IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

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To cite this article: D I Stom *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **990** 012073

IOP Conf. Series: Earth and Environmental Science **990** (2022) 012073 doi:10.1088/1755-1315/990/1/012073

Methodological approaches to assessing the toxicity of compounds by changing the behavioral response of soil oligochaetes

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Abstract. The methodological aspects of assessing the toxicity of surface-active substances (SAS) by changing the behavioral response of avoiding the substrate by earthworms Eisenia fetida andrei Bouche, 1972 were studied. The conditions for biotesting were selected, under which the method is more sensitive to the tested pollutants. In this method, 30 worms. placed in a Petri dish with a substrate unfavorable for worms (dry sand). Around it, Petri dishes with test samples of moistened sand contaminated with individual toxicants in various concentrations and their mixtures were placed radially. A control sample (wet sand) was also placed among the prototypes. When the worms selected the most favorable of the tested contaminated substrate samples, their movement from dry sand was observed. After 4 h from the start of the experiment, the number of worms in each of the compartments was counted. The toxic effect of the cationic surfactant cetyltrimethylammonium bromide (CTAB) was manifested when its content in the soil sample was from 0.001 g / kg; nonionic surfactant Tween-80 – from 1 ml / kg; anionic surfactant sodium lauryl sulfate – from 20 mg / kg.

1. Introduction

Biotesting of contaminated soils has a number of features, which is why there is a significant lag in the development of environmental standards in relation to soils in comparison with other media, primarily water. This is due to the complexity and heterogeneity of the object. The soil is a multi-component system. This greatly complicates the regulation of the content of pollutants in soils and their adequate ecological assessment. Particular complications arise in the case of complex technogenic pollution. Namely, it is most often the case [1].

The above indicates the demand for research aimed at developing new methods for soil biotesting. The most important requirements for biotesting methods are speed, simplicity and cost-effectiveness of experiments (both in material terms and in terms of labor costs); test object availability; reproducibility and reliability of the results obtained [2].

Currently, soil biotesting in most cases is carried out by assessing the toxicity indicators of aqueous extracts from soils. However, a number of authors have shown that the results of determining the toxicity of soils and water extracts from them by the biotesting method very often differ significantly. The difference in results is especially significant when soil is contaminated with toxicants that are poorly soluble in water, for example, oil products, oil and its hydrocarbons. Thus, the most promising methods for bioanalysis of soil media are those that are based on the reactions of soil organisms and, accordingly, make it possible to assess the toxicity of soil samples directly. Soil oligochaetes are one of these test objects. In this case, such test reactions of earthworms are used as survival, reproduction. Earlier, we also proposed to evaluate the toxicity of soil samples by the thickness of the coprolite layer formed in the process of vermit transformation by worms of the substrate [3-7].

The aim of this work was to consider some methodological aspects in assessing the biological effects of surfactants on the behavioral response of soil oligochaetes E. fetida andrei Bouche, 1972.

2. Materials and methods

The object of the study was the red Californian hybrid *E. fetida andrei* Bouche, 1972. This worm belongs to *Annelida*, class *Oligochaeta*, family *Lumbricidae*. In the laboratory, the worms were bred in boxes at a temperature of 20-25 °C and a humidity of 80-85%; vegetable leftovers (potatoes, carrots, cabbage) were used for feeding. In the experiments, we used sexually mature worms with a girdle zone (clitellum) 8-10 cm long [8-11].

The tested substances in the work were surfactants – representatives of 3 main types of different groups: 1) cetyltrimethylammonium bromide (CTAB, $C_{19}H_{42}BrN$) – quaternary ammonium cationic surfactant; 2) sodium lauryl sulfate (sodium dodecyl sulfate SDS, $C_{12}H_{25}SO_4Na$) – sodium salt of lauryl sulfuric acid, anionic surfactant; 3) tween-80 (Tween-80, polysorbate 80, $C_{64}H_{26}O_{124}$) – polyoxyethylene derived from sorbitan and oleic acid, nonionic surfactant.

The criterion for assessing the effect of toxicants was the behavioral response of the choice of substrates by soil oligochaetes.

When using the worm preference reaction as a test parameter. The main substrate was sand (particle size 0.5-1 mm), which was preliminarily washed with distilled water. The test substances were added to it. For this, two modifications of the method were used, which was used to select the most and least favorable substrates for worms or feed mixtures.

Modification of method No. 1. The rectangular container was divided into 2 compartments using a partition. Its height was 1.5-2 times lower than the height of the sides of the container. These compartments were filled with test samples (in equal volumes): 1 – control (sand moistened to 80%); 2 – experience (wet sand + pollutant). In each of the compartments, 10 individuals of *E. fetida andrei* were placed (figure 1).

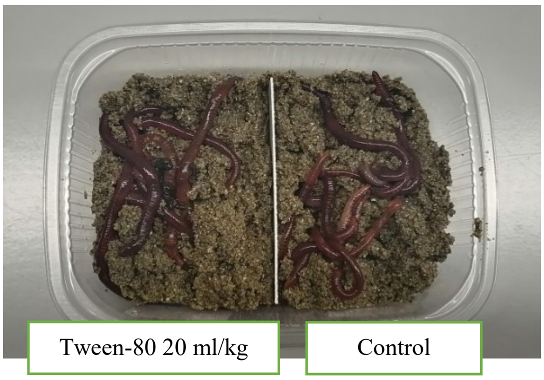


Figure 1. Setting up an experiment to assess the toxicity of test samples of sand contaminated with the studied toxicants by determining their reaction of preference or avoidance by worms *E. fetida andrei* Bouche, 1972 (modification of method No. 1).

After 4 h from the start of the experiment, the number of worms in each of the compartments was counted. The toxicity of the test sample in relation to the behavioral response of the worms was calculated by the formula (1):

$$T = \frac{K - O}{K} \times 100\% \tag{1}$$

where T is toxicity (% in relation to control); K is the initial number of worms placed in one compartment (10 individuals) at the beginning of the experiment; O – the number of worms found in the experimental compartment at the time of the recording of the results of the experiment.

The toxicity values calculated in this way reflect both the inhibitory (positive T value) and the stimulating (negative T value) effects of the test samples on the *E. fetida andrei* Bouche, 1972.

Modification of method No. 2. Another variant of experimental design was used in the work. It is especially useful in the selection of the optimal formulations of feed mixtures for worms). In this method, 30 worms. placed in a Petri dish with a substrate unfavorable for worms (dry sand). Around it, Petri dishes with test samples of moistened sand contaminated with individual toxicants in various concentrations and their mixtures were placed radially. A control sample (wet sand) was also placed among the prototypes (figure 2).

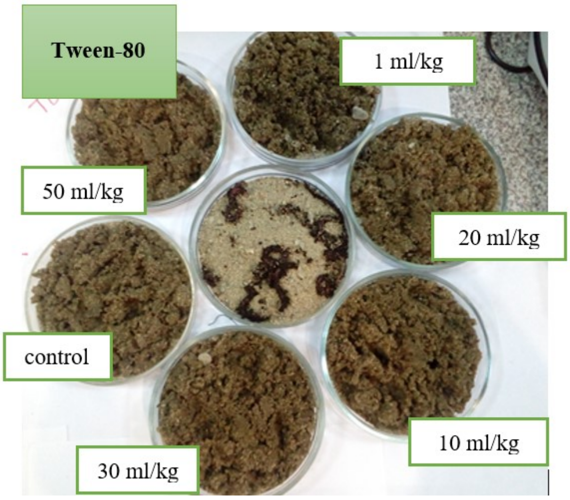


Figure 2. Setting up an experiment to assess the toxicity of the test samples of sand contaminated with the studied toxicants, by determining the reaction by their preferences or avoiding the worms *E. fetida andrei* Bouche, 1972 (modification of method No. 2).

When the worms selected the most favorable of the tested contaminated substrate samples, their movement was observed from the less preferred samples for worms. After 4 h from the start of the experiment, the number of worms in each of the compartments was counted. The toxicity of the test sample in relation to the behavioral response of the worms was calculated by the formula (2):

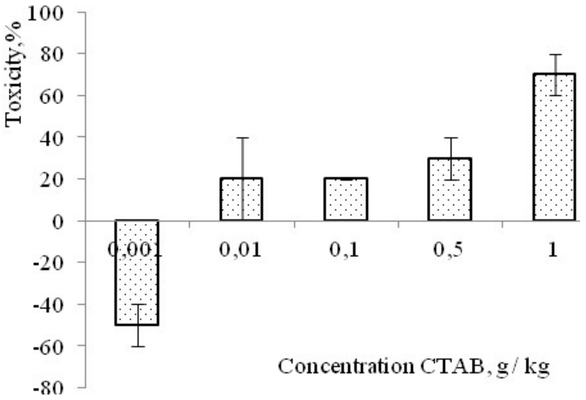
$$T = \frac{K - O}{K} \times 100\% \tag{2}$$

where T is toxicity (% in relation to control); K is the number of worms detected at the moment of taking the result in the control compartment; O – the number of worms found at the time of the removal of the result in the test compartment.

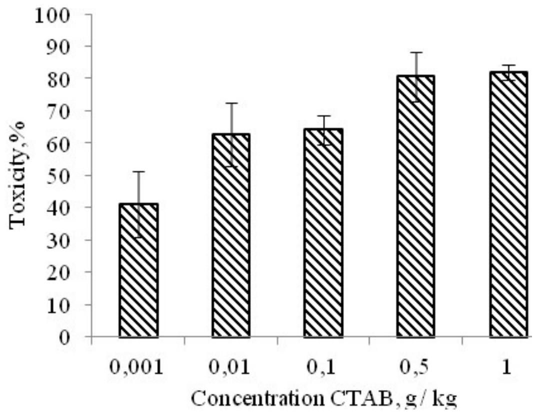
Statistical data processing was performed using the Microsoft Office software package. The experiments were carried out in 5 independent experiments, 3 replicates in each experiment. The conclusions were made with the probability of an error-free prediction $P \geq 0.95$.

3. Results

When using modification No. 1 of the method for assessing the choice of feed mixtures by earthworms, the following results were obtained. CTAB at a concentration of 0.001 g / kg had a stimulating effect on the studied behavioral response of oligochaetes. A sand sample contaminated with 0.001 g / kg CTAB attracted 50% more worms than a control sample (sand without toxicant added). CTAB at a concentration of 0.01 g / kg had no visible effect on the test reaction of E. fetida andrei Bouche, 1972. An increase in the content of CTAB in sand samples to 0.1 g / kg led to the manifestation of a toxic effect. The toxicity index in this case was 20%. The toxicity of the sample containing 0.5 g / kg CTAB reached 30%, and with the addition of 1 g / kg of the tested surfactant – 70% (figure 3a).



a



b

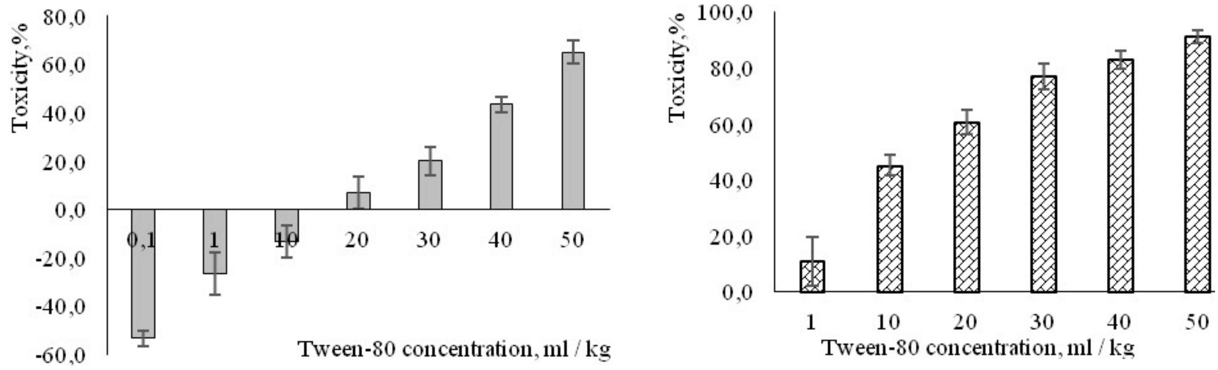
Figure 3. The results of assessing the effect of CTAB on the behavioral response of substrate avoidance by earthworms *E. fetida andrei* Bouche, 1972, obtained using a modification of method No. 1 (a) and modification of method No. 2 (b).

The reaction of preference for test samples by worms, assessed by modification No. 2, was found to be more sensitive to CTAB. Thus, the toxicity of CTAB was manifested already in its presence at a concentration of 0.001 g / kg and amounted to 41%. With the introduction of 0.01 and 0.1 g / kg surfactant, the toxicity index was 63 and 64%, respectively. With an increase in the content of CTAB to 0.5 and 1 g / kg, the toxicity increased to 81-82% (figure 3b).

Evaluation of the effect of the nonionic surfactant Tween-80 also showed a higher sensitivity of the modification of method No. 2 compared to method No. 1. Thus, when tested by method No. 1, the studied surfactant showed a toxic effect on the behavioral response of E. fetida worms at a concentration of 30 ml / kg – the toxicity of soil samples containing the indicated surfactant content was 20%. When soil samples were contaminated with 0.1 and 1.0 ml / kg Tween-80, a stimulating effect was observed (figure 4a).

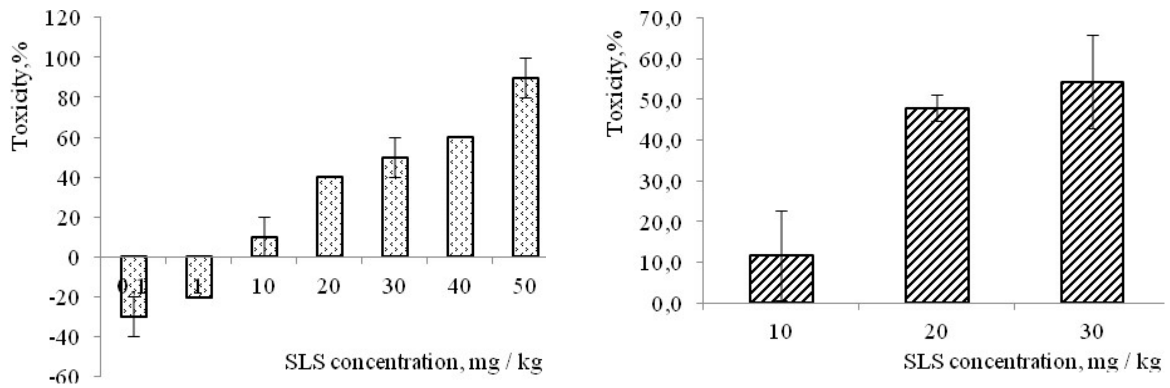
In the case of using modification method No. 2, it was shown that the medium with tween-80 had a toxic effect on the behavioral response of worms even at a concentration of 1 ml / kg. In this case, the toxicity of the substrates was 11% when tested for 4 h. A further increase in the surfactant concentration in the substrates (up to 50 ml / kg) led to a sequential increase in toxicity (figure 4b).

The anionic surfactant sodium lauryl sulfate had a toxic effect on the behavioral response of *E. fetida* at a concentration of 20 mg / L or more when tested by both the first and the second methods (figure 5).



a b

Figure 4. The results of evaluating the effect of Tween-80 on the behavioral response of substrate avoidance by earthworms *E. fetida andrei* Bouche, 1972, obtained using a modification of method No. 1 (a) and modification of method No. 2 (b).



a b

Figure 5. The results of assessing the effect of sodium lauryl sulfate on the behavioral response of substrate avoidance by earthworms *E. fetida andrei* Bouche, 1972, obtained using a modification of method No. 1 (a) and modification of method No. 2 (b).

4. Conclusion

Thus, the behavioral avoidance response of the earthworms *E. fetida andrei* Bouche, 1972 is acceptable for assessing surfactant toxicity. At the same time, this method had the greatest sensitivity when it was executed in modification No. 2. In this method, worms in the amount of 30 pcs. placed in a Petri dish with a substrate unfavorable for worms (dry sand). Around it, Petri dishes with test samples of moistened sand contaminated with individual toxicants in various concentrations and their mixtures were placed radially. A control sample (wet sand) was also placed among the prototypes. When the worms chose the most favorable of the tested contaminated substrate samples, their movement from the samples unfavorable for the worm was observed. After 4 h from the start of the experiment, the number of worms in each of the compartments was counted. The toxicity of the test sample in relation to the behavioral response of the worms was calculated by the formula (2):

$$T = \frac{K - O}{K} \times 100\% \quad (3)$$

where T is toxicity (% in relation to control);
K is the number of worms detected at the moment of taking the result in the control compartment;
O – the number of worms found at the time of the removal of the result in the test compartment.

The toxic effect of the cationic surfactant CTAB was manifested when its content in the soil sample was from 0.001 g / kg; nonionic surfactant Tween-80 – from 1 ml / kg; anionic surfactant sodium lauryl sulfate – from 20 mg / kg.

Acknowledgments
This work was supported by the RFBR grant 19-29-05213 "Mechanisms of the complex interaction of soils with oil, oil products and surfactants in the processes of oil pollution and bioremediation."

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