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Environmental Fate and Effects of Foaming Agents Containing Sodium Lauryl Ether Sulphate in Soil Debris from Mechanized Tunneling

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Abstract: A wide use of foaming agents as lubricants is required in mechanized tunneling. Their main component, the anionic surfactant sodium lauryl ether sulphate (SLES), can remain in residual concentrations in soil debris, influencing their potential reuse as by-product. This study aimed at evaluating the environmental fate and effects of a foaming product used for conditioning soils collected from real excavation sites, in the presence/absence of an anti-clogging polymer, both containing SLES. Soil microcosm experiments were set-up and incubated for 28 days. Over time, soils and their water extracts (elutriates) were collected to perform both ecotoxicological tests (*Vibrio fischeri*, *Lepidium sativum*, *Eisenia foetida*, *Hetereocypris incongruens*, *Danio rerio*) and SLES analysis. The results showed that, just after conditioning, SLES did not exert any hazardous effect on the organisms tested except for the bacterium *V. fischeri*, which was the most sensitive to its presence. However, from day seven the toxic effect on the bacterium was never observed thanks to the SLES decrease in the elutriates (<2 mg/L). SLES degraded in soils (half-lives from 9 to 25 days) with higher disappearance rates corresponding to higher values of microbial abundances. This study highlights the importance of site-specific studies for assessing the environmental reuse of spoil materials.

Keywords: TBM-EPB excavation; SLES; spoil material; ecotoxicity

1. Introduction

Transport is a key point for the overall economy and its efficient services are essential in a globalized world. For this reason, the past decade has seen a wide development of roads and railways for improving it. Tunnel construction (i.e., for underground or subsoil roads) can play an important role in minimizing surface impacts, in reducing urban congestion and in avoiding deforestation in the rural areas [1–3] and, in some cases, tunnels are necessary for completing railing and road webs when a mountain area is present. However, tunneling excavation projects require the assessment of their sustainability before, during and after their implementation.

In the last few years, tunnel mechanized excavation with EPB-TBMs (Earth Pressure Balance-Tunnel Boring Machines) made it possible to perform more efficient, rapid and safe works. The use of foaming agents (FAs) and additives, such as anti-clogging or anti-wear polymers, as lubricants is necessary for enhancing the mechanized excavation process with EPB-TBMs, reducing the hard rock abrasiveness and facilitating excavated material transportation [4,5]. FAs are generally water solutions of anionic surfactants and are typically used in tunneling excavations between 0.1 and 3 L/m^3 of soil [6]. Sodium lauryl ether sulfate (SLES) is the main component of most FAs, in percentages variable from 5 to 50%. In some cases, other minor components are also present [7,8].

The management of many millions of tons of excavated soil mixed to FAs containing SLES residues (spoil material) can be an environmental issue for both the excavation industry and society if it is not well planned. In fact, if spoil material cannot be reused, it has to be treated as a waste [9]. On the contrary, its possible reuse as a by-product for industrial (aggregate for concrete, building and infrastructure construction) or green area use (e.g., land covering, public green areas, including with artificial ponds) and has the undoubted advantages of lowering project costs, recycling a non-renewable natural resource (soil), and avoiding occupying large areas for waste disposal [8]. On the other hand, SLES and other minor chemicals occurring in soil debris may pose an environmental risk at concentrations which are toxic for biota [6,10–12]. Although at the FA amounts generally used in tunneling SLES residual concentrations in the spoil material are not toxic for terrestrial organisms, they can be potentially hazardous for aquatic ones because of their sensitivity to surfactant presence [6,12,13]. In particular, if at the final destination site (e.g., renaturation areas or refilling of roads and quarries close to water bodies) aquatic contamination is possible, the leaching of SLES from soil into underlying aquifers or nearby rivers has to be taken into consideration in the tunnel project plan [14]. Consequently, a correct evaluation of SLES residual concentrations and ecotoxicity of spoil material is definitely required for assessing its possible reuse and protect human and ecosystem health [15].

Recent studies conducted in microcosm experiments using real soil from excavation sites, showed that the anionic surfactant SLES is degradable in spoil material and its depletion can occur during the temporary storage at the construction site. These studies showed that SLES can be degraded by environmental bacteria, with variable rates which depend on site-specific biotic (bacterial abundance) and abiotic (e.g., soil type, temperature) factors [6,8,16]. In principle, whenever possible, the storage in temporary deposit areas of spoil material makes the natural degradation of SLES possible and, consequently, reduces or blanks its possible toxic effects on the environment. The use of ecotoxicological tests on target organisms is an effective approach for assessing the storage time required for spoil material before establishing any reuse of spoil material, in order to avoid potential environmental risks [6,12,13,17]. Moreover, the ecotoxicological responses make it possible to evaluate the overall effects of a chemical or a mixture of chemicals occurring in soil treated with FAs, including minor or unknown ones [18–21].

The aim of this work was the evaluation of the environmental fate and possible effects of a foaming agent in soil debris from an underground EPB-TBM excavation. The foaming agent was tested at different real concentrations on four different soils alone or together with a polymer. For this purpose, the soils were collected from an excavation area and were conditioned with the least ecotoxic foaming agent (consisting of a water solution of SLES at 5–10% of the product) previously selected using a toxicity screening with the bacterium *V. fischeri*. The FA conditioned soils were maintained for 28 days

in laboratory microcosms for simulating spoil material storage at the construction site. Soil sub-samples were collected from each microcosm to carry out chemical (SLES residual concentration in soils and elutriates), microbiological (microbial abundance) and ecotoxicological (a battery of tests using five different species) analyses. The tests were performed both on conditioned soils (with the ostracod *Heterocypris incongruens* and the worm *Eisenia foetida*) and the corresponding soil water extracts (with the luminescent bacteria *Vibrio fischeri*, the plant *Lepidium sativum* and the fish embryo *Danio rerio*). In this study, the following hypothesis were tested: (i) If the concentration of SLES in the soil water extracts was lower/equal to 2 mg/L, soil debris was not toxic for any organism tested and could be used as a by-product; (ii) The *Vibrio fischeri* test was the most sensitive to the anionic surfactant [17].

2. Materials and Methods

2.1. Toxicity Screening of Foaming Agents with V. fischeri

In order to select the less toxic product, an ecotoxicological screening of three commercial FAs (F, F1, F2) was performed with the luminescent bacterium *Vibrio fischeri*, in accordance with the UNI EN ISO 11348-3:2019 protocol [22], reported in detail in the following Section 2.6.1.

The FA toxicity was evaluated and expressed as the Effective Concentration (EC), the concentration that sources the effect (as percentage) on the tested individuals (i.e., EC_{20} and EC_{50} on 20 or 50%, respectively). A higher EC value corresponds to a lower ecotoxicity effect. The EC_{20} and EC_{50} values of F, F1 and F2 were determined, exposing *V. fischeri* to various foaming agent concentrations prepared with subsequent dilutions (using distilled water) from a stock solution of each product (438, 245 and 120 mg/L for F, F1 and F2, respectively). The test (81.9% Basic Test) was performed three times and 7–9 diluted solutions for each foaming agent (in the range 2.80–358.7; 0.8–200.7 and 0.4–98.3 mg/L for F, F1 and F2, respectively) were tested. The EC_{20} and EC_{50} were statistically estimated (Microtox Omni® software V 4.2, Milan, Italy). Based on the obtained results (Section 3.1), the foaming agent F was selected for the subsequent degradation and ecotoxicity microcosm experiments, as described in the following paragraphs.

2.2. Soil Conditioning and Design of Microcosm Experiments

Four different soils (S1, S2, S3, S4) representative of three tunnels for a railway line to be implemented, were collected from the excavation area at 200–280 m depth, in a mountainous area in Southern Italy. The soil texture, classification, water content and pH are reported in Table 1.

Table 1. Soil texture, classification (USDA), water content and pH of the four soils (S1, S2, S3, S4).

Soil	Composition of Soil (%)			Classification	Water Content (%)	pH
	Clay	Silty	Sand			
S1	32	63	5	Silty clay soil	15	9.17
S2	36	46	15	Silty clay soil	27	9.35
S3	75	25	1	Clay soil	29	9.57
S4	44	53	3	Silty clay soil	30	8.82

All the soils were conditioned in the laboratory with the commercial foaming agent F, in the presence or absence of a polymer (P); the latter was used for increasing foam persistence in the case of S3 and S4. Both commercial products F and P contained the anionic surfactant SLES as the main component at concentrations of 5–10% and 25–50%, respectively.

Foam generation systems, specifically designed at the Department of Structural and Geotechnical Engineering (DISG) of Rome Sapienza University and Department of Environment, Land and Infrastructure Engineering (DIATI), Polytechnic of Turin, were used to simulate in the lab soil conditioning with EPB-TBM. The systems are similar in all their parts to those inside a TBM machine [23,24], which makes it possible to control the foam parameters (e.g., concentration factor of

foaming agent, foam expansion ratio, foam injection ratio, treatment ratios) and to reproduce correctly conditioning activities accomplished on site [23,25,26]. The soils conditioning was performed using the specific parameters obtained after geotechnical analyses (slump cone tests) on each soil. The theoretical values of SLES expected in the spoil material (mg/kg soil), as shown in Table 2, were calculated on the basis of the foaming agent (F) and the polymer (P) treatment ratios (TR, L/m^3) suggested for each soil (S1, S2, S3 and S4) to be conditioned. In particular, the amount of SLES was determined considering its percentage in the F commercial product and P polymer (if present), the specific product density and an average soil density of 1.54 t/m^3. In the case of soil S4, the conditioning was performed considering two possibilities, one with only the F foaming agent and the second one with both F and P polymer.

Table 2. Experimental conditions and treatment ratio values (TR, L/m^3) of the foaming agent (F) and polymer (P) used for soil conditioning of the four different soils (S1, S2, S3 and S4); minimum and maximum SLES (%) in each product; theoretical SLES concentrations calculated for each soil conditioned (mg/kg).

Soil Type	Conditioning	TR (L/m^3)	SLES (%)	Theoretical SLES Concentration in Soil (mg/kg)	
				Min	Max
S1	F	1.89	5–10	47	94
S2	F	1.26	5–10	55	110
S3	F + P	0.94 + 0.2	5–10 + 25–50	75	150
S4	F	0.72	5–10	25	49
S4	F + P	0.72 + 0.2	5–10 + 25–50	58	116

Each microcosm (2 L capacity) was filled with 1.5 kg of each F or F + P conditioned soil. Finally, five (S1 + F, S2 + F, S3 + F + P, S4 + F and S4 + F + P) conditions and the corresponding unconditioned soils (C1, C2, C3 and C4) were set up. Each condition was performed in duplicate. The overall microcosms were kept in the lab for 28 days and maintained at environmental temperature (about 20 °C) under natural light to simulate their storage at the construction site. The soil water content and pH were monitored during the experimental time. At 0, 7, 14 and 28 days, soil subsamples (about 150 g, in duplicate) were collected from each microcosm (S1 + F; S2 + F; S3 + F + P, S4 + F; S4 + F + P; C1; C2; C3; C4) and were analyzed for chemical, microbiological (microbial abundance) and ecotoxicological tests. Moreover, from each soil sample the corresponding elutriates were produced (see Section 2.3) for the chemical determinations and other ecotoxicological tests.

2.3. Preparation of Elutriates from Soil Samples

The elutriates (soil water extracts) were produced from soil samples in a 1:10 (solid/liquid) ratio, as reported in Grenni et al. [12], following the procedure described in UNI EN 2004 [27]. Briefly, an aliquot (100 g) of fresh soil sample was put into a 1 L bottle and the calculated amount of distilled water (also considering the moisture of the soil sample) was added. The suspension was shaken for 24 h at 20 °C in the dark, settled, and the supernatant was then centrifuged for 15 min at 9000 rpm.

2.4. Chemical Analysis

2.4.1. Chemicals

Methanol and chloroform of high-performance liquid chromatographic grade hydrochloric acid (37%), sulphuric acid (98%) and methylene blue were purchased from VWR (Radnor, PA, USA). Sodium hydrogen carbonate and anhydrous sodium carbonate were obtained from Carlo Erba reagents (Milano, Italy). Water was purified (18 MΩ/cm quality) by a Milli-Q system Millipore (Bedford, MA, USA). The anionic surfactant sodium lauryl ether sulphate (SLES) of technical grade purity, purchased from BOC Sciences (New York, NY, USA), was used as the reference compound for the MBAS analysis.

The stock solution of SLES (1000 mg/L) was prepared in methanol and stored at −20 °C. The calibration curve (in the range from 0.05 to 4 mg/L of SLES) was obtained using a dilution of stock solution with ultrapure water. For the Pressurized Liquid Extraction (PLE) procedure, diatomaceous earth was purchased from Thermo Scientific Inc. (Waltham, MA, USA).

2.4.2. Analytical Determination of SLES in Soils and Elutriates

The extraction of SLES from soil was performed by Pressurized Liquid Extraction (PLE) using the Accelerated Solvent Extractor system (Thermo ScientificTM DionexTM ASE 150, Thermo Fisher Scientific Inc., Waltham, MA, USA), as reported in Barra Caracciolo et al. [8]. The PLE methanolic extracts and the aqueous elutriates were analyzed for SLES content using the spectrophotometric MBAS method [28]. In brief, the method is based on three consecutive chloroform extractions of the ionic-pair reaction between SLES and the methylene blue. Following, the absorbance of the SLES–MBAS complex was measured by spectrophotometry at a wavelength of 650 nm (Lambda 25 UV–VIS spectrophotometer, Perkin–Elmer, Waltham, MA, USA). SLES concentrations were calculated using the equations obtained with the standard calibration curves (0.05–4 mg/L SLES), as detailed in Barra Caracciolo et al. [8]. The limit of detection (LOD), calculated following the IUPAC method [29], and the PLE extraction recovery were 0.013 mg/L and 96.5 ± 1.6%, respectively.

2.5. Microbiological Analysis

Microbial Abundance

The total microbial abundance (No. cells/g soil) was quantified in soil (1 g each, three replicates for each conditions), at each experimental time in previously formaldehyde fixed samples. The method applied was the epifluorescence direct count using DAPI (4′,6-diamidino-2-phenylindole) as the DNA fluorescent intercalant. The epifluorescence microscope used was a Leica DM 4000B (Leica Microsystems GmbH, Wetzlar, Germany). Further details are reported in Di Lenola et al. [30]

2.6. Ecotoxicological Tests

The overall toxicity of an environmental matrix can be evaluated with a test battery, which is composed of three or more organisms belonging to different trophic levels in order to represent the main environmental compartment (aquatic or terrestrial one) [12,31–33]. In this work, the battery selection depended on the final destination site of the spoil material and its possible contact with water bodies. The ecotoxicities of the four soils conditioned with the foaming agent F, alone or combined with the polymer P, were evaluated with a precautionary approach by testing soils and the elutriates with five organisms belonging to both terrestrial and aquatic compartments, as summarized in Table 3.

Table 3. Lists of organisms used for performing tests on conditioned soils and corresponding elutriates from the various microcosm experiments (S1 + F, S2 + F, S3 + F + P, S4 + F, S4 + F + P).

Test Organisms	Matrix	Experimental Conditions				
		S1 + F	**S2 + F**	**S3 + F + P**	**S4 + F**	**S4 + F + P**
Vibrio fischeri	Elutriate	x	x	x	x	x
Lepidium sativum	Elutriate	x	x	x	x	x
Heterocypris incongruens	Soil		x	x	x	x
Eiseniafoetida	Elutriate/Soil	x	x	x	x	x
Danio rerio	Elutriate	x				

In particular, the S1 conditioned soil should be placed in an area where below groundwater is present. Although the leaching to the water body is not probable, this hypothesis cannot be excluded. For this reason, two aquatic species were considered, such as the bacterium *Vibrio fischeri* (selected for its known sensitivity to SLES residues) [17] and the fish embryo *Danio rerio*. In the case of the other soils, the spoil material should be used to fill a completely waterproof hollow with no nearby water

bodies. Consequently, the bacterium *V. fischeri* was always selected and the fish test was substituted with that of the crustacean *H. incongruens*. The latter is an aquatic species which lives in the interface between water and soil, representing a species useful to assess the presence of toxicants both in soil and its interstitial water [34].

Unconditioned soil (C1, C2, C3 and C4) and relative elutriates were used as test controls to measure the net toxicity due only to the conditioning. Elutriates were filtered (0.45 µm, cellulose acetate Whatman) for ecotoxicological analysis following the standardized procedure [35].

2.6.1. *Vibrio fischeri* Acute Toxicity Test

The acute toxicity test with *Vibrio fischeri* was performed using a Microtox® analyzer (Model 500, Ecotox LDS, Milan, Italy) according to the UNI EN ISO 11348-3: 2019 standard protocol [22]. This test is based on the inhibition of the luminescence naturally emitted by the marine bacterium *Vibrio fischeri* after its exposition to a toxic substance. Light output of the test organism, compared with a blank (toxic-free solution: distilled water containing 22% NaCl), was measured after three exposure times (5, 15 and 30 min) and was calculated as percentage of inhibition using specific software (Microtox Omni® software V 4.2, Milan, Italy). The difference in light output (between the sample and the blank) was ascribed to the matrix (elutriate) effect on the organisms. Before carrying out the tests, the pH value of each elutriate was recorded and eventually corrected (range 6.0–8.0) using an HCl 0.1 M solution [36], as required by the standard procedures. The coefficient of variation (CV%: standard deviation/mean × 100) as validity criterion (has to be <20%) was also calculated. The effect (% luminescent inhibition) is considered toxic if it is more than 20% [37,38], according to the UNI EN ISO 11348-3:2019 protocol [22].

2.6.2. *Lepidium sativum* Seed Germination Test

The test, performed in accordance with the OECD Guideline No. 208 [39] and the US EPA Ecological effects test guidelines [40] with *Lepidium sativum* seeds, evaluated the effects of aqueous elutriates on germination and on the lengthening of roots, hypocotyls and epicotyls, expressed as the percent germination index (GIx%). For further details, refer to Galli et al. [13].

2.6.3. *Heterocypris incongruens* Test

The *Heterocypris incongruens* tests were performed with the Ostracodtoxkit F (MicroBioTests Inc., Mariakerke, Belgium), in accordance with ISO 14371: 2012 [41]. The acute (mortality) and sub-chronic (growth inhibition) effects on organisms tested were assessed in a 6 day contact with conditioned and unconditioned soil of the test organism. In brief, cysts (dormant stage) of *H. incongruens* were hatched and then 10 neonates were added in each cup of the test plates (six cups in total) with 2 mL of algal suspension, 2 mL of standard water, and 1 g of sample soil. The test plate was performed for each condition (soil conditioned; soil unconditioned; the standard sediment as blank). At 6 days of exposure at 25 °C in the dark, the mortality and growth inhibition of the surviving organisms were determined. The mortality values were expressed as mean mortality (%) with the Abbott's correction [42], which normalizes the effects compared to a standard sediment (blank, provided by the kit) or to an unconditioned matrix. The growth inhibition, evaluated in percentage comparing data with the blank, was determined if the mortality was <30% in the sample, in accordance with standardized procedure. The cut-off value of 20% was considered as toxic for both the endpoints of mortality and growth inhibition [37,43]. The growth inhibition (GIn%) of *H. incongruens* was calculated as follows:

$$GIn(\%) = 100 - \left(\frac{A}{B} \times 100\right) \quad (1)$$

where A is the length increment of the ostracods in the soil samples and B is the increment of the ostracods in the standard sediment.

All measures were performed using a Leica S9i microscope (and the LAS V4.12 software Leica, Wetzlar, Germany). The test validity criteria were a mortality <20% in the reference sediment and the mean growing of organisms >1.5 times compared with neonates at the start of test. The coefficient of variation (CV%) for test reproducibility has to be 20–30% [43,44].

In this test, it may happen that the chemical-physical and mineralogical characteristics of the soil samples tested can falsify the final test result (interference). The sample lithology composition (e.g., the presence of clays of type Kaolinite) can sometimes affect with the growth of *H. incongruens* [45]. Therefore, in order to link the chronic effect detected to only to the presence of the foaming agent/polymer, the toxic response of organisms was calculated at the net of that found in the unconditioned soil (Control).

2.6.4. Earthworm *Eisenia foetida* Acute and Chronic Tests

The ecotoxicological tests with the earthworms *E. foetida* were performed following the methods described in the OECD Guidelines N. 222 and 207 [46,47]. The acute toxicity test was performed with soil elutriates following the filter paper contact test method [47] and using 1 mL of the elutriates in vials. The chronic test on conditioned and unconditioned soil was performed in glass boxes filled with 700 g soil taken from the excavation site. At day 14, the percentages of earthworm mortality were measured, whereas the reproduction effects were determined at 56 days. For further details see Galli et al. [13].

2.6.5. *Danio rerio* Acute Toxicity Test

The Fish Embryo Acute Toxicity (FET) test was performed according to OECD [48–50]. The test is based on a 96-h exposure of newly fertilized eggs of the *Danio rerio* fish (Zebrafish) to a liquid sample and is expected to reflect acute toxicity in fish in general. The test was performed in 24-well plates (1 embryo per well) and used 20 embryos per sample tested and 4 embryos as an internal negative control. Each well was filled with 2 mL of elutriate. The plates were covered with lids and incubated at 26.0 ± 1.0 °C for 96 h with a 14:10 h light–dark photocycle. At the end of the exposure period, the acute toxicity was determined on a positive outcome in any of the four apical endpoints. The results were expressed as mortality (%). For further details, see Grenni et al. [12].

2.7. Statistical Analysis

Correlations between variables (e.g., microbial abundance and DT_{50}) were calculated using the Pearson test. Possible differences among the data were analyzed using the one-way ANOVA. All statistical analyses were performed using R-project software [51].

3. Results

3.1. Toxicity Screening of Foaming Agents with V. fischeri

The dose-response relationships between the concentration of each foaming agent tested (F, F1 and F2) and the corresponding response of *V. fischeri* (bioluminescence inhibition %) were determined. The test with the bacterium *V. fischeri* was used because previous studies have demonstrated it as very sensitive to SLES residues in spoil material [12,17].

The EC_{20} and EC_{50} mean values, together with the standard deviations and the CV%, are reported in Table 4. The foaming agent F was less toxic to the bacterium. In fact, higher values of effective concentrations (EC_{20}: 32.7 mg/L; EC_{50}: 82.2 mg/L) than F1 (EC_{20}: 3.22 mg/L; EC_{50}: 10.29 mg/L) and F2 (EC_{20}: 2.35; EC_{50}: 12.32 mg/L) were found. For this reason, it was selected for the soil conditioning and microcosm experiments (as described in Section 2.2).

Table 4. Ecotoxicity of the three commercial foaming agents (F, F1 and F2) as EC_{20} and EC_{50}, expressed as Bioluminescence inhibition (%) of the bacterium *V. fischeri*. s.d. = standard deviation; CV% = coefficient of variation.

Foaming Agents	EC_{20} (mg/L)	s.d.	CV%	EC_{50}(mg/L)	s.d.	CV%
F	32.73	4.63	14.13	82.20	8.11	9.87
F1	3.22	0.60	18.65	10.29	1.65	16.01
F2	2.35	0.44	18.74	12.32	1.95	15.84

3.2. SLES Concentration in Soils and Elutriates

The residual concentrations of SLES measured over the experimental time (0, 7, 14 and 28 days) in the various conditioned soils and in the corresponding elutriates, are shown in Tables 5 and 6, respectively. The initial SLES values in the various soil microcosms ranged from 52.2 to 105.8 mg/kg. In all cases, the anionic surfactant degraded substantially at the end of the experiment and with different degradation rates. The degradation pathways followed a first order kinetic and the theoretical values of the disappearance time of 50% of the initial SLES concentration (DT_{50s}) were calculated from correlations (r = 0.95; *p*-value < 0.01) between concentrations (expressed as ln C_t/C_0, where C_t = SLES concentration at the sampling time and C_0 = SLES concentration at day 0) versus time. Interestingly, the initial SLES amounts were not correlated with the DT_{50}s, suggesting that degradation rates did not depend only on the concentrations of the anionic surfactant in soil. For example, for similar values of SLES at the conditioning (e.g., 76 mg/kg in S2 + F and 69.4 in S4 + F + P), the DT_{50s} were quite different (17 and 9 days, respectively).

Table 5. SLES concentrations (mg/kg) in soils collected from the various conditioned microcosms over 28 days; SLES decreased (%) at day 28 and calculated disappearance times of 50% of the initial SLES concentration (DT_{50}). (s.e. = standard error).

Experimental Conditions	SLES in Soil (mg/kg ± s.e.)				SLES Decrease at Day 28 (%)	DT_{50} (days)
	0 day	7 days	14 days	28 days		
S1 + F	88.2 ± 1.6	74.5 ± 2.0	55.2 ± 1.4	43.1 ± 2.2	51	25.3
S2 + F	76.1 ± 7.4	48.6 ± 3.4	37.4 ± 0.7	28.7 ± 0.2	62	16.8
S3 + F + P	105.8 ± 1.2	59.5 ± 5.7	39.4 ± 0.1	34.4 ± 6.3	67	22.6
S4 + F	52.2 ± 8.4	25.7 ± 0.27	23.6 ± 0.8	18.5 ± 0.6	65	14.0
S4 + F + P	69.4 ± 12.8	24.8± 1.2	24.5 ± 1.1	20.4 ± 0.04	71	9.0

Table 6. SLES concentrations (mg/L) in elutriates obtained from the various conditioned microcosms (S1, S2, S3, S4) over 28 days (s.e. = standard error).

Experimental Conditions	SLES in Elutriate (mg/L ± s.e.)				SLES Decrease at Day 28 (%)
	0 day	7 days	14 days	28 days	
S1 + F	2.31 ± 0.49	2.28 ± 0.58	1.62 ± 0.18	1.27 ± 0.11	45
S2 + F	1.40 ± 0.29	0.99 ± 0.00	0.85 ± 0.02	0.55 ± 0.11	61
S3 + F + P	2.60 ± 0.56	1.45 ± 0.00	1.22 ± 0.01	0.78 ± 0.25	70
S4 + F	0.74 ± 0.01	0.40 ± 0.01	0.10 ± 0.00	<LOD *	100
S4 + F + P	1.22 ± 0.01	0.18 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	91

* Limit of detection.

A positive correlation (r = 0.99) between SLES in soils and the corresponding value in elutriates was found. The initial SLES concentration in the elutriates was in the range of 0.74–2.60 mg/L, with the highest value in S3, as shown in Table 6. At the end of the experiment, very low concentrations of SLES were detected and, in some cases (e.g., S4 + F), it was never found. The amount of SLES washed out from different soils in the corresponding elutriates was not constant and varied from 8 to 28%.

3.3. Microbial Abundance, Water Content and pH in Soils

The microbial abundance, as shown in Table 7, was initially quite low in all soils; however, its values increased over the experimental time in all conditions. Interestingly, significant differences (one-way ANOVA, p-value < 0.05) were found among the various soils. The average lowest values were in S1 + F and the highest ones in S4+F. Finally, a significant negative correlation (r = 0.85, p-value < 0.05) was found between the average microbial abundance in conditioned soils and the DT_{50} values.

Table 7. Microbial abundance (N. cells/g soil) in soil samples collected from the various microcosms (S1 + F, S2 + F, S3 + F + P, S4 + F, S4 + F + P).

Experimental Conditions	Microbial Abundance (No. Cells/g Soil)			
	0 day	7 days	14 days	28 days
S1 + F	$4.6 \times 10^4 \pm 1.7 \times 10^3$	$1.2 \times 10^5 \pm 8.9 \times 10^3$	$1.5 \times 10^5 \pm 1.6 \times 10^4$	$7.0 \times 10^4 \pm 8.8 \times 10^3$
S2 + F	$4.2 \times 10^6 \pm 5.3 \times 10^4$	$2.0 \times 10^7 \pm 7.3 \times 10^5$	$2.6 \times 10^7 \pm 3.9 \times 10^5$	$1.9 \times 10^6 \pm 2.5 \times 10^5$
S3 + F + P	$5.8 \times 10^5 \pm 2.0 \times 10^4$	$8.6 \times 10^5 \pm 5.0 \times 10^4$	$2.5 \times 10^5 \pm 2.4 \times 10^4$	$6.3 \times 10^5 \pm 1.2 \times 10^4$
S4 + F	$3.1 \times 10^6 \pm 1.7 \times 10^6$	$3.4 \times 10^7 \pm 1.7 \times 10^6$	$5.1 \times 10^7 \pm 1.3 \times 10^6$	$6.4 \times 10^7 \pm 1.7 \times 10^6$
S4 + F + P	$3.8 \times 10^6 \pm 9.0 \times 10^5$	$1.8 \times 10^7 \pm 1.5 \times 10^6$	$2.5 \times 10^7 \pm 1.7 \times 10^6$	$3.2 \times 10^7 \pm 2.1 \times 10^6$

The soil water content was different among the various soils: 15% in S1 + F; 27% in S2 + F; 29 in S4 + F + P; 30% in S4 + F; 31% in S3 + F + P. Finally, the pH was basic in all soils (9.17 in S1; 9.35 in S2; 9.57 in S3; 8.82 in S4) and its values did not change after soil conditioning.

3.4. Ecotoxicological Tests

The toxicity results of the soils and corresponding elutriates of the various conditions (S1 + F, S2 + F, S3 + F + P, S4 + F and S4 + F + P) are reported below. All validity criteria for all the tests were met and all the data reported can therefore be considered valid.

The *V. fischeri* tests were performed on elutriates obtained from all soil conditions over the experimental time (0, 7, 14 and 28 days) and the results are shown in Figure 1. The results indicate the conditioned soils were not toxic (inhibition of luminescence <20%) for the bacteria *V. fischeri*, with the exception of S3 conditioned with both the foaming agent and the polymer (S3 + F + P) at day 0 (bioluminescence inhibition of 40.63%). However, this effect was transient and from day 7 the value of inhibition of luminescence was always under 20%.

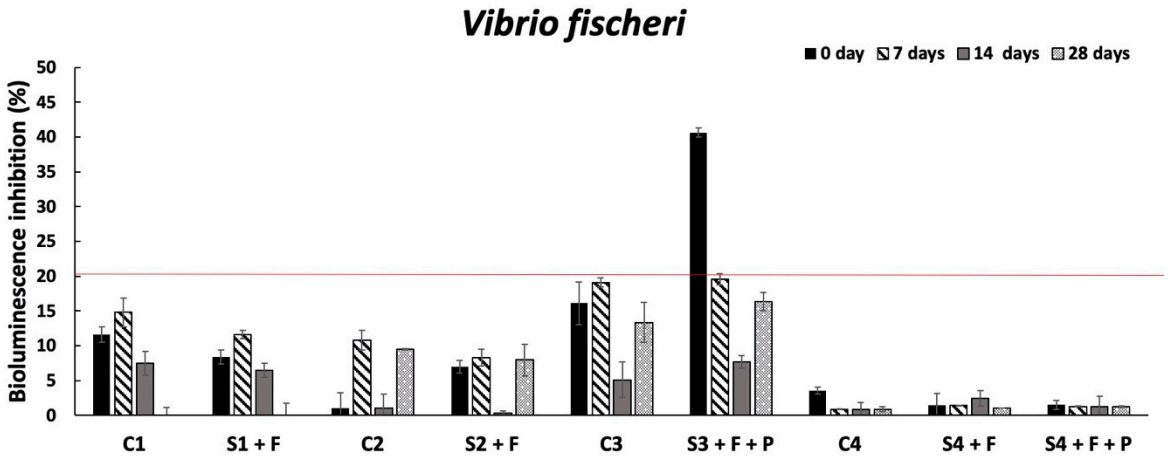

Figure 1. Inhibition of bioluminescence (%) of *Vibrio fischeri* exposed to elutriates from conditioned (S1 + F, S2 + F, S3 + F + P, S4 + F, S4 + F + P) and control (C1, C2, C3, C4) soils at different experimental times (0, 7, 14 and 28 days). The bars represent the standard errors. The red line represents the threshold of toxicity (20%).

Figure 2 shows the results of the cress germination test. The results were analyzed considering the germination index in the control as 100%. No toxic effect was observed in all soil elutriates from day 0 and during the experiment; in fact, the germination index (GIx%) was always comprised between 80 and 120%.

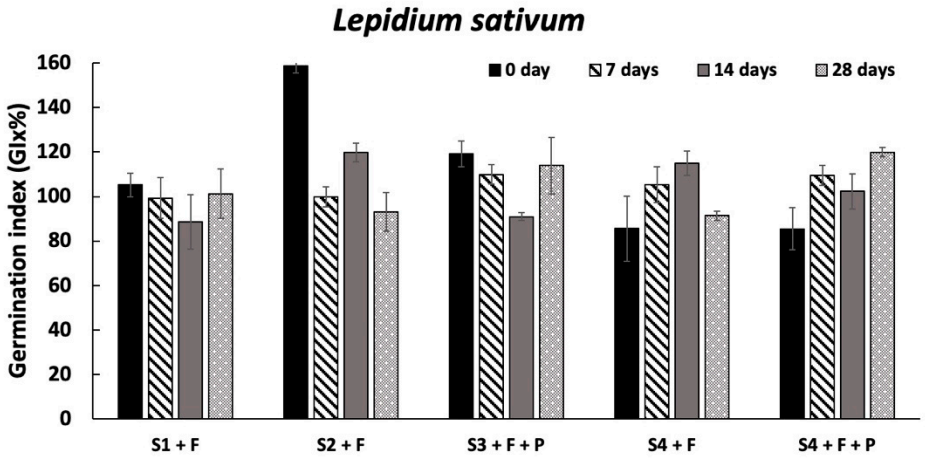

Figure 2. Germination index of *Lepidium sativum* seeds (GIx%) exposed to elutriates from conditioned soils (S1 + F, S2 + F, S3 + F + P, S4 + F, S4 + F + P) at different experimental times (0, 7, 14 and 28 days). The bars represent the standard errors.

The highest GIx% value (158%) was observed at day 0 in the S2 + F microcosms, suggesting in some cases a stimulation effect on seed germination with the presence of the foaming agent.

The acute and sub-chronic test with the ostracod *Heterocypris incongruens* was performed on S2, S3 and S4, both in the conditioned and in the control soils (C2, C3, C4). The results of the acute effects, (mortality) normalized to the control conditions, are shown in Figure 3. The Abbott's mortality of the test organisms was always lower than 20% in all conditions and sampling times. Indeed, the survival of the organisms in S3 seem to be favored by the presence of the foaming agent (Abbott's mortality value normalized to the unconditioned soil was always negative).

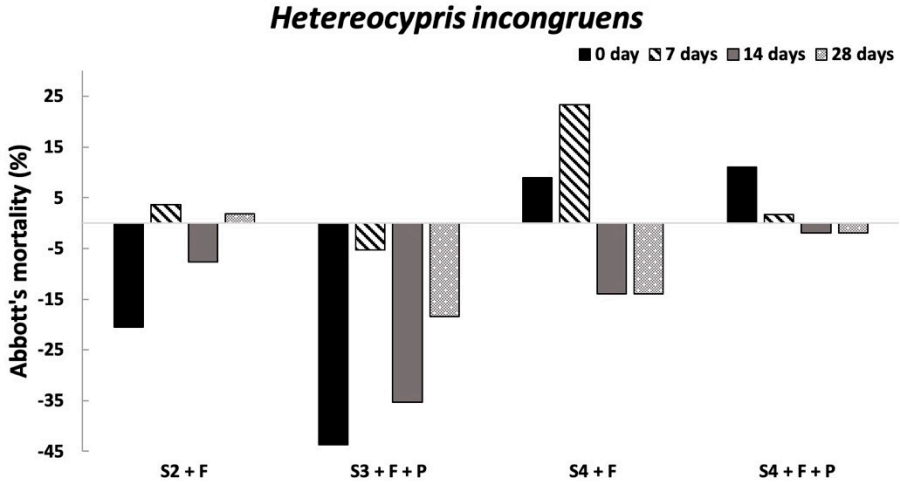

Figure 3. Abbott's Mortality (%) of *Heterocypris incongruens* exposed to conditioned soils (S2 + F, S3 + F + P, S4 + F, S4 + F + P) at 0, 7, 14 and 28 days. The data are normalized to the controls (C2, C3, C4).

The results of growth inhibition (GIn%) of *H. incongruens* are shown in Figure 4. The data show no significance effects (one-way ANOVA, p-value > 0.05) on growth in all conditions (value lower than

20%) and sampling times; on the contrary, at day 7 the presence of the conditioning agent seems to favor the growth of the ostracod. However, the mean growth of the organisms was comparable to that in the standard reference sediment.

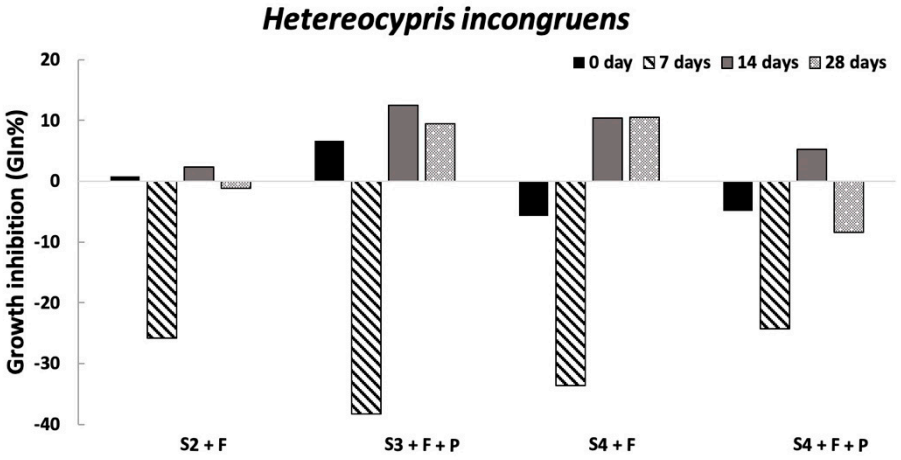

Figure 4. Growth inhibition (GIn%) of *Heterocypris incongruens* exposed to conditioned soils (S2 + F, S3 + F + P, S4 + F, S4 + F + P) at 0, 7, 14 and 28 days. The data are normalized to the controls (C2, C3, C4).

The earthworm toxicity test (chronic tests) performed with the various conditioned soils did not show any mortality (at days 14 and 28) or reproduction effect at 56 days (data not shown). Moreover, the acute test carried out on soil elutriates at different experimental times showed no toxic effect at any experimental time (0, 7, 14 and 28 days). In fact, in all the tests conducted on elutriates, the percentage of living organisms was 100%.

The *Danio rerio* acute toxicity test was only performed on the elutriates obtained from S1, both on conditioned soil (S1 + F) and the control (C1). The results of the FET test did not show any acute toxicity effects, from the start of the experiment (0d) (data not shown). In fact, the mortality of the embryo of *Danio rerio* was about 0% for control soil and 2.5% for the S1 + F conditions during all the experimental time (0, 7, 14 and 28 days).

4. Discussion

The present work shows the application of an ecological approach for the evaluation of environmental fate and the effects of foaming agents in soil debris from underground EPB-TBM excavation. It combined the analysis of SLES and the performances of ecotoxicological tests at different times (0, 7, 14 and 28 days) in both FA-conditioned soils and the corresponding elutriates, simulating the storage of the spoil material at the construction site.

The anionic surfactant decreased in soils at different rates (DT_{50} ranged from 9 to 25 days). Since the overall experimental conditions (temperature, light, pH values) were similar. The fact that SLES was degraded more (S4 + F, S4 + F + P and S2 + F) in some soils than in others (S1 + F and S3 + F + P) can be ascribed to the different soil lithological characteristics and, above all, the microbial abundance. In fact, a positive correlation was found between average microbial numbers and SLES half-lives (DT_{50s}), confirming the role of microorganisms in degrading this anionic surfactant [6,8,16]. The slowest disappearance rate in S1 + F can be also ascribed to the low water content of this soil (15% lower than in the other ones), which is known to influence biodegradation.

Following the SLES decrease in soils, lower amounts of surfactant were gradually also found in the corresponding elutriates. Elutriates were obtained in accordance with a standard procedure [27], which is used for simulating the leaching of a substance from soil to water and representing the worst scenario (no degradation) at day 0. In reality, SLES was leached from soil to water even at

concentrations quite low at day 0 and negligible from day 7. Moreover, the percentage of SLES washed out from soils was not constant and comprised the range 8–28%, indicating a high adsorption capacity of the surfactant in these soils, which is ascribable to their high fine fraction percentages. The overall results are in accordance with Finizio et al. [6], which affirm that SLES concentration in elutriates depends not only on the surfactant amount in soils, but also on their adsorption capacities, which, in turn, are influenced by soil texture and mineralogical composition [8]. The different adsorption capacity of each soil indicates the usefulness of performing site-specific studies for evaluating SLES mobilization in the aqueous phase. In fact, previous studies showed that foaming agents at the amounts used in tunneling operations are generally not toxic to terrestrial organisms, but can have a potential impact on the aquatic compartment, which is known to be very sensitive to any anionic surfactant residues [6,7,12,52].

As mentioned above, the results of the ecotoxicological tests at day 0 represent a scenario which excludes any SLES degradation in soils, by taking into account instantaneous leaching or run-off processes, simulating SLES transfer directly into the aquatic compartment.

Overall, the ecotoxicological results evidenced the absence of harmful effects on the organisms already tested at the conditioning time, supporting the first hypothesis that if SLES in soil elutriates is not higher than 2 mg/L [17], soil debris can be re-used as a safe by-product. At the concentrations used in these experiments (from 52.2 to 105.8 mg/kg), the spoil material did not cause any acute or sub-chronic effect on the organisms tested. Although, in some cases (e.g., *H. incongruens* in all conditioned soils and experimental times and *L. sativum* in S2 + F), an increase in the organism survival and growth was observed, these results cannot be considered significant because the values were in the range of those observed in the standard reference sediment. Interestingly, as assumed, in the second hypothesis, the most sensitive organism to SLES residues was confirmed to be the bacterium *V. fischeri*. In fact, at day 0 in S3 + F + P, where the highest SLES concentration was detected both in the soil (105.8 mg/kg) and in the corresponding elutriate (2.6 mg/L), a harmful effect on the bacterial bioluminescence (40% inhibition) was recorded. Anyway, this effect was transient and, in the following sampling (7 days), in line with SLES decrease in the elutriate (1.45 mg/L), it was never observed. These results are in accordance with previous ones, which showed 2 mg/L as a toxic SLES threshold in spoil material for this bacterium [17]. In fact, the authors proved this concentration value for a significant toxic effect (>20%) occurring on *V. fischeri* in soil elutriates and proposed the bioluminescence inhibition test as a suitable tool for evaluating the environmental compatibility of foaming agent-treated soils during the excavation phase. This bacterium was demonstrated to be an effective test for real matrices in other works [53] and its reliability lies in its reproducibility [54] and high sensitivity to several contaminants.

However, although SLES concentration in elutriates, which negatively affects the *V. fischeri* bacterium, is currently known, its corresponding value in soil cannot be generally established because, as mentioned above, the soil adsorption capacity can vary by several orders of magnitude among different soils. This suggests the need for site-specific studies for evaluating SLES in elutriates and confirms that the use of biological assays is the most reliable approach to assess the environmental impact of chemicals. In fact, ecotoxicological responses reflect the overall effects of contaminants, also considering 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. Moreover, the use of ecotoxicological tests on site-specific environmental matrices (soil and water extract) makes it possible to overcome the legislative gaps in this context. In fact, currently neither at EU level nor in Italy (Italian Decree 161/2012 and 120/2017) are there specific threshold limits in environmental regulations for spoil material containing additive components (e.g., SLES) used for excavation.

For these reasons, knowledge on SLES's environmental fate in realistic use conditions, together with studies on the ecotoxicological effects on terrestrial and aquatic compartments, need to be performed to ensure a correct management of the large quantity of soil debris produced during excavation works and to support the decision-making processes of stakeholders involved in tunneling projects (as performed in this specific case-study).

5. Conclusions

It is not possible to establish a priori if the soils conditioned with foaming agents used with EBP-TBM excavation can be considered a safe by-product to be promptly re-used. The application of an ecological approach for the evaluation of the environmental fate of the anionic surfactant (amount that can leach to the aqueous phase) and effects (ecotoxicological tests) is useful and effective for ensuring a safe reuse as a by-product of soil debris. In the context of a circular economy, soil debris reuse has been proven to be environmentally and economically beneficial (i.e., reduction in waste disposal) and therefore its characterization as a by-product for different purposes with the precautionary approach shown here is desirable.

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