



# Toxic response of the bacterium *Vibrio fischeri* to sodium lauryl ether sulphate residues in excavated soils

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## Abstract

Sodium lauryl ether sulphate (SLES) is the main chemical component in several lubricant products used for soil conditioning in the mechanized excavation industry using Earth Pressure Balance-Tunnel Boring Machines. During the tunnelling process, huge amounts of excavated soil are produced and the SLES presence can affect the subsequent re-use of this material as a by-product. Currently, there is still no regulatory indication of reliable and sensitive bioassays for monitoring soil quality during the excavation process. The main objective of this work was to verify if the *Vibrio fischeri* screening test was suitable as a consistent and precautionary tool for this specific purpose. Firstly, the ecotoxicity (EC<sub>20</sub> and EC<sub>50</sub>) of the SLES standard solution and three commercial products (SLES content from 10 to 50%) were evaluated to select the most environmental friendly product. Subsequently, soil samples from about 2 years of tunnelling in a real construction site, conditioned with the selected product, were evaluated for their environmental compatibility with the prescriptions of an Italian site-specific protocol. The latter established 2 mg/L as a threshold value for SLES concentration in soil water extracts and a no toxic response (≤20%) for the *Vibrio fischeri* test. The comparison of the bacterium bioluminescence inhibition values (%) with analytical determinations showed an ecotoxicity when SLES was >2 mg/L. The toxicity was directly related to SLES concentration, indicating that the *V. fischeri* test and the SLES analyses are suitable tools for assessing excavated soil as a by-product, ensuring its safe reuse in accordance with a green production process (circular economy).

**Keywords** Anionic surfactant · TBM-EPB tunnelling · Soil re-use management · Site-specific protocol · Environmental compatibility · Circular economy

## Introduction

The widespread use of Earth Pressure Balance-Tunnel Boring Machines (EPB-TBMs) in the excavation industry (for highway, railway and metro tunnel construction) is due to the various advantages compared to the traditional drill-and-blast methods. In fact, mechanized tunnelling makes it

possible to reduce the damage at surface level, lower the environmental impact on groundwater circulation, increase tunnelling speed and have safer working conditions, while lowering project costs (EFNARC 2005; Huang et al. 2018). EPB-TBM performance involves the use of commercial lubricant products (foaming agents) to condition the soil, reducing hard rock abrasiveness and facilitating excavated material transportation (Vinai et al. 2008; Peila et al. 2016). The type and amount of products depend mainly on both the TBM and excavated material characteristics (Peila et al. 2007; Peila 2014; Talebi et al. 2015). The EPB-TBM mechanized process produces huge amounts of spoil material (soil debris) which can contain commercial soil-conditioning products. Several of these consist of water solutions of the anionic surfactant sodium lauryl ether sulphate (SLES) in percentages varying from 5 to 50%; in some cases, other minor components are also present (Barra Caracciolo et al. 2017). SLES is an anionic surfactant (ANS) that can be composed by several homologues, with the hydrocarbon and the ethoxy tails that can differ in

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composition and length. ANS are considered by the European Chemical Agency as Unknown or Variable composition, Complex reaction products. SLES can have several synonyms (such as Alcohols, C10-16, ethoxylated, sulfates, sodium salts; Alcohols, C12-14, ethoxylated, sulfates, sodium salts; 2-dodecoxyethyl hydrogen sulfate), and can have different CAS (e.g. CAS#: 68585-34-2; 68891-38-3; 9004-82-4) and EC numbers (e.g. EC#: 500-223-8; 500-234-8; 618-398-5), (Barra Caracciolo et al. 2017; Finizio et al. 2020). High concentrations of SLES in soil debris (ranging from 26 to 350 mg/kg soil; Finizio et al. 2020) may pose a risk for the environment, including the aquatic one if there is a direct contact with water bodies (Mininni et al. 2018; Grenni et al. 2019; Finizio et al. 2020).

In the framework of EU policies on environmental protection such as the 7<sup>th</sup> Environment Action Programme to 2020 (European Commission 2019) one of the main objectives is a “circular economy where nothing is wasted and where natural resources are managed sustainably”. Consequently, to avoid production of millions of tonnes of waste as spoil material, the reuse of excavated soil as a by-product is desirable and implemented in most EU Countries, in line with the circular economy model (D’Aloia Schwartzentruber et al. 2019; Murr et al. 2019; Padulosi et al. 2019). Currently, at EU level there are no specific regulations for soil (European Commission 2012) or even for this specific matrix, although the use of excavated soil as a by-product is potentially regulated by the EU Directive 98/2008 relating to waste (D’Aloia Schwartzentruber et al. 2019; ITA-AITES 2019). In Italy, spoil material from excavation processes can currently be re-used as a by-product if it meets environmental legislative criteria. In particular, the chemical thresholds for several organic and inorganic contaminants should not be exceeded (Italian Decrees 161/2012, 120/2017 and 152/2006). The chemicals to be investigated are some inorganic elements (As, Cd, Cb, Ni, Pb, Cu, Zn, Hg, total Cr and Cr VI), Hydrocarbons (C>12), asbestos, BTEX (Benzene, Toluene, Ethylbenzene and Xylene) and PAHs (Polycyclic Aromatic Hydrocarbons). However, there are currently no threshold limits for SLES concentrations in either European or Italian legislation, although SLES residues in re-used excavated materials could pose an environmental risk (Finizio et al. 2020). In the case of lubricant products containing chemicals not listed in the above-mentioned Decrees, a technical document showing that the excavated soils do not represent a risk for the environmental and human health has to be approved by the Italian authorities (Padulosi et al. 2019).

Indications on the assessment of the environmental compatibility of soil debris, applying an ecological approach as regards both the foaming agent degradation and ecotoxicological effects, are lacking in the regulatory framework. An ecotoxicological approach could be very useful for evaluating the overall effects of chemical mixtures

occurring in soil treated with foaming agents (Meistro et al. 2019; Padulosi et al. 2019; Grenni et al. 2019). In fact, ecotoxicological tests overcome the limitations of chemical analysis by detecting only what is searched for, making it possible to identify both the additive and synergic effects of all environmental contaminants, including unknown ones, in an environmental matrix (Bispo et al. 1999; Ma et al. 2014). In this context, site-specific degradation studies were recently performed in microcosms, using different soils conditioned with two different commercial foaming agents, and demonstrated that SLES is a biodegradable compound (Grenni et al. 2018; Barra Caracciolo et al. 2019; Finizio et al. 2020). In fact, this anionic surfactant halved from initial concentrations of 70–100 mg/kg in 6–9 days, depending on the soil considered (Barra Caracciolo et al. 2019). Moreover, in another study, ecotoxicological tests on the terrestrial plant *Lepidium sativum* (OECD 2006), using the same spoil material conditioned with the same foaming agents, but with higher (191–350 mg/kg) concentrations, showed that SLES was non-toxic for the target species tested (Grenni et al. 2018). Furthermore, a battery test (using the *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio* embryo) was also performed on the same conditioned soils to estimate if the overall toxicity of soil water extracts decreased over time. These aquatic species were selected in order to hypothesize a worst-case scenario at the final destination site, with SLES from the soil debris being transported via runoff or leaching to a water body. The ecotoxicological tests were performed at different times from the soil conditioning in order to reproduce the temporary storage of the spoil material in the construction area, where SLES degradation can occur naturally, making the spoil material non-toxic. The results showed that the initial SLES ecotoxicity for the aquatic species decreased over time, in line with the reduction in SLES concentration in soil water extracts (Grenni et al. 2018). Finally, another recent experiment (Galli et al. 2019) performed with both *L. sativum* and *Eisenia foetida* (OECD 2006; 1984) confirmed that SLES concentrations in spoil materials are generally not toxic for terrestrial organisms, suggesting that aquatic species are more sensitive to SLES residues, as reported for surfactants in other studies (Barra Caracciolo et al. 2019). Among all the organisms tested, the bacterium *V. fischeri* (UNI EN ISO 2019) was one of the most sensitive to residual concentrations of the anionic surfactant SLES (Grenni et al. 2018). This species was also found to be highly sensitive to other surfactants (Farré et al. 2001b), including the anionic Sodium Dodecyl Sulphate (SDS), (Mariani et al. 2006), also commonly used as a reference toxicant.

The bioluminescent bacteria *Vibrio fischeri* is currently considered a useful biosensor in environmental risk assessments for chemicals (Parvez et al. 2006; Lopez-Roldan et al.

**Table 1** Foaming agents initially considered. The amounts of FAs to be used for each soil lithology (SS, MM and MIX) are reported as treatment ratio (TR) in L/m<sup>3</sup>. SLES concentrations expected in the soils are reported in mg/kg

	Density <sup>a</sup> (g/mL)	SLES (%)	TR (L/m <sup>3</sup> soil)		SLES (mg/kg) expected in soil	
			SS	MM or MIX	SS	MM or MIX
FA 1	1.04	10–20	0.59	1.46	40–80	99–197
FA 2	1.03	<30	0.35	1.12	71	227
FA 3	1.01	10–30	0.53	1.46	65–195	116–348

SS sandy-silty-clay soil, MM gravel in a sandy-silty soil, MIX a mixture of SS and MM

<sup>a</sup>The density of the FAs and % SLES are those reported in the chemical safety data sheet of the commercial products.

2012; Abbas et al. 2018). This test has also been successfully used with various environmental matrices containing chemicals, such as metals (Guéguen et al. 2004; Hsieh et al. 2004; Ishaque et al. 2006; Rosen et al. 2008; Tsybulskii and Sazykina 2010; Yang et al. 2016), PCBs and PAHs (Salizzato et al. 1998; Bispo et al. 1999; Serafim et al. 2013). The test made it possible to evaluate the harmful impacts of various complex matrices such as effluents (Reemtsma et al. 1999; Wang et al. 2002; Farré et al. 2008), sewage sludge (Farré et al. 2001a; Farré and Barceló 2003), landfill leachate (Thomas et al. 2009) and even soil, sediments and air (Girotti et al. 2008; Chen et al. 2017; Abbas et al. 2018). The test can be performed on soil water extracts or by directly exposing bacteria to a contaminated matrix and provides an accurate toxicity assessment (Bláha et al. 2010; Jarque et al. 2016).

This paper reports a data set of *V. fischeri* tests and SLES chemical determinations in soil water extracts from a real case study of a tunnel excavation in Central Italy. The ecotoxicological and chemical analyses were performed in accordance with a site-specific protocol (ISPRA 2016), agreed with the Italian Ministry of the Environment, which defined the criteria for classifying the spoil material as a by-product. For this purpose, the acute toxicity screening test with *Vibrio fischeri* and the analytical determinations of residual SLES concentrations, using the MBAS method on elutriates from each sample, were selected as the suitable parameters for evaluating the environmental compatibility of the spoil material from a tunnelling construction site. The overall results of more than 2 years of data collection are reported and discussed here.

## Materials and methods

### Study area and selection of the commercial lubricant product (foaming agent)

The geographical location of the EBP-TBM tunnel excavation was in Central Italy. Three main soil lithologies were found: gravel in a sandy-silty-clay soil (SS), gravel in a sandy-silty soil (MM) and a mixture of both (MIX). For these lithologies, three commercial lubricant products

(foaming agents, FA) were identified as suitable for the tunnelling. The amount of each product (Treatment Ratio, TR, L/m<sup>3</sup> soil) for conditioning the excavated material was selected with geotechnical tests (slump cone tests) based on each soil type (EFNARC 2005). The slump tests were performed at Polytechnic University of Turin (Italy) where a laboratory foam generation system, reproducing the conditioning activity carried out by an EPB-TBM, was available (Borio and Peila 2011; Peila 2014; Peila et al. 2016).

The three foaming agents, termed FA1, FA2 and FA3, contained a water solution where SLES was present from 10 to 30% (Table 1). In order to select the most environmental friendly FA for use at the excavation site, ecotoxicological evaluations, using the *Vibrio fischeri* test, were performed. For this purpose, the effective concentrations (EC<sub>20</sub> and EC<sub>50</sub> values) of a SLES standard solution and of the three foaming agents were determined.

The SLES concentrations expected in the conditioned soil were also calculated for each foaming agent (FA1, FA2 and FA3) by converting the TR values (L/m<sup>3</sup>) of each FA into mg SLES/kg soil, considering a soil density of 1.54 t/m<sup>3</sup> and each specific product density (Table 1). Each product had a range of SLES concentration (%) indicated in the chemical safety data sheet. The same soil samples used for the slump cone tests were also analysed for determining the actual SLES concentrations.

### Site-specific protocol for monitoring the environmental compatibility of excavated soil

In compliance with the Italian legislation, if the spoil material produced from each tunnelling construction work is used as a by-product, it has to be demonstrated that it is not waste. Owing to the lack of legal threshold limits for surfactant anions, the Italian Ministry of the Environment required specific ecotoxicological studies for the selection of the most environmental friendly foaming agent. For this purpose, a “site-specific protocol” was designed on the basis of the ecotoxicological evaluations of the foaming agents FA1, FA2 and FA3, the maximum concentrations of SLES expected in the soils conditioned (Table 1) and other site-specific environmental considerations mentioned in the “Report on the final

destination and use of the excavated soil". The Engineering contractor is required to produce this Report by the national legislation (Italian Decree 120/2017). The site-specific protocol was drawn up by the Italian Institute for Environmental Protection and Research together with the Water Research Institute of the National Research Council, the Italian National Institute of Health and the Tuscany Regional Environmental Agency (ISPRA 2016). In the study case reported here, the final destination of the excavated soil was a landfill in an area to be used for an industrial building and unrelated to any water bodies. The protocol also established in detail the operating procedures for sampling the spoil material and performing both the chemical analysis of the anionic surfactant SLES and the *V. fischeri* test on the soil elutriates. In particular, it established 2 mg/L as a threshold value for SLES concentration in soil water extracts and a no toxic response ( $\leq 20\%$ ) for the ecotoxicological test with the bacterium *Vibrio fischeri*. Both parameters needed to be satisfied to classify the spoil material as a by-product.

### Sampling and preparation of excavated soil for analysis

During the tunnelling, spoil material was directly transported by a conveyor belt to the temporary deposit areas inside the construction site where it was stocked for some days before its final use. The provisional storage of excavated soil was generally 7 days and in some cases more, depending on the results of the environmental compatibility analyses foreseen in the site-specific protocol mentioned above.

After the storage period, a composite sample was collected from the deposit area, in accordance with the operating procedures set out by specific guidelines from the Tuscany Regional Environment Agency. The sample was dried at the environmental temperature, sieved (2 mm) and then used for both the chemical analyses (foreseen in Italian Decree 152/2006, Annex 1, Section V) and for evaluating the environmental compatibility, as specified in the "Site-specific protocol" (ISPRA 2016). In this regard, the analyses were performed on the soil water extracts (elutriates), which were prepared in a solid/liquid ratio of 1:10 with distilled water (taking the moisture already in the sample into account). The elutriates were obtained following standardized procedures for waste characterization (UNI EN 2004) and in accordance with other authors (Hubálek et al. 2007; Marguá et al. 2016). In brief, each soil sample (3 replicates, 100 g each) was put into a bottle (1 L) with distilled water. The bottles were shaken to simulate the leaching process (in the dark for 24 h, 20 °C). After the solid particles fell to the bottom (15 min), the supernatant phase was then centrifuged (15 min at 9000 rpm). The elutriates were subsequently processed for SLES determination or filtered (0.45 µm, cellulose acetate *Whatman*) for ecotoxicological analysis in accordance with UNI EN 2005. The elutriates

obtained were stored in polyethylene vials at 4 °C until ecotoxicological and chemical analysis. The environmental compatibility analyses were performed on soil water extracts of about 150 soil samples collected during more than 2 years of tunnel excavation.

### Chemicals and SLES determination

Chemical Chloroform and methanol of HPLC grade, sulphuric acid (98%) and methylene blue were obtained from VWR (Radnor, PA, USA). Sodium hydrogen carbonate and anhydrous sodium carbonate were purchased from Carlo Erba Reagents (Milano, Italy). The water was purified (18 MΩ/cm quality) using a Milli-Q system (Millipore, Bedford, MA, USA). The SLES standard (CAS#: 68585-34-2, technical grade purity of 71.92%) was purchased from BOC Sciences (US, Canada) and used as the MBAS (Methylene Blue Active Substances) reference compound. The stock solution of SLES was prepared at 1000 mg/L in methanol and stored at -20 °C. Two calibration curves for SLES were obtained by analysing working standard solutions in lower (0.05–0.5 mg/L) and higher (0.5–4.0 mg/L) concentration ranges.

The SLES extraction from conditioned soil was performed by Pressurized Liquid Extraction (PLE), as described in detail in Barra Caracciolo et al. (2019). The PLE extracts and the soil water extracts (elutriates) were analysed for SLES content with a quick and easy method that could be used by the national and regional agencies in charge of environmental monitoring. In particular, the optimized MBAS spectrophotometric method, reported in detail elsewhere (Grenni et al. 2018), was used. SLES was extracted three times with solvent chloroform to obtain a blue salt. The intensity of the blue colour of the SLES-MBAS complex was then measured using a UV/Vis spectrophotometer (Perkin Elmer 25) at a wavelength of 650 nm. The SLES concentration was finally calculated using the previously determined calibration curve. The limit of detection (LOD) (Thompson et al. 2002) was 0.013 mg/L. SLES was determined in both the FA solutions prepared for evaluating EC<sub>20</sub> and EC<sub>50</sub> values and in soil water extracts produced from the spoil material over 2 years of the tunnelling.

### Ecotoxicological tests

The *Vibrio fischeri* acute toxicity test was performed using a Microtox® analyser (Model 500, Modern Water, UK) in accordance with both the manufacturer's instructions and the standard method (UNI EN ISO 2019). This test is based on the inhibition of the luminescence naturally emitted by the bacterium *Vibrio fischeri* after its exposure to a sample. The difference in light output between the sample and the blank (saline solution, 20 g/L NaCl) is due to the sample effect on the organisms, measured at 30 min of exposure. The endpoint



(luminescence inhibition %) is calculated using the Microtox calculation software (Microtox Omni® software V 4.2).

The lyophilized, freeze-dried *Vibrio fischeri* (strain NRRL B-11177) and the reconstitution solution (RS) for the bacteria were purchased from Ecotox LDS s.r.l. (Milan, Italy). To achieve a ready-to-use bacterial suspension, the lyophilized bacteria (batch number 17H4227) were rehydrated with the RS. Before starting the test, an osmotic adjustment was performed, using a saline solution (22% w/v NaCl in Milli-Q water) to make the sample salinity optimal (2%) for *V. fischeri*.

The toxicity of the SLES standard solution and foaming agents (FA1, FA2 and FA3) was expressed as the effective concentration (EC) causing 20% (EC<sub>20</sub>) and 50% (EC<sub>50</sub>) of luminescence inhibition. The EC<sub>20</sub> and EC<sub>50</sub> values were determined with various dilutions (using distilled water) of a stock solution (50 mg SLES standard/L water) of SLES; 7 solutions (0.6, 1.3, 2.6, 5.1, 10.2, 20.5, 40.9 mg/L) were obtained and tested. In a similar way, a stock solution (127.6, 130.5, 127.6 mg FA/L water for FA1, FA2 and FA3, respectively) for each foaming agent was diluted to obtain 7 solutions (1.6, 3.3, 6.5, 13.1, 26.1, 52.3, 104.5 mg/L) to be tested. The concentrations of SLES in the solutions were analysed with the MBAS method above described. The test was repeated three times; the EC<sub>20</sub> and EC<sub>50</sub> (average values) were calculated using the Microtox software.

The screening tests on spoil material were performed on 6 replicates of soil water extract (elutriates) using a 1:10 ratio soil/water following the procedure described in UNI EN 2005. At the same time, the saline solution (blank, 20 g/L NaCl) and the reference toxicant (3,5-Dichlorophenol aqueous solution, 3.5 mg/L) were used in all tests as negative and positive controls, respectively. The pH value was recorded and eventually neutralized, as required by the standard procedures for the *V. fischeri* test. The coefficient of variation ratio (CV<sub>R</sub>% = standard deviation/mean × 100) for test reproducibility was also calculated. If necessary, a correction of the colour or turbidity of the elutriates was applied in accordance with the standardized method and with procedural adaptations by other authors (Lappalainen et al. 2001; Volpi Ghirardini et al. 2009; Jarque et al. 2016). The colour correction was useful for avoiding interference factors that could cause false toxic responses.

## Results

### Toxicity of SLES standard solution and foaming agents

Figure 1 shows the dose-response relationship for SLES standard solution. The bioluminescence inhibition of the bacterium *V. fischeri* expressed as EC<sub>20</sub> and EC<sub>50</sub>

corresponded to average SLES concentrations of  $1.3 \pm 0.1$  mg/L and  $4.00 \pm 0.1$  mg/L, respectively (Table 2). The coefficient of variation ratio was always  $\leq 20\%$ , see Table 2 (Environment Canada 2007).

The dose-response relationships for the three commercial lubricant products (foaming agents, FA) considered in this study are reported in Fig. 2a–c for FA1, FA2 and FA3, respectively.

The EC<sub>20</sub> and EC<sub>50</sub> average values estimated for the foaming agent FA1 (5.8 and 17.3 mg/L, respectively) were almost two times higher than those of FA2 and FA3 (2.2 and 6.9 mg/L, respectively), demonstrating that FA1 was less toxic than the other two FAs (Table 2). Moreover, the lowest SLES concentrations expected in soils were also those of FA1 (Table 2). The latter result was confirmed by the actual SLES concentrations; in fact, the average values measured for the three lithologies (SS, MM and MIX) in conditioned soil samples were: FA1: 177.5 mg/kg < FA2: 232.3 mg/kg < FA3: 250.5 mg/kg.

For these reasons, FA1 was selected as the commercial product to be used for the tunnelling and included in the site-specific Protocol for conditioning the soil at the actual excavation site.

### Environmental compatibility of excavated soil in accordance with the site-specific protocol

As mentioned above, the tunnel excavation was performed using the FA1 commercial product as the conditioning agent at the treatment ratios indicated in Table 1. The site-specific protocol established that the spoil material produced weekly during the tunnelling should be put in temporary deposit containers constructed at the excavation site.

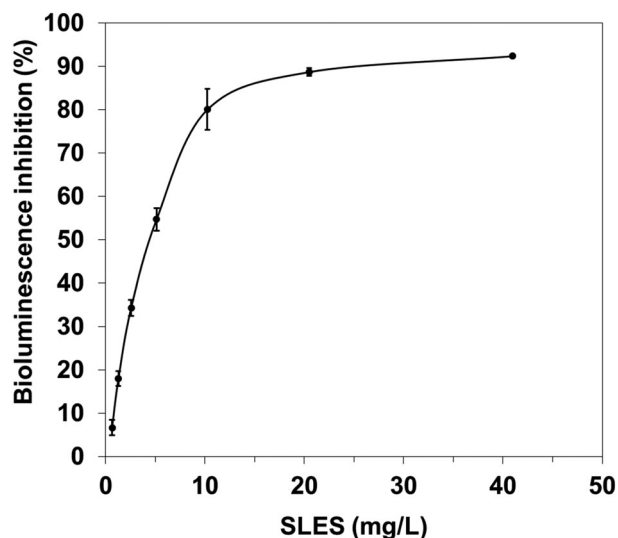


Fig. 1 Dose-response relationship between the concentration (mg/L) of SLES standard solution and the effect (% bioluminescence inhibition) in *V. fischeri*

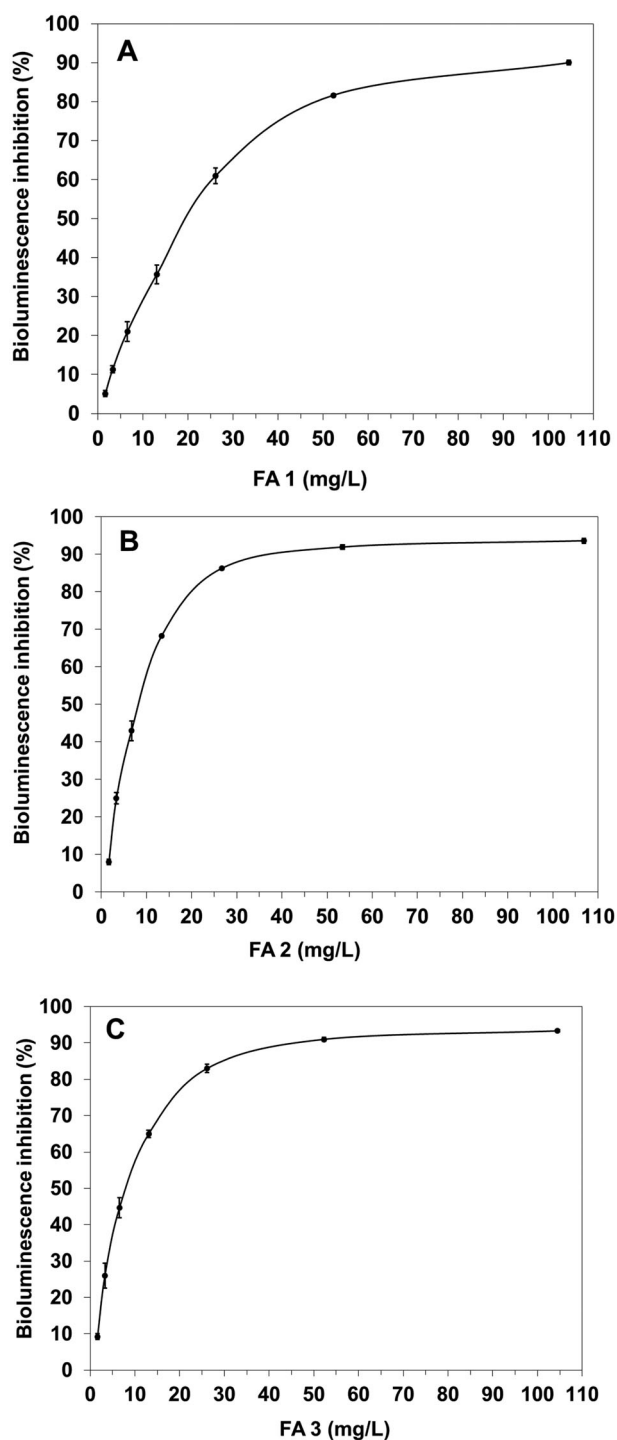
**Table 2** Toxicity of the SLES standard solution and of the three commercial products (FA 1, FA2, and FA3) expressed as EC<sub>20</sub> and EC<sub>50</sub> ± standard errors (s.e.) CV<sub>R</sub>: Coefficient of variation ratio

	EC <sub>20</sub>		EC <sub>50</sub>	
	mg/L ± s.e	CV <sub>R</sub> (%)	mg/L ± s.e.	CV <sub>R</sub> (%)
SLES (standard solution)	1.3 ± 0.1	8.0	4.0 ± 0.1	5.20
FA 1	5.8 ± 0.6	18.0	17.9 ± 1.5	14.82
FA 2	2.2 ± 0.3	17.9	6.9 ± 0.9	14.36
FA 3	2.2 ± 0.3	18.0	6.9 ± 0.9	14.51

Before its final destination, the environmental compatibility analyses foreseen in the site-specific protocol (ecotoxicological test and SLES concentration in elutriates) were performed 7 days after the temporary storage. The *V. fischeri* toxic responses (% bioluminescence inhibition at 30 min) and the SLES residual concentrations (mg/L) measured in soil elutriate samples over time, are reported as monthly averages in Fig. 3. The CV<sub>R</sub> values for *V. fischeri* tests were always ≤20% (Environment Canada 2007). SLES concentration in the spoil material and its effect percentage did not exceed the threshold limit of 2 mg/L and bioluminescence inhibition of 20% in most cases. In a few cases the samples required more time (ranging from 15 to 18 days) to be in line with the Protocol thresholds. Interestingly, when plotting the chemical (SLES residual concentrations) versus the ecotoxicological data, a significant ( $r = 0.8$ ;  $p < 0.01$ ) correlation was found. This means that the bacterium bioluminescence inhibition was directly correlated to SLES residues detected in the elutriate, as shown in Fig. 4. However, there were a few samples where, although the concentration of SLES was less than 2 mg/L, a bioluminescence inhibition higher than 20% was found.

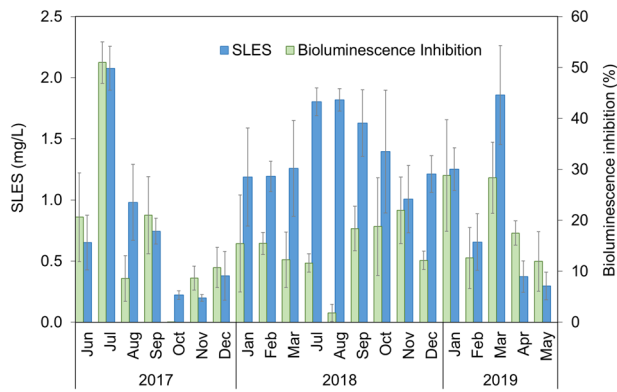
## Discussion

In the coming years, due to the numerous tunnelling projects planned in Europe and worldwide, several hundreds of millions of tons of spoil material containing SLES will be produced. A recent paper (Finizio et al. 2020) reports the environmental risk assessment of SLES in 12 foaming agents used for tunneling in site-specific conditions. Owing to the presence of residual SLES concentrations in the material excavated and to the fact that it can be in contact with aquatic bodies at some final destination sites, potential threats to the natural environment have to be taken into account. This work reports the application of the *Vibrio fischeri* test to the SLES compound on its own, to three commercial products containing SLES as the main component in a variable percentage and finally to soil treated

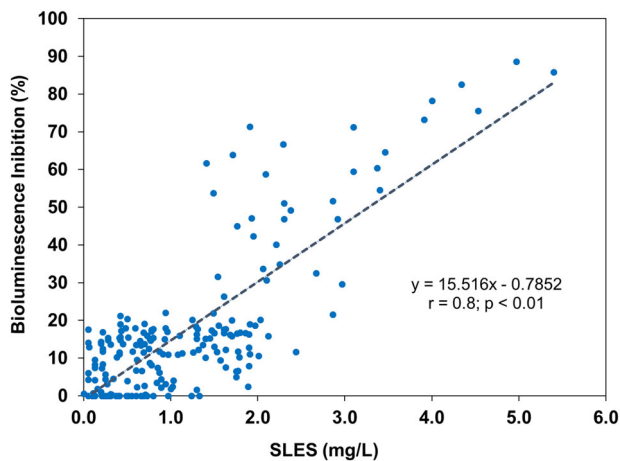


**Fig. 2** Dose-response relationship between the concentration (mg/L) of each commercial foaming product (A: FA 1; B: FA 2; C: FA 3) and the effect (% bioluminescence inhibition) in *V. fischeri*

with a foaming agent (FA1) from a real tunnelling project. *V. fischeri* is a gram-negative luminescent bacterium and its exposure to toxic substances causes the inhibition of enzymatic activity, corresponding to a decrease in bioluminescence. The light intensity reflects the bacterium's



**Fig. 3** SLES residual concentrations (mg/L) and bacterium bioluminescence inhibition (%) in soil water extract samples from the tunnelling excavation site using FA1 as the conditioning foaming agent. Each datum is reported as monthly average value of 4–5 elutriates



**Fig. 4** Bioluminescence inhibition values (%) of the bacterium *V. fischeri* versus SLES concentrations (mg/L) from the tunnelling excavation site (c.a. 150 samples). SLES concentrations are the residual ones found in elutriates obtained from soil conditioned with FA 1

metabolic status and is related to the toxicity of a xenobiotic (Meighen 1991, 1993; Ma et al. 2014). The theoretical effective concentrations evaluated using SLES on its own showed *V. fischeri* to be very sensitive to the anionic surfactant and confirm that it is potentially harmful for aquatic organisms (Ying 2006; Mariani et al. 2006; Karray et al. 2016). Other comparisons performed with pharmaceuticals and personal care products containing surfactants reported that *V. fischeri* exhibited greater sensitivity than assays involving other aquatic test organisms (Ortiz de García et al. 2014). A review reported the effective concentrations of SLES for various aquatic organisms and found  $EC_{50}$  values higher (less toxicity) for crustaceans, fish and other invertebrates and a comparable value for a microalga (Barra Caracciolo et al. 2017). The ecotoxicological analysis of the foaming agents showed how their intrinsic toxicity ( $EC_{20}$  and  $EC_{50}$ ) is ascribable to their percentage content of SLES

(Table 1). The SLES percentage in FA1 (10–20%) was lower than in the other FAs (FA2 < 30% and FA3 10–30%). In accordance with these data, the effective concentrations ( $EC_{20}$ : 5.8 mg/L and  $EC_{50}$ : 17 mg/L) for *V. fischeri* in the case of FA1 were higher than the other two commercial products, owing to a lower SLES concentration (Table 2). The SLES expected in the spoil material depended on the TR used for the soil conditioning and the percentage of the anionic surfactant in each foaming agent (see TR values and SLES % for each FA in Table 1). Consequently, in the FA1 conditioned soil lower SLES concentrations would be expected, as detected in this study.

Surfactants are known to be toxic for microorganisms at a critical micelle concentration (CMC) and SLES is reported to form micelles at a concentration higher than 300 mg/L (Aoudia et al. 2009). Surfactant micelles can solubilize the membrane lipid of bacterial cells and lead to cell lysis (Glover et al. 1999; Li and Chen 2009). On the other hand, the bacterium *Vibrio fischeri* was particularly sensitive to SLES, showing a toxic response at concentrations that were very low and much below the CMC.

The overall data sets (150 samples over 2 year of analyses) of the FA1-conditioned soil samples from the tunnelling construction site definitely confirmed the relationship between SLES residues and its toxicity (Fig. 4). The amount and degradation of the anionic surfactant in the spoil material during its storage in temporary deposit areas is a key factor for its potential toxicity in the corresponding soil water extract, as found in a previous work (Grenni et al. 2018). In all cases where the bioluminescence inhibition was less than 20%, SLES was present at residual concentrations  $\leq 2$  mg/L in the elutriates. The fact that some samples were not in compliance with the threshold of 2 mg/L after 7 days from the conditioning depended on a delay in the SLES degradation, presumably due to abiotic factors (e.g. temperature, water soil content). Moreover, in another few cases, although SLES concentrations were very low (much less than 2 mg/L), a significant effect (>20%) on the *V. fischeri* was found. This could be ascribable by other chemicals in the soil (not included in the Italian decrees on spoil materials from tunnelling) which can negatively affect the bacterium response, showing how this test is more reliable than the chemical analysis alone of a single component such as SLES. In fact, the ecotoxicological response refers to effects of overall chemicals of a sample. Other studies used *V. fischeri* as an effective screening test for soil samples (Parvez et al. 2006; Abbas et al. 2018). However, this work is the first one with a huge number of soil debris samples, produced from a long time real excavation work, which has demonstrated a significant correlation between SLES content and *V. fischeri* response.

The overall results showed that *V. fischeri*, beyond its sensitivity to low SLES concentrations, was a useful, reliable and reproducible tool for meeting tunnelling operational needs (e.g. short time for obtaining the results) and making it

possible to recycle spoil material as a safe by-product in line with a circular economy strategy.

The test organism has a high sensitivity to various contaminants (Parvez et al. 2006; Yang et al. 2016; Wiczerzak et al. 2016; Abbas et al. 2018) and the test procedure makes it possible to obtain reliable and reproducible data thanks to its standardized method, involving an automatic equipment. The test is cost-effective and can be performed quickly, since bacterial metabolism responds promptly to the presence of a toxic compound (Coz et al. 2007). All these characteristics make the test a suitable tool for evaluating the environmental compatibility of foaming agent conditioned-soil. For this reason, we suggest the bioluminescence inhibition test be included as a precautionary tool in a regulatory framework for evaluating the environmental compatibility of spoil material.

## Conclusions

The successful use, in support of analytical determinations, of *V. fischeri* as an ecotoxicological tool for assessing the environmental compatibility of spoil materials containing FAs was demonstrated thanks to the significant correlation between the anionic surfactant SLES and ecotoxicity. Moreover, the effectiveness of the site-specific protocol used for assessing the environmental compatibility of a huge amount of soil debris from the real excavated site was validated.

The bioluminescence inhibition test made it possible to obtain information in a real case study about the different interactions among the mixture (soil debris and foaming agent) and the specific matrix (different soil lithologies conditioned with different FA amounts), and the possible ecotoxicological effects. If in the soil excavated there was an unknown element, the test showed a higher toxicity than expected, as occurred in some samples. The test characteristics in terms of SLES sensitivity, reliable response and fast and easy application made it possible to quickly assess if the spoil material could be used as a by-product.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any research using humans or (vertebrate) animals performed by any of the authors and it is in compliance with ethical standards.

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