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Effect of cationic, anionic and non-ionic surfactants on soil oligochaetes *Eisenia fetida andrey* (Bouche, 1972)

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Results of studying the main types of surfactants effect on earthworms *Eisenia fetida andrey* (Bouche, 1972) are presented. The worms were tested for survival and preference-avoidance behavioral reactions. Earthworms avoided sodium dodecyl sulfate (SDS) at 0.02 g/kg and above, cetyltrimethylammonium bromide – at 0.1 g/kg and above, and polysorbate 80 – at 30 mL/kg and above. The 100% mortality of earthworms occurred at 0.001, 0.01 and 0.02 g/kg of SDS on day 30, at 1 g/kg – on day 25, and at 1 g/kg – on day 2 of the experiment. Cetyltrimethylammonium bromide at 0.001 and 0.01 g/kg caused the 100% mortality of worms on day 30; at 0.1 g/kg – on day 25; at 0.5 g/kg – on day 5; and at 1.0 g/kg already on day 2 of the experiment. The 100% mortality of worms occurred at 0.1, 1 and 10 mL/kg of polysorbate 80 on day 30 of incubation; at 20 or 30 mL/kg – on day 20; at 50 mL/kg – on day 10 of experience. The following toxicity series (both in terms of g/L and in terms of g/mol) of the tested surfactants for earthworms was constructed on the basis of the results obtained (increasing toxicity): polysorbate 80 (non-ionic surfactant) < cetrimonium bromide (cationic surfactant) < sodium dodecyl sulfate (anion-active surfactant).

Keywords: surfactants, soil pollution, sodium dodecyl sulfate, cetyltrimethylammonium bromide, polysorbate 80, toxicity tolerance, survival of earthworms, bioassay.

УДК 573.6

Влияние катионоактивного, анионоактивного и неионогенного поверхностно-активных веществ на почвенные олигохеты *Eisenia fetida andrey* (Bouche, 1972)

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Изучено действие основных типов поверхностно-активных веществ (ПАВ) на дождевых червей *Eisenia fetida andrei* (Bouche, 1972). Тест-реакциями червей при этом служили выживаемость и поведенческая реакция предпочтения-избегания. Дождевые черви избегали додецилсульфат натрия при его содержании от 0,02 г/кг почвы, цетилтриметиламмония бромид – от 0,1 г/кг, твин-80 – от 30 мл/кг. Гибель 100% особей происходила при концентрации додецилсульфата натрия 0,001; 0,01 и 0,02 г/кг на 30-е сут, в присутствии 0,1 г/кг – на 25 сут, а при 1 г/кг – уже через 2 сут эксперимента. Цетилтриметиламмония бромид вызывал гибель всех червей на 30 сут опыта – при содержании 0,001 и 0,01 г/кг, на 25 сут – при внесении 0,1 г/л, на 5 сут – 0,5 г/кг, и уже на 2 сут опыта – при 1,0 г/кг. В присутствии 0,1; 1 и 10 мл/кг твина-80 отмирание 100% особей червей наступало на 30 сут инкубирования, при добавлении 20 и 30 мл/кг – на 20 сут, при 50 мл/кг ПАВ – на 10 сут опыта. Полученные результаты позволили выстроить следующий ряд токсичности (как в пересчёте на г/л, так и в пересчёте на г/моль) испытуемых ПАВ для дождевых червей (по возрастанию степени токсичности): твин-80 (неионогенный ПАВ) – цетилтриметиламмония бромид (катионоактивный ПАВ) – додецилсульфат натрия (анионоактивный ПАВ).

Ключевые слова: поверхностно-активные вещества, загрязнение почвы, додецилсульфат натрия, бромид цетилтриметиламмония, полисорбат 80, толерантность к токсичности, выживаемость дождевых червей, биотестирование.

Surfactants comprise a highly prevalent class of pollutants of various media. In particular, soils are subject to pollution with surfactants. Along with the infiltration of domestic wastewater through the soil, a significant amount of surfactants entering soil horizons is associated with their various applications in the oil industry [1–3]. In addition, surfactants are increasingly used in the reclamation of oil-contaminated media [4–10].

Once in the ground, surfactants can affect the soil flora, fauna and microbiota. In this connection, a fairly extensive body of research has been devoted to studying the effect of surfactants on microorganisms [11–17]. However, the effect of surfactants on soil worms is a less studied issue, as evidenced by the much smaller number of works [18–20]. At the same time, it is difficult to overestimate the role of these invertebrates in maintaining fertility to support normal life-sustaining activity and allow revegetation of polluted or disturbed soils.

In connection with the above, the purpose of the present work is to study the biological effects of representative anionic, cationic and non-ionic surfactants on earthworms *Eisenia fetida andrei* (Bouche, 1972).

Materials and research methods

The object of research was the red Californian earthworm *Eisenia fetida andrei* (Bouche, 1972). The worms were bred in boxes in a laboratory at a temperature of ~ 20 °C and a humidity of 80–85%. The worms were fed by introducing vegetable waste (potatoes, carrots, cabbage). In the experiments we used sexually mature worms of the same age with a girdle zone (clitellum), 8–10 cm in length.

The studied surfactants are representatives of three main classes of surfactants: cationic – CTAB (High Purity Grade, >99.0%); anionic – sodium dodecyl sulfate (puriss grade); non-ionic – polysorbate 80 (imp).

CTAB is a cationic surfactant of the quaternary ammonium compounds class. It comprises the main component of the topical antiseptic cetrimide, an effective remedy against bacteria and fungi.

Sodium dodecyl sulfate (sodium lauryl sulfate) is a sodium salt of lauryl sulfuric acid.

This anion-active surfactant is widely used as a strong detergent in industry, pharmacology, and cosmetology.

Polysorbate 80 (Tween-80) is a polyoxyethylene (POE) derived from sorbitan and oleic acid. This non-ionic surfactant is an emulsifier and solubilizer of fats. It is used in the cosmetics and food industries, as well as in remediation of oil-contaminated soils, etc.

In our experiments contamination of the test substrates (soil, sand) was conducted by applying liquid surfactants and thoroughly mixing them in a Waring 800G laboratory blender (Conair Corporation, USA).

To assess the survivability of earthworms 10 individuals were placed in containers with 50 g of a test sample of river sand contaminated with surfactants. The sand was calcined and thoroughly washed with distilled water prior to the experiments. The sand without added pollutants was the control. The worms were exposed in the experiment for 30 days in the same conditions as their cultivation. During this time the number of surviving individuals of *E. fetida andrei* was periodically calculated.

In parallel the toxic effect of the tested compounds was evaluated by the behavioural reaction of preference-avoidance of the substrate by *E. fetida* earthworms [21]. Rectangular polyethylene containers (10.8 × 8.2 × 4.6 cm) were divided into 2 compartments using a plastic partition (Fig. 1). The height of the latter was 1.5–2 times lower than the height of the container sides. These compartments were filled with equal volumes of test samples of substrates: 1 – control (moistened up to 80% sand); 2 – experiment (moistened up to 80% sand contaminated with surfactants). 10 individual *E. fetida andrei* were placed in each of the compartments.

After 4 hours from the start of the experiment, the number of worm individuals in each of the compartments was counted. The biological effect of the test sample on the earthworm behavioural reaction was calculated using the formula:

$$T = \frac{K_0 - K_i}{K_0} \cdot 100\%$$

where T – biological effect (%); K_0 – initial number of worms placed in one compartment (10 individuals) at the beginning of the experiment; K_i – the number of worms observed in the experimental compartment at the time of taking down the results of the experiment.

The parameter calculated by such formula can reflect both preference (negative T value) and avoidance (positive T value) reactions of the *E. fetida* specimens.

The experiments were carried out in at least 5 independent experiments, with three repetitions in each. Statistical data processing was carried out using the Microsoft Office software package. The conclusions are made with the probability of an error-free forecast P≥0.95.

Results and discussion

Studying the CTAB effect on earthworms. At 0.001 g/kg CTAB stimulated the studied behavioural response of oligochaetes. A sand sample contaminated with CTAB of 0.001 g/kg attracted 50±8% more worms than the control sample (sand without added pollutants). CTAB at a concentration of 0.01 g/kg did not cause significant changes in *E. fetida* test reaction in comparison with the control. However, an increase in

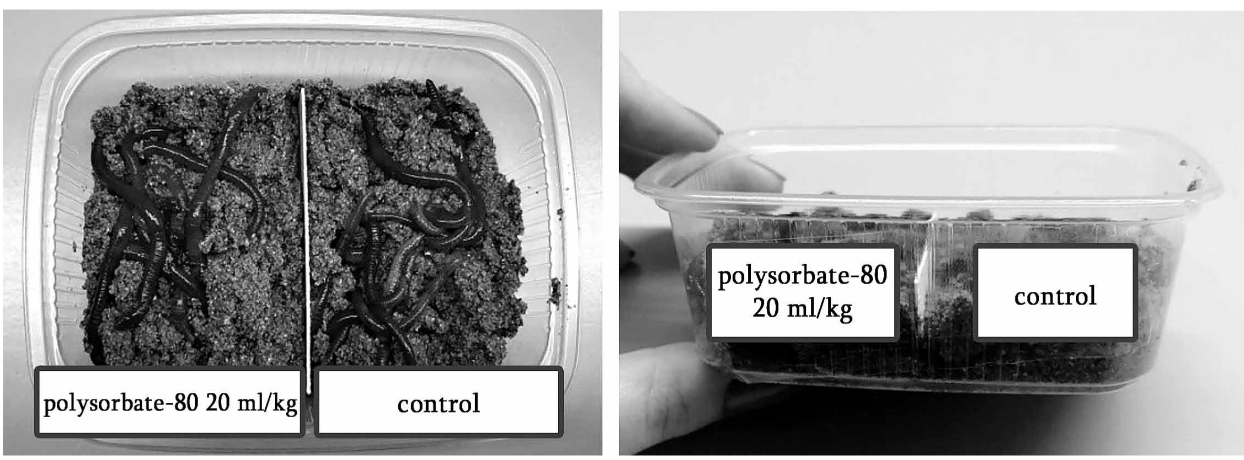

Fig. 1. Setup of experiment to evaluate the effects of tested sand samples contaminated with test toxicants using a preference-avoidance reaction with worms *Eisenia fetida andrei* (Bouche, 1972)

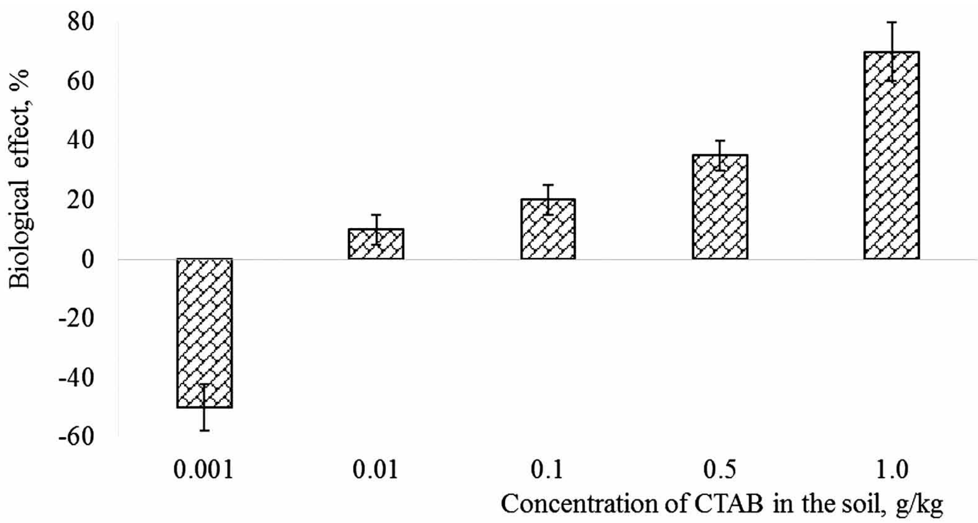

Fig. 2. The cationic surfactant CTAB effect on the preference-avoidance reaction of earthworms (experiment duration – 4 h)

Table 1

The CTAB cationic surfactant effect on the earthworms' viability

CTAB content, g/kg	Earthworms mortality, % of baseline							
	Day 1	Day 2	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	0	15±5	100
0.01	0	0	0	0	0	0	55±8	100
0.1	0	0	0	0	0	15±5	100	100
0.5	0	0	100	100	100	100	100	100
1.0	0	100	100	100	100	100	100	100

the CTAB content in the substrate samples up to 0.1 g/kg induced a worm avoidance reaction. In this case, the *T* indicator was 20%. The increase in CTAB up to 0.5 g/kg increased *T* to 35±5%; when 1 g/kg of the test surfactant was added *T* was 70±10% (Fig. 2).

Incubation of earthworms for 30 days in STAB contaminated sand samples revealed the following. The complete death of worms in the presence of 0.001 and 0.01 g/kg of CTAB was observed on day 30 of the experiment. The proportion of dead individuals in these samples on day 25 was 15±5 and 55±8%, respectively. At application of 0.1 g/L all worm individuals died on day 25; at 0.5 g/kg – on day 5; at 1.0 g/kg – already on day 2 of the experiment (Table 1).

Studying the sodium dodecyl sulfate (SDS) effect on earthworms. The anion-active surfactant sodium dodecyl sulfate negatively affected the preference response of earthworms at 0.02 g/kg content. A sand sample with the above content of surfactant was avoided by 40±7% of worms. With a subsequent increase in the SDS content the avoidance response increased. Thus, at a surfactant content of 0.03 g/kg worms avoidance of the sample was 50±10% of the total number of individuals taken in the experiment; at 0.04 g/kg – 60±8%; at 0.05 g/kg – 90±9 % of worms (Fig. 3).

Conversely, the lower SDS content – 0.0001 and 0.001 g/kg – attracted the earthworms. Thus, the samples with 0.0001 g/kg of SDS were preferred by 30±5%, and with 0.001 g/kg – 20±6% of the individuals taken in the experiment. The SDS addition of 0.01 g/kg did not affect the behavioural response of earthworms (Fig. 3).

In parallel, the SDS effect on the survivability of earthworms was evaluated. This surfactant caused 100% mortality of individuals at 0.001, 0.01 and 0.02 g/kg for 30 days; at 0.1 g/kg – for 25 days; at 1 g/kg – for 2 days of the experiment (Table 2).

Studying the polysorbate 80 effect on earthworms. The non-ionic surfactant polysorbate 80 also differently affects the behavioural

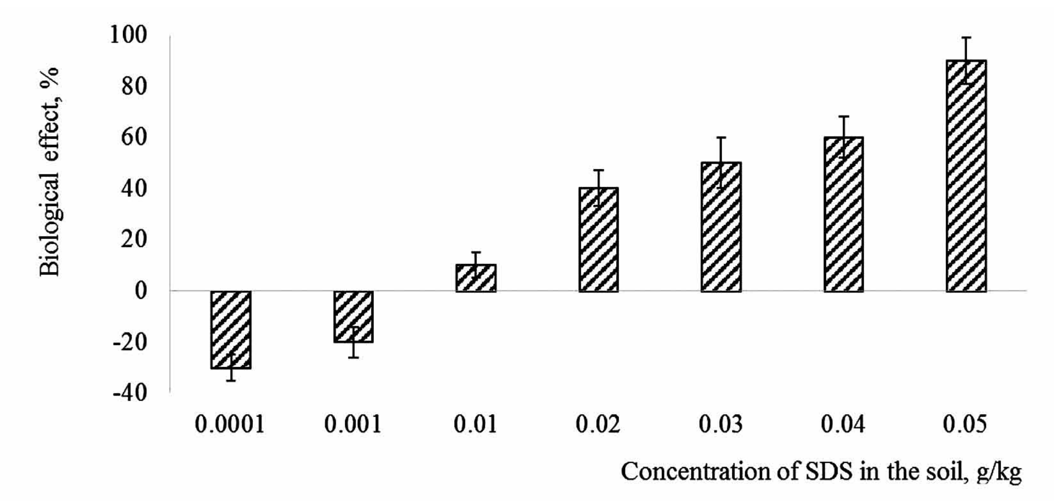

Fig. 3. The anion-active surfactant SDS effect on the preference-avoidance reaction of earthworms (experiment duration – 4 h)

Table 2

The SDS anion-active surfactant effect on the earthworms' viability

SDS content, g/kg	Earthworms mortality, % of baseline							
	Day 1	Day 2	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0	0	0	0	0	0	0	0
0.001	0	0	0	0	0	20	45±8	100
0.01	0	0	0	0	0	10	30	100
0.02	0	0	0	0	0	5	15±3	100
0.1	0	0	0	20	35±5	65±5	100	100
1	0	100	100	100	100	100	100	100

reaction of earthworms depending on the concentration. In concentration ranging from 0.1 to 10 mL/kg polysorbate 80 caused a preference reaction in worms, while 30 mL/kg and above led to an avoidance response to the tested substrates. For example, sand samples with 0.1, 1 and 10 mL/kg of polysorbate 80 preferred respectively 53±3, 27±9 and 13±6% more worms than control samples. The worms' behaviour was not significantly affected by polysorbate 80 in the substrate at 20 mL/kg. The number of individuals that chose this substrate was comparable to that in the control. 20±6, 43±3 and 65±5% of *E. fetida* individuals escaped from samples supplemented with 30, 40 and 50 mL/kg polysorbate 80, respectively (Fig. 4).

The 100% mortality of earthworms occurred on day 30 of incubation at 0.1; 1 and 10 mL/kg of polysorbate 80; with the addition of 20 and 30 mL/kg – on day 20, with 50 mL/kg of surfactant – on day 10 of experience (Table 3).

Thus, based on the concentrations at which the tested surfactants had a negative effect on earthworms, it can be concluded that the anion-active SDS had a higher toxicity (in terms of both g/L and g/mol). It caused an avoidance reaction in earthworms at 0.02 g/kg content. The 100% mortality of individuals occurred when this surfactant was added at concentrations of 0.001, 0.01 and 0.02 g/kg on day 30; at 0.1 g/kg – on day 25; at 1 g/kg – on day 2 of the experiment.

The cationic surfactant cetyltrimethylammonium bromide (CTAB) turned out to be somewhat less toxic than sodium dodecyl sulfate (SDS). The CTAB negatively affects the behavioural reaction of oligochaetes at 0.1 g/kg. The 100% mortality of earthworms was observed at 0.001 and 0.01 g/kg on day 30 of experience; at 0.1 g/kg – on day 25; at 0.5 g/kg – on day 5; at 1.0 g/kg – already on day 2 of experience.

The non-ionic polysorbate 80 was the least aggressive of the three tested surfactants. Its effect on the behavioural reactions of earthworms depends on the concentration. Polysorbate 80 positively affected the studied behavioural reaction at a range from 0.1 to 10 mL/kg, and negatively affected – at 30 mL/kg and above.

The 100% mortality of worms occurred at 0.1, 1 and 10 mL/kg of polysorbate 80 on day 30 of incubation; at 20 or 30 mL/kg – on day 20; at 50 mL/kg – on day 10 of experience.

The resulting toxicity ratio of the surfactants (belonging to three different types) used in the work in relation to *E. fetida* turned out to be quite similar to that obtained by us and other authors earlier using other test organisms. For example, in studies to evaluate the effect of surfactants on the survival of *Paramecium caudatum* and *Daphnia magna* [22], as well as on changes in the intensity of bioluminescence of a cell culture of marine luminous bacteria *Photobacterium phosphoreum* and their enzyme system NAD(P)H:FMN oxidoreductase + luciferase (Red + Luc) [23]. The similar results were shown in the works [24, 25] to study the effect of surfactants on the release of electrolytes from cells of plants – *Beta vulgaris* L. and *Elodea canadensis*. Other authors also reported lower toxicity of non-ionic surfactants compared to ionic ones [26, 27].

T. Pescatore et al. [28] studied the effect of sodium laureth sulfate (SLES) on *E. fetida*. It was shown that 40% of worms were attracted to soil contaminated with SLES at a concentration of 4 mg/kg. At the highest surfactant concentration in soil tested by the authors (201 mg/kg), the avoidance reaction increased significantly (up to 67%). This suggests that earthworms will avoid such contaminated soil.

It was observed that Tween-80 additions to soil changed the fractional distribution of fluoranthene in soil and had a positive effect on fluoranthene removal and vermiaccumulation [19]. Some researchers have used worm survival rate as a test to evaluate the effect of detergents based on various surfactants [20, 29].

However, it should be noted that such works are quite small in number and are not comprehensive. In addition, a comparison of the toxicity of surfactants belonging to different classes towards *E. fetida* has not previously been carried out.

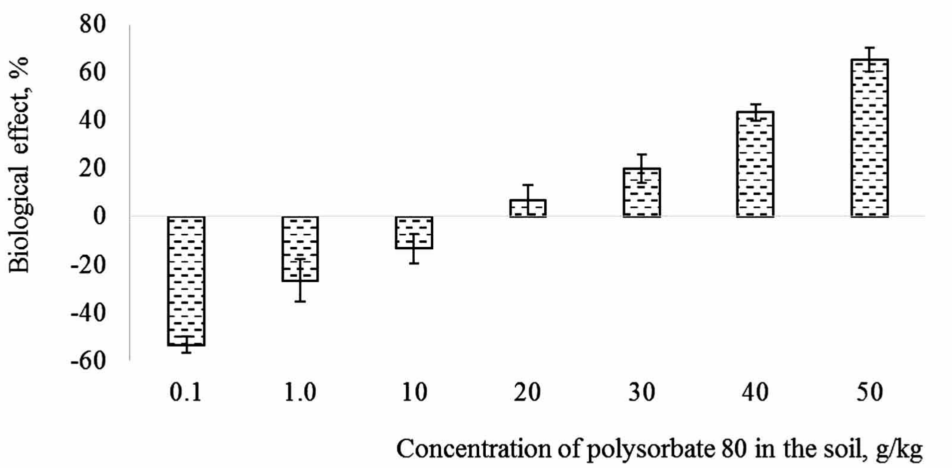

Fig. 4. The non-ionic polysorbate 80 surfactant effect on the preference-avoidance reaction of earthworms (experiment duration – 4 h)

Table 3

The non-ionic surfactant polysorbate 80 effect on the earthworms' viability

Polysorbate 80 content, mL/kg	Earthworms mortality, % of baseline							
	Day 1	Day 2	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	5±1	100
1	0	0	0	0	0	0	20±6	100
10	0	0	0	0	0	0	50	100
20	0	0	0	0	30	100	100	100
30	0	0	0	0	40	100	100	100
50	0	0	10±5	100	100	100	100	100

Conclusion

The following toxicity series of test surfactants in relation to survival and preference-avoidance behavioural reaction of *E. fetida* was constructed on the basis of the experimental results (increasing the degree of toxicity): polysorbate 80 (non-ionic surfactant) < CTAB (cationic surfactant) < SDS (anion-active surfactant).

The obtained data demonstrated that the preference-avoidance behavioural test reaction of *E. fetida* provides valid information about the toxicity of the tested surfactants at higher concentrations than in the assessment of worm survival. This is due to the difference in the contact time of the test organism with the toxicant (4 hours and 30 days, respectively). The sensitivity of acute biotests is always lower than in chronic experiments. However, despite the lower sensitivity of the behavioural response compared to survival, the significant advantage of the former is its rapidity. The possibility of obtaining a test response in a short time (4 hours) makes the preference-avoidance worms reaction promising for certain tasks of biological analysis, especially when conducting a preliminary assessment of contamination. In addition, the described test reaction makes it possible to detect not only the negative effect (toxicity) of the tested compounds, but also their stimulating effect (biological activity).

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