the overall commercial product and at present, there are no SLES threshold limits in EU and Italian legislation for excavated soil (Mininni et al., 2018; Barra Caracciolo et al., 2019). Setting a threshold limit for the concentration in soil of chemicals contained in every commercial product is a difficult goal to achieve, but at the same time there is concern about the lack of a complete knowledge of the environmental risk due to the reuse of soils conditioned with additives injected during excavation. In principle, the possibility that the spoil material can be considered a by-product or a waste is related to SLES degradability. Recent studies show that SLES in real soil debris produced during an excavation processes is biodegradable, although the degradation rate depends on site specific abiotic and biotic conditions (Barra Caracciolo et al., 2017; 2019). Moreover, the complete composition of a commercial product is often unknown and the presence of minor components can influence the ecotoxicity of foaming agents. For all these reasons, the ecotoxicological approach (i.e. evaluation of the effects of foaming agent conditioned soils on target terrestrial and aquatic organisms) saves the time needed to design new analytical strategies and allows more information on the different interactions between the foaming mixture and the specific matrix (Barra Caracciolo et al., 2017; Di Paolo et al., 2016; Grenni et al., 2019). Biological assays prove the most reliable approach to assessing the chemical environmental impact, not least because they combine the overall effects of the contaminants, considering also the additive, antagonistic and synergistic effects, and taking into account the bioavailable fraction of all the chemicals in solid or semi-solid samples, such as soil debris.

Currently, only few studies have adopted this ecotoxicological approach for assessing if spoil materials can be classified as a suitable by-product for different purposes (including covering of green areas). Baderna et al. (2015) tested the effects of five concentrations of three foaming agents containing SLES added to OECD standard soils on germination of cress (*Lepidium sativum*). IC_{20} and IC_{50} values were determined for each conditioning agent. To date, only Grenni et al. (2018) evaluated the ecotoxicity of real excavated soils conditioned with different foaming products containing SLES as the main component, in order to verify the eco-compatibility of soil debris. Five test species (*P. subcapitata, D. magna, D. rerio, V. fischeri* and *L. sativum*) were utilised in order to represent both the aquatic and terrestrial environmental compartments. Overall ecotoxicity was assessed through a bioassay battery index and compared to SLES residual concentrations in elutriates produced from soils. This study showed that the ecotoxicological approach is very sensitive to SLES residual concentrations and supports the need to perform site-specific environmental studies on excavated material from construction works for stakeholders' decision making processes (Grenni et al., 2019).

In this context, the aim of the present work was to evaluate the ecotoxicity of two soils with different lithological characteristics. The soils were collected *in situ* from a tunnel excavation site and separately conditioned with three different foaming agents (P1,

P2, P3), containing the anionic surfactant SLES, at the real concentrations used for the mechanized drill and put into 1 m^3 mesocosms for simulating temporary storage at a construction site for 28 days. Moreover, as use of lime is a possible soil debris treatment in excavation areas, some mesocosms were set up with soil treated with a foaming agent and lime. At different times after conditioning, soil samples from mesocosms and their aqueous extracts (elutriates) were analysed to determine the SLES residual concentrations over time and used for some ecotoxicological tests. For this purpose, two organisms were tested, cress (*Lepidium sativum)* and the earthworm (*Eisenia foetida)*, following standard protocols.

2. Materials and methods

2.1. Soil conditioning with foaming agents

Two soils with different geopedological characteristics were collected at 50 m depth in the tunnel excavation area: Soil 1: gravel 56.7% sand 23.1%, silt 14.1%, clay 6.1%; Soil 2: gravel 88.3%, sand 9.1%, silt 2.0%, clay 0.6%. The soils were conditioned with three common commercial foaming agents (P1, P2, P3) at the treatment ratios (TR, L/m^3) used for the site specific mechanized drill. In Table 1 the different soil treatments with each foaming agent used are reported. A polymer (PR) was also added to Soil 1 conditioned with the foaming agent P2. Lime was added to Soil 1 alone or together with P1. All the three foaming products and the polymer contained SLES, as the main chemical component, water and unknown minor substances. In particular, SLES percentages in the different products were: P1: 10-20%; P2: 10-30%; P3: <30%; PR: 25-50%. The conditioned soils were placed in 1 m^3 mesocosms to simulate the temporary storage of soil debris produced during tunnel excavation at a construction site.

2.2. SLES concentrations in soil and elutriates

SLES was analysed at t=0 d and t= 28 d both in soil samples and in the elutriates produced from the same soils following the method reported in Grenni et al. (2018). The SLES extraction from soils was performed with ASE (Accelerated Solvent Extraction) by using methanol as an extraction solvent. For elutriates production, distilled water (1:10 w:v, UNI EN 14735, 2005) was added to aliquots of soil (about 80 g, in three replicates) from the mesocosms. The suspension was shaken for 24 h at 20°C in the dark and then centrifuged for 15 min at 9000 rpm.

The aqueous elutriates produced from soils and the final extracts from ASE were analysed using the spectrophotometric method MBAS-Methylene Blue Active Substances (Standard Methods, 2012), based on the formation of an ionic-pair reaction between the anionic surfactants and the methylene blue and three successive extractions with chloroform as a solvent. The absorbance of the chloroform extract was then measured at 650 nm wavelength (Perkin–Elmer Lambda 25 UV-VIS spectrophotometer).

The SLES concentration was calculated using the calibration curve determined in the range 0.05–4 mg/L SLES. The limit of detection (LOD), calculated in accordance with the IUPAC method (IUPAC, 1999), was 0.013 mg/L and the ASE extraction recovery was 96.5±1.6%.

2.3. Ecotoxicological tests on soils

The Seedling Emergence and Seedling Growth tests (OECD, 2006) with cress (*Lepidium sativum)* and the acute (mortality) and chronic (reproduction) tests with earthworms (*Eisenia foetida)* (OECD, 1984, 2016) were performed on soil sub-samples collected from mesocosms after 0, 7, 14, and 28 days after soil conditioning.

The ecotoxicological tests with plants were performed in a greenhouse under the following conditions: T=25°C±10°C, Relative Humidity=70%± 25%, photoperiod= 16 h light and 8 h dark; light intensity=350±50 µE/m^2/s. Twenty cress seeds (Fratelli Ingegnoli, Milano) were grown in pots (10x10x22 cm) filled with untreated/treated soils from the mesocosms. A control soil was used according to OECD (2006). At day 21, the plant dry weight was measured and the Yield Index % was calculated with Eq. (1).

$$Yl(\%) = Yl_{treated} \div Yl_{control} x100 \quad (1)$$

Table 1. Soil treatment conditions in the different mesocosms

Mesocosm set up	*Treatment*
Soil 1	No treatment (only water)
Soil 1 + Lime	Lime (20 kg/m^3)
Soil 1 + P1	Foaming agent 1 (0.59 L/m^3)
Soil 1 + P1 + Lime	Foaming agent 1 (0.59 L/m^3) + Lime (20 kg/m^3)
Soil 1 + P2	Foaming agent 2 (0.53 L/m^3)
Soil 1 + P2 + PR	Foaming agent 2 (0.53 L/m^3) + polymer (0.165 L/m^3)
Soil 1 + P3	Foaming agent 3 (0.35 L/m^3)
Soil 2	No treatment (only water)
Soil 2 + P1	Foaming agent 1 (1.46 L/m^3)
Soil 2 + P2	Foaming agent 2 (1.46 L/m^3)
Soil 2 + P3	Foaming agent 3 (1.12 L/m^3)

The ecotoxicological tests with earthworms were performed in glass boxes filled with 700 g of soil taken from the different mesocosms. In addition, an artificial soil prepared according to OECD guideline n. 207 (1984) was used as a control. At the start of the tests, 10 worms were put in each box and incubated at 20°C. The percent of earthworm mortality in the soils was measured at day 14; the reproductive effects were determined at day 56, in accordance with the OECD (2016) guideline.

2.4. Ecotoxicological tests on elutriates

At the same sampling times used for the ecotoxicological tests on soils (0, 7, 14, 28 days), elutriates were produced as described in paragraph 2.2. The germination test was performed in Petri dishes (90 mm diameter) adding 5 mL of elutriate (pre-filtered through 0.45 μm cellulose acetate filters) and 10 seeds of *L. sativum* (Fratelli Ingegnoli, Milano).

After 72 h in a growth chamber at 25°C, the number of germinated seeds and the length of seedlings were measured (US EPA, 1996). The Germination Index (GI) for each treatment was calculated with Eq. (2).

$$GI = N.germinated\ seeds\ x\ mean\ seedling\ lenght \quad (2)$$

The percent germination index (GI%) was calculated with Eq. (3).

$$GI(\%) = GI_{treated} \div GI_{control} x100 \quad (3)$$

The acute toxicity test was performed on the earthworms following the method described in OECD guideline n. 207 (OECD, 1984) and using 1 mL of the elutriates in a filter paper contact test in vials. Briefly, round filter paper (Whatman No. 1) was cut to a suitable size and placed in such a way that the sides of the vials were lined with filter paper. 1 mL of the test solution was pipetted into each vial in order to wet the filter paper. Blank tests were performed with 1 mL of deionized water.

Each test consisted of ten replicates. Adult earthworms, which possessed clitellum and had an individual wet weight of 250-350 mg, were selected for testing. Earthworms were washed briefly with deionized water, and were kept on moist filter paper for 3 h to eliminate the gut content, after which they were rinsed again with deionized water, put on the filter paper and placed in a test vial. One earthworm was introduced to each vial and the latter was covered with plastic film that was punched with small holes using a needle. Tests were done in the dark for 48 h. After this period, each earthworm was monitored for mortality by a gentle mechanical stimulus to the front part.

2.5. Statistical analysis

The data from the cress and earthworm toxicological tests were processed with a one-way analysis of variance (ANOVA). Statistical significance of the differences between means was assessed by a post hoc Tukey's Test with the level of significance established at $p \leq 0.05$.

3. Results and discussion

3.1. SLES concentration in soils and elutriates

In Table 2 SLES concentrations in conditioned soils and elutriates produced at the initial and final experimental times (t=0 and 28 days) are shown. The initial SLES concentration in soil ranged from 77.8 to 368.5 mg/kg, corresponding to real concentrations used for tunnelling excavation at a construction site. A reduction in concentration of the surfactant both in soils and in elutriates was detected at day 28, corresponding to an overall decrease (expressed as percentage) in the range of 3-56% in soil and 7-100 % in elutriates depending on the SLES initial concentration and soil type. As reported in recent studies (Grenni et al., 2018; Barra Caracciolo et al., 2019) the soil lithological composition can affect the surfactant adsorption into the soil matrix and, consequently, its release in the water phase. Indeed, in this study lower SLES concentrations were found in the elutriates produced from Soil 1 (Table 2), in which the silty-clay composition (the fine fraction, with higher sorption properties) was higher than that in Soil 2. Moreover, this result was also due to the initial lower SLES concentrations in soil.

The presence of the polymer (PR) in Soil 1 conditioned with P2 foaming agent did not influence the SLES decrease in soil and, at the end of the experiment, SLES was not detected in the corresponding elutriates. The highest SLES values were found in Soil 2 (276.3-368.5 mg/kg) due to the initial higher foaming agent dosages applied (TR, L/m^3, Table 1) and in the corresponding elutriates, where the surfactant concentration was in the range of 8.92-16.50 mg/L.

In the case of Soil 2, where the gravel composition prevailed, the anionic surfactant was adsorbed into the soil to a lesser extent and it was more easily released into the aqueous interstitial phase. Finally, the presence of lime in Soil 1 conditioned with P1 foaming agent did not produce any effect on the SLES concentration decrease in soil, compared to that without lime (34 and 30 of percentage decrease at day 28, respectively); in the corresponding elutriates, SLES concentration values were 1.2 mg/L and below the detection limit, respectively (Table 2).

Table 2. SLES concentration detected in soil (mg/kg) and elutriate (mg/L) samples at the initial (t0) and final (t28) experimental times

Treatment	*SLES concentration in soil (mg/kg)*	*SLES concentration in soil (mg/kg)*	*SLES decrease in soil (%)*	*SLES concentration in elutriate (mg/L)*	*SLES concentration in elutriate (mg/L)*	*SLES decrease in elutriate (%)*
	t0	*t28*		*t0*	*t28*	
Soil 1	nd	nd		nd	nd	
Soil 1 + Lime	nd	nd		nd	nd	
Soil 1 + P1	77.8±0.9	54.1±0.8	30	1.23±0.05	nd	100
Soil 1 + P1 + Lime	157.9±1.2	103.6±1.3	34	2.64±0.12	1.2±0.13	55
Soil 1 + P2	158.5±1.8	69.9±1.1	56	3.64±0.35	nd	100
Soil 1 + P2 + PR	154.7±2.1	67.4±0.9	56	4.91±0.32	nd	100
Soil 1 + P3	132.6±2.9	78.8±1.3	41	3.36±0.15	0.18±0.06	95
Soil 2	nd	nd		nd	nd	
Soil 2 + P1	276.3±2.5	201.4±2.2	27	9.62±0.95	8.92±0.98	7
Soil 2 + P2	305.9±3.2	296.9±1.9	3	20.79±1.21	9.78±1.20	53
Soil 2 + P3	368.5±3.5	248.1±2.3	33	23.26±1.33	16.5±2.05	29

nd= not detectable

3.2. Ecotoxicological tests on soils

In order to evaluate the possible ecotoxicity effects of the conditioned soils, the cress seedling growth test and earthworm mortality test were performed by using soil sub-samples collected over time from unconditioned and conditioned mesocosms (Soil 1 and Soil 2; days 0, 7, 14, 28). The ecotoxicological results at 0 and 28 days are reported in Tables 3 and 4 and in Fig. 1.

Tables 3 and 4 show the results obtained from the *L. sativum* growth tests (the number of germinated plants and the Yield Indexes) at the initial and final sampling time. The yield index (YI%) for plant grown in conditioned and unconditioned soils was very low in comparison with the control, because few plants germinated in the soils and the growth of them was reduced by the absence of organic matter and the high gravel concentration. The overall results obtained therefore showed that both soils used were not suitable for plant growth. This concealed the possible effect of the foaming products (Tables 3 and 4). In the pots containing Soil 1 and Soil 2 without any treatment and at day 28 (Table 4), the number of germinated seeds increased.

However, the plants grew suffering and the YI percentage was very low. Moreover, comparing data for Soil 1 without any treatment with that of the same soil plus lime (Soil 1+ P1 +Lime) it was evident that adding lime completely inhibited germination. Fig. 1 shows the results of the ecotoxicological test performed on conditioned soils samples using the terrestrial organism *E. foetida* at 0 and 28 days.

The mortality in the artificial OECD soil (Control) was very low, indicating the validity of the experimental conditions. The overall results showed that in the unconditioned and conditioned S1 soil mesocosms (both t0 and t28), the mortality was very low, without any significant difference (Tukey test: $p \leq 0.05$). The soil treated with lime (S1 + lime and S1 +P1 + lime) showed a 100% mortality, highlighting the high toxicity of lime for earthworms, whether SLES was present or not, presumably due to the very high soil pH. No significant differences were found among unconditioned and conditioned S2 soil mesocosms at day 0 (Tuckey test: $p \leq 0.05$), indicating that the mortality did not depend on the presence of SLES (the highest % mortality was recorded in S2, untreated mesocosm).

Table 3. *L. sativum* growth test in soil debris at the initial time (t0). Number of total germinated plants and calculated Yield Index (YI%±Standard Error) were assessed after 21 days of growth. YI data are the mean of three replicates

Mesocosms (t0)	*Plants (No.)*	*YI ± S.E. (%)*
Control	45	100
Soil 1	5	2.09±1.47
Soil 1 + Lime	0	0.0
Soil 1 + P1	13	4.80±0.23
Soil 1 + P1 + Lime	0	0.0
Soil 1 + P2	9	5.59±3.91
Soil 1 + P2 + PR	37	13.93±1.83
Soil 1 + P3	33	13.82±1.26
Soil 2	11	1.71±0.86
Soil 2 + P1	0	0.0
Soil 2 + P2	0	0.0
Soil 2 + P3	1	0.04±0.04

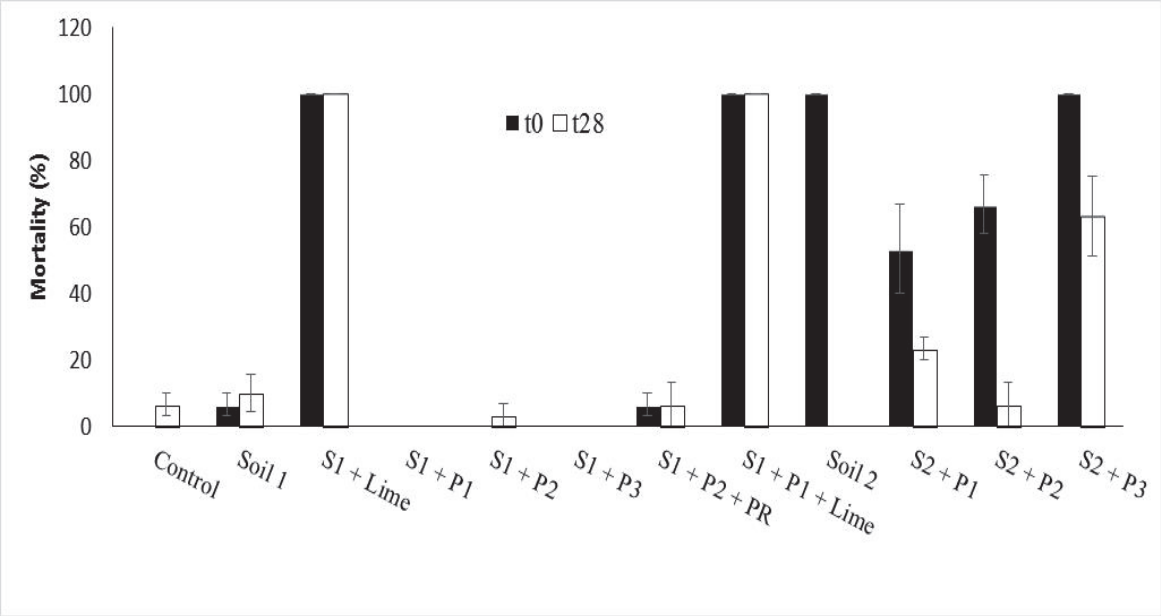

Fig. 1. Mortality (%) of *E. foetida* grown for 14 days in soil subsamples taken from mesocosms at 0 and 28 days (for the explanation of the acronyms see Table 1)

Table 4. *L. sativum* growth test in soil debris at the final time (t28). Number of total germinated plants and calculated Yield Index (YI%±Standard Error) were assessed after 21 days of growth. YI data are the mean of three replicates

Mesocosms (t28)	***Plants (No.)***	***YI ± S.E. (%)***
Control	50	100
Soil 1	30	6.98±2.41
Soil 1 + Lime	18	2.69±0.13
Soil 1 + P1	27	6.54±0.09
Soil 1 + P1 + Lime	9	1.22±0.59
Soil 1 + P2	42	9.64±1.63
Soil 1 + P2 + PR	45	11.30±0.78
Soil 1 + P3	30	6.89±0.69
Soil 2	48	5.32±0.19
Soil 2 + P1	0	0.0
Soil 2 + P2	9	0.50±0.50
Soil 2 + P3	0	0.0

At day 28 (t28 in Fig. 1), significant differences in earthworm mortality were found among the unconditioned (S2) and conditioned (S2+P1, S2+P2 and S2+P3) soils (Tukey test: $p \leq 0.05$). Indeed, no dead organisms were found in S2, whereas a mortality ranging from 7 to 63% was recorded for the S2 soil conditioned with the foaming agent containing SLES. However, these differences were mainly due to the casual lack of mortality in the unconditioned S2 soil test, which should be regarded as an outlier. Indeed, 100% of earthworm mortality was recorded in all the other unconditioned S2 soil in tests performed at 0, 7 and 14 days (data for 7 and 14 d are not shown in Fig. 1). Overall, it can be concluded that the S2 soil was not suitable for the survival of earthworms, whether SLES was present or not.

Finally, the reproductive tests (OECD, 2016) using the S1 and S2 soil mesocosms showed that after 8 weeks no cocoons were present in all treated and untreated mesocosms. These negative effects could be attributed to the intrinsic characteristics of both soils, and probably to the scarce presence of organic matter, because they were collected from a deep soil layer.

3.3. Ecotoxicological tests on elutriates

With regard to the cress germination tests, no significant differences were found among the control (distilled water) and elutriates obtained from soil mesocosms at day 0, apart from the sample Soil 1 + P2, with a GI% of 55.42%. The value grew to 89.81% after 7 days of conditioning. The tests performed at 7, 14 and 28 days after the soil conditioning showed that no sample was toxic for germination (data not shown).

With regard to the earthworm tests, the elutriate produced from the conditioned soils 1 and 2 did not cause any toxic effect on this organism at all sampling points. Indeed, in almost all the elutriate tests the percentage of survival was equal to 100%. These findings confirmed the results found for the

ecotoxicity of soils (paragraph 3.2), that is to say that SLES at the concentrations tested did not produce any acute toxicity effects on *E. foetida*. The only exception was the result obtained when elutriates derived from soils added with lime were tested; in this case the mortality measured was about 70%, and this was presumably due to the high pH value (>11) of the elutriates. In fact the optimal pH for earthworm life is from 6.2 to 7.8.

The soils analysed in this study had a low organic matter and nutrients content due to the fact that they were excavated at a depth of about 50 m and thus proved inadequate for the plant and earthworm growth. However the addition of the foaming agents did not substantially influence their survival negatively. The overall results give an indication of the unsuitability of these soils for green areas, but the use of the soil debris for industrial purposes can be contemplated.

4. Conclusions

The assessment of the ecotoxicological effects of excavated soils containing foaming agents (as well as of their aqueous elutriates) is a very promising approach for evaluating their possible re-use as a by-product in the context of the so-called circular economy.

The choice of terrestrial organisms can be suited to a final destination use of soil debris such as filling a waterproof depression area close to a construction site, as in the case of this study. If the destination site is underground and with no possible interactions with water bodies, the ecotoxicological tests on the water compartment are not necessary. In any case, the conduction of ecotoxicological tests on soil aqueous elutriates was performed for precautionary reasons and to achieve environmental protective goals in the best possible way.

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