Toxic effects of Tween-80 and its mixtures with oil on oligochaetes

K D Zyuzina¹, M M Gelman¹,², G O Zhdanova¹, A B Kupchinsky² and D I Stom¹,²,³

¹ Irkutsk State University, 1, st. Karl Marx, Irkutsk, 664003, Russia
² Baikal Museum of the Irkutsk Scientific Center, 1, st. Akademicheskaya, Listvyanka, 664520, Russia
³ Irkutsk National Research Technical University, 83, st. Lermontov, Irkutsk, 664074, Russia

E-mail: stomd@mail.ru

Abstract. This study investigates the toxicity of the nonionic surfactant Tween-80, both its pure form and mixtures with oil, against the Baikal oligochaetes Mesenchytraeus bungei Michaelsen, 1901. It was experimentally shown that mixtures of the surfactant under study and oil have a toxic effect on oligochaetes at concentrations, at which the action of individual substances is harmless. In addition, this negative effect intensifies with an increase in the concentration of both the surfactant and oil in the mixtures.

1. Introduction
The remediation of oil-contaminated media is increasingly performed using various reagents. Among them, polysorbate 80 (Tween-80, C₆₄H₁₂₄O₂₆) is attracting practical attention [1-5] due to its low cost and low toxicity. The use of polysorbate 80 for removing oil and other hydrophobic pollutants determines the possibility of its presence, along with oil products, in the as-decontaminated soils. All these components can enter water bodies with thawing and rain water. Despite this potential danger, the biological effects caused by the combined presence of various pollutants have been poorly investigated. In this regard, the aim of this work was to study the toxicity of the nonionic surfactant Tween-80, both its pure form and mixtures with oil, against the Baikal oligochaetes Mesenchytraeus bungei.

2. Materials and methods
The object of the study was the Baikal oligochaetes Mesenchytraeus bungei Michaelsen, 1901. Systematics: type: Annelida Lamarck, 1809; class: Clitellata Michaelsen, 1919 (girdle); subclass: Oligochaeta (small-bristle annulus (worms)); order: Enchytraeida; family: Enchytraeidae Vejdovsky, 1879; genus: Mesenchytraeus Eisen, 1878; species: Mesenchytraeus bungei Michaelsen, 1901.

Oligochaetes (figure 1) were collected in July–August 2020 along the coastal zone of Lake Baikal near the village of Bolsheie Koty (South Baikal, 51°54'25" N 105°04'14" E).

Prior to experiments, the collected oligochaetes underwent adaptation for 2 days under laboratory conditions using an aeration system equipped with a micro-compressor. Their incubation was carried out in glass or plastic cages filled with non-sterile Baikal water, at a temperature of 8–10°C, illumination 250–300 lux. River sand was placed at the bottom, with the layer thickness of 0.8–1.5 cm.
Figure 1. Baikal oligochaetes *Mesenchytraeus bungei* Michaelsen, 1901.

Investigated toxicants.
1) Tween-80 (polysorbate 80, C_{64}H_{26}O_{124}) – polyoxyethylene (figure 2).

![Figure 2. Structural formula of Tween-80.](image)

This non-ionic surfactant is an emulsifier and solubilizer of hydrophobic products, widely used in cosmetics [2] and the food industry, as well as for remediation of oil-contaminated soils. In our study, the effect of this surfactant was evaluated at concentrations of 1; 10; 20; 30; 40 and 50 mM.

2) Oil from the Markovskoye field of the Irkutsk region.

Estimation of the survival rate of oligochaetes.

In order to study effects of the Tween-80 and oil mixtures on the survival of Baikal oligochaetes, 20 ml of both the oil emulsion and surfactant solution under study were almost simultaneously poured into a Petri dish. Baikal water was added to the solutions. The experiment was started immediately after mixing the components.

10 individuals of oligochaetes with a length of about 1.8–2.0 cm were placed into each dish. The experimental dishes were maintained for 4 days under the same conditions at which the oligochaetes were cultivated. The criterion for death was complete immobilization.

Statistical data processing.

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 under the P ≥ 0.95 probability of error-free prediction.

3. Results

During the experiment (4 days), Tween 80 solutions did not lead to the death of Baikal oligochaetes across the entire concentration range (1; 10; 20; 30; 40 and 50 mM). However, Tween 80 mixed with oil or diesel fuel (at their initial concentrations having no visible effect on oligochaetes) caused a toxic effect. This negative effect became more pronounced upon an increase in the Tween-80 concentration.
For example, in mixtures of 10 ml/L of oil and 1 mM of Tween-80, the death of 20% of oligochaetes was noted. When the content of Tween-80 was increased to 10–20 mM and 30–40 mM, 30% and 60–70% of the individuals died, respectively. The addition of 50 mM of Tween-80 to 10 ml/L of oil resulted in the death of all the Baikal oligochaetes under study (figure 3).

![Figure 3](image)

**Figure 3.** Effects of oil (10 ml/L), Tween-80 (1; 10; 20; 30; 40 and 50 mM) and their mixtures on the death of Baikal oligochaetes (experiment time – 4 days).

Similar and even stronger effects were produced by an increase in the oil concentration up to 25 ml/L. Thus, in mixtures containing 25 ml/L of oil and Tween-80 at the concentrations of 1 and 10 mM, the mortality rate of oligochaetes comprised 40 and 50%, respectively. In similar variants characterized by a lower oil content of 10 ml/L, 20 and 30% of individuals died, respectively.

The addition of Tween-80 to 25 ml/L of oil at concentrations of 20-40 mM led to the death of 60% of oligochaetes. When the content of Tween-80 in the mixture was increased to 50 mM, 100% of the individuals under study died (figure 4).

![Figure 4](image)

**Figure 4.** Effects of oil (25 ml/L), Tween-80 (1; 10; 20; 30; 40 and 50 mM) and their mixtures on the death of Baikal oligochaetes (experiment time – 4 days).
Similar experiments were carried out with the addition of oil at an even higher concentration of 50 ml/L to Tween-80 (1; 10; 20; 30; 40 and 50 mM) solutions. This increase in the oil content further enhanced the toxic effect of its mixtures with Tween-80, as compared to the above-described combinations of Tween-80 (1; 10; 20; 30; 40 and 50 mM) and oil (10 and 25 ml/L). Thus, the death of 100% oligochaetes occurred in the presence of 30 mM Tween-80 in the mixture; however, at lower oil contents (10 and 25 ml/L), this effect was observed only with the addition of 50 mM of Tween-80 (figure 5).

![Figure 5](image5.png)

**Figure 5.** Effects of oil (50 ml/L), Tween-80 (1; 10; 20; 30; 40 and 50 mM) and their mixtures on the death of Baikal oligochaetes (experiment time - 4 days).

An increase in the content of Tween-80 and oil in the mixture increased the toxic effect (figure 6).

![Figure 6](image6.png)

**Figure 6.** Effects of Tween-80 mixtures (1; 10; 20; 30; 40 and 50 mM) with oil (10; 25 and 50 ml/L) on the survival rate of Baikal oligochaetes (survival of oligochaetes in individual oil emulsions and in solutions of Tween-80 in all investigated concentrations was 100%).
4. Discussion
It was shown that mixtures of oil and the nonionic surfactant Tween-80 have a toxic effect on oligochaetes at concentrations, at which the action of individual substances is harmless. An increase in the concentrations of Tween-80 and oil in the mixtures under study intensified the toxic effect.

Presently, it is premature to talk about the mechanism underlying the observed phenomenon. Among possible explanations is the suggestion that the increased toxicity is associated with an increase in the bioavailability of oil hydrocarbons under the action of surfactants, due to an increase in the solubility (solubilization) of hydrocarbons in the presence of Tween-80 [6-11]. However, the observed effects could also have been caused by weakening of the barrier properties of biomembranes under the action of Tween-80, which facilitated the entrance of oil.

5. Conclusion
In this study, such concentrations of oil emulsions and Tween-80 solutions that caused no visible toxic effect on the Baikal oligochaetes Mesenchytraeus bungei Michaelsen, 1901 for 4 days were selected. At the same time, the toxicity of oil and Tween-80 mixtures at concentrations, at which the action of individual substances was harmless to oligochaetes, was observed. Notably, the observed negative effect intensified with an increase in the concentration of both the detergent and oil in the mixtures.

Acknowledgments
This work was supported by the RFBR grant No. 19-29-05213 MK “Mechanisms of complex interaction of soils with oil, oil products and surfactants in the processes of oil pollution and bioremediation”. The studies were carried out using the resources of the Center for Collective Use of the Baikal Museum of the Irkutsk Scientific Center (http://ckp-rf.ru/ckp/495988/).

References
[1] Zvereva Yu M 2016 What do Baikal worms breathe? Science First Hand 68(2) 654
[2] Trellu C, Mousset E, Pechaud Y, Huguenot D, van Hullebusch E D, Esposito G and Oturan M A 2016 Removal of hydrophobic organic pollutants from soil washing/flushing solutions: A critical review. J. Hazard. Mater. 306 149-174
[3] Yang S, Liu L, Han J and Tang Y 2020 Encapsulating plant ingredients for dermocosmetic application: an updated review of delivery systems and characterization techniques. International Journal of Cosmetic Science 42 16–28
[4] Gao Y Z, Ling W T, Zhu L Z, Zhao B W and Zheng Q S 2007 Surfactant-enhanced phytoremediation of soils contaminated with hydrophobic organic contaminants: Potential and assessment. Pedosphere 17(4) 409–418
[5] Lu H, Wang W, Li F and Zhu L 2019 Mixed-surfactant-enhanced phytoremediation of PAHs in soil: Bioavailability of PAHs and responses of microbial community structure. Science of The Total Environment 653 658-666
[6] Cheng M, Zeng G, Huang D, Yang C, Lai C, Zhang Ch and Liu Y 2017 Tween 80 surfactant-enhanced bioremediation: toward a solution to the soil contamination by hydrophobic organic compounds. Critical Reviews in Biotechnology 13 11296
[7] Cheng M, Zeng G, Huang D, Yang C, Lai C, Zhang Ch, and Liu Y 2017 Advantages and challenges of Tween 80 surfactant-enhanced technologies for the remediation of soils contaminated with hydrophobic organic compounds. Chemical Engineering Journal 314 98-113
[8] Prasanthi D, Jyothish K and Lakshmi P K 2016 Optimization of Alfuzosin Hydrochloride Organogels for Transdermal Delivery. Journal of Pharmaceutical Research International 11(2) 1-16
[9] Mao X, Jiang R, Xiao W and Yu J 2015 Use of surfactants for the remediation of contaminated soils: A review J. Hazard. Mater. 285 419-435
[10] Sun X, Pan Ch, Ying Zh, Yu D, Duan X, Huang F, Ling J and Ouyang X 2020 Stabilization of
zein nanoparticles with k-carrageenan and tween 80 for encapsulation of curcumin. International. *Journal of Biological Macromolecules* **146** 549-559

[11] Stom D I, Boyarova N A, Dagurov A V, Vyatchina O F and Saksonov M N 2008 Possible mechanisms of biological action of humic substances. *Siberian Medical Journal* **6** 76-79