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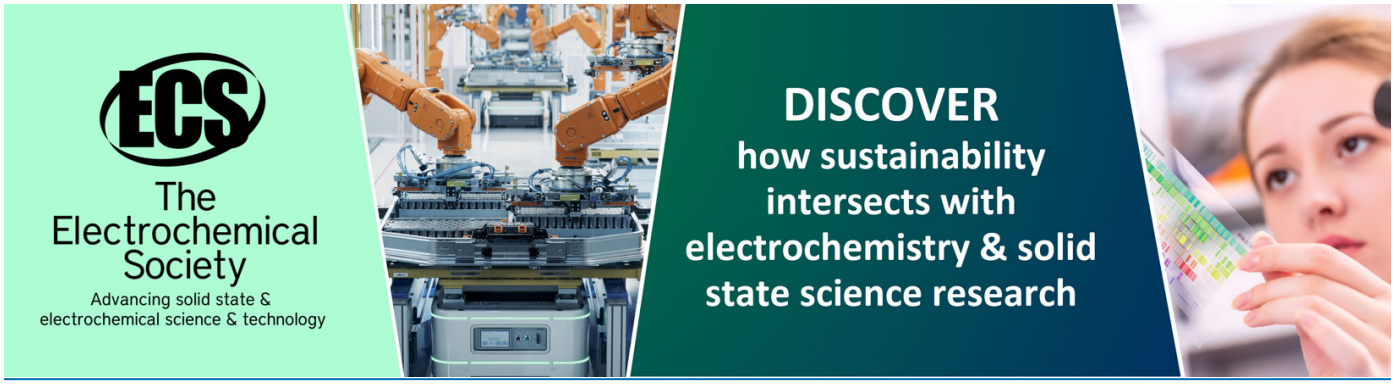

Methods for determining the toxicity of technogenic materials used in road construction

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Abstract. A method for determining the toxicity of soil and technogenic materials used in road construction has been developed. It is proposed to determine the toxicity of technogenic substances used as building materials for the construction of road surfaces by using biotesting on Colpoda steinii infusoria based on the test reaction of the survival rate of these infusoria. Soil samples for toxicity determination were taken at different distances from the roadbed (5, 10, 20, 25, 50 m from the edge of the road surface). During the experiment, it was found that anyone of the tested soil samples taken at a distance of 25 and 50 m from the roadway is not toxic to soil infusoria, and even after three hours of water extraction in all experimental samples, the test objects survived. These data on toxicity confirm additional experiments conducted by another method of biotesting on test cultures. In this case, soft spring wheat (Triticum vulgare L.) variety Novosibirsk 29 and watercress (Lepidium sativum) variety Donskoy were used as test crops. Experiments with these test cultures showed complete identity of the results obtained for toxicity according to the proposed method using Colpoda steinii infusoria.

When using waste as a road construction material, the existing problem of environmental pollution may become more complicated [1]. The impact of roads on the natural environment in Russia has not yet been sufficiently studied. This is especially true for contamination of the soil in the roadside strip and adjacent water bodies when using road construction materials of technogenic origin [2]. According to the results of previous studies, it was found that the 100-meter strip along roads with high traffic intensity is most polluted. But in some cases, the problem of developing rapid methods for determining the toxicity of technogenic materials used is very important for road organizations. When using industrial waste in road construction, it is necessary to confirm the absence of toxic substances that can migrate to adjacent environments from road surface materials to soil [3,4]. It is necessary to take into account their aggressiveness and toxicity to the environment by toxicity categories.

The purpose of this work is to develop a method for determining the toxicity of soil and technogenic materials used in road construction, using biotesting C based on the use of Colpoda steinii infusoria.

Nepheline sludge of JSC "RUSAL Achinsk" is a large waste of alumina production, obtained after extraction of alumina and soda products in the processing of Kiya-Shaltyr of nepheline together with

Mazul limestone quarry. Electron microscopic studies have shown that the nepheline sludge is mainly represented by porous particles of bicalcium silicate β-2CaO×SiO_2 (more than 80 %). (300-500 microns) (Figure1). It was also found to contain sodium and calcium aluminosilicates ($Na_2O×Al_2O_3×SiO_2$, $2CaO×Al_2O_3×SiO_2$), iron compounds in the form of ferrites and oxides ($CaFe_2O_4$, Fe_2O_3), calcite ($CaCO_3$) and sodium aluminate ($Na_2O×Al_2O_3$).

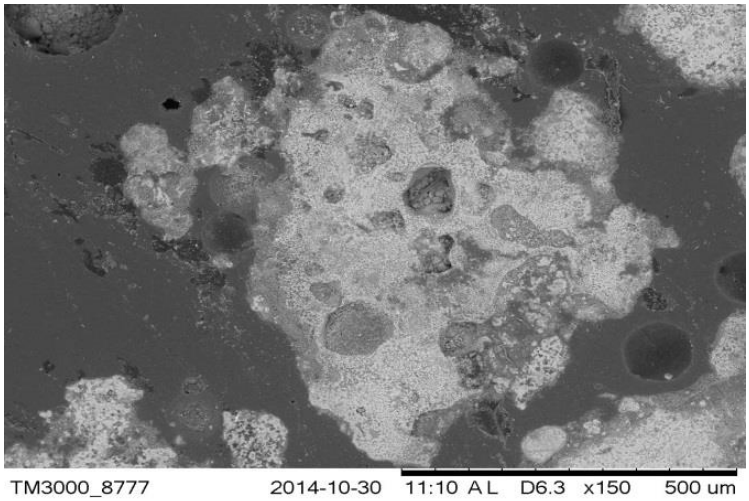

Figure 1. Microstructure of nepheline sludge particles: magnification ×150.

Using biotesting on Colpoda steinii infusoria, the toxicity of technogenic substances used as building materials for road construction is determined by the test reaction and survival of these infusoria. Infusoria, being single-celled microscopic organisms, due to their size, absorb mainly dissolved substances.

The implementation of this method of rapid bio-testing and identification of toxicants will allow you to get a quick preliminary result and correct the further course of road construction and prevent contamination of the soil and the environment [4].

Experimental sites were selected on the reconstructed Krasnoyarsk – Zheleznogorsk highway in the area of Sosnovoborsk (a section using a base with nepheline sludge) and Zheleznogorsk (without nepheline sludge). The expansion of the reconstructed section of the highway shows in figure 2.

The experimental site was located when the roadbed was expanded along the reconstructed Krasnoyarsk – Zheleznogorsk highway 2 km from the highway intersection in Sosnovoborsk. The second control section was selected on the same highway 10 km closer to the city of Zheleznogorsk, which was not expanded and reconstructed using nepheline sludge.

Figure 2. Reconstruction of the Krasnoyarsk – Sosnovoborsk highway.

The method of sampling soil along the roadbed included the following. Soil sampling was carried out using the "envelope" method (combined samples consisting of 5 point samples from an area of 5×5 m) from a depth of 0-20 cm. Fresh samples were poured on a plastic tape, thoroughly mixed, quartered 3 – 4 times (manually crushed soil was leveled on a plastic tape in the form of a square, divided into four parts, two opposite parts were discarded, the remaining two parts were mixed). After quartering, fresh soil was used for testing.

The selection of soil samples at test sites was carried out taking into account the vertical structure of the soil layer, as well as the edaphic and orographic conditions of the area. Test sites were planned on a grid with a distance from the roadbed of 0, 5, 10, 50, 100 m.

Determination of the toxicity of soil and technogenic materials used in road construction using infusoria was carried out as follows. An automatic pipette with a replaceable tip was used to select 20 microliter of the medium with infusoria and placed in each of the five microaquariums or slide holes. Then 20 microliter of the water extract of the test sample was added to the same place using an automatic pipette with a clean tip. Water extract from the soil for biotesting was prepared in the ratio: 1 part of the soil and 5 parts of distilled water (pH 7.0 - 7.5). For preparation, the average sample of the test sample was crushed before passing through a sieve with cells 1 mm in diameter. The homogeneous sample obtained in this way was used to obtain an aqueous extract.

To prepare an aqueous extract of the studied soil, a sample weighing 50 g was introduced into a 500 ml flask and 250 ml of distilled water was poured. The flasks containing the contents were shaken on the apparatus for 20 minutes, after which the mixture was filtered through a paper filter or centrifuged at a speed of 1000 rpm for 5 minutes and the supernatant was separated. Each sample was analyzed five times. When the test object was placed, the amount of culture fluid in the well did not exceed 0.02 cm. Five holes were used as control holes. Then the time of the beginning of the biotesting was noted and the number of individuals in each well was counted under a microscope. After this time has viewed the entire volume of microaquarium or pit has viewed and took into account the presence of the living Colpoda under a microscope. The survivors were considered infusoria that moved freely in the water column. Immobilized individuals were classified as dead.

The toxicity of the soil was determined by the example of soil selected at a distance from the object of technogenic soil contamination. The Krasnoyarsk-Sosnovoborsk highway, built using technogenic materials (nepheline slurries), was taken as an object of technogenic soil pollution. Soil samples for toxicity determination were taken at different distances from the roadbed (5, 10, 20, 25, 50 m from the edge of the road surface). The toxicity of soil samples (in %) was determined by the formula

$$T = \frac{\mathrm{Qtot} - \mathrm{Qs}}{\mathrm{Qtot}} \times 100\%,$$

where: Qtot – the total initial number of infusoria, pieces
Qs –the number of surviving infusorias at the end of the experiment, pieces

Then the toxicity category was determined for each sample. The analyzed materials were classified as practically non-toxic (toxicity within 20%), low-toxic (toxicity within 21-40%), moderately toxic (toxicity within 41-60%), dangerously toxic (toxicity within 61-80%), and highly dangerously toxic (toxicity within 81-100%). The results of studies on the use of the proposed method for determining toxicity are shown in table 1.

The conducted experiments have shown that the proposed method for determining the toxicity of soil contaminated with technogenic materials is the most acceptable for practical application. According to the proposed method, reliably accurate data on soil toxicity were obtained, so a soil sample taken at a distance of 10 m from the roadbed is considered moderately toxic (table.1 experiment 1), at 20 m, respectively, low-toxic, and at a distance of 25 and 50 m is considered non-toxic (table.1 experiments 4 and 5). During the experiment, it was found that anyone of the tested soil samples taken at a distance of 25 and 50 m is not toxic to soil infusoria, and even after three hours of water extraction in all experimental samples, the test objects survived.

Table 1. Results of determining the toxicity of contaminated soil at different distances from the roadway using Colpoda steinii infusoria.

№ experience	Conditions for conducting the experiment	Define the parameters of toxicity		
		Toxicity in water extraction 3h, %	Toxicity with a 1% aqueous-acetone extract 1,%	Category of the toxicity of soils
1.	Soil sampling at a distance of 5 m from the roadbed	72	78	Dangerously toxic
2.	Soil sampling at a distance of 10m from the roadbed	45	48	Moderately toxic
3.	Soil sampling at a distance of 20m from the roadbed	23	24	Low-toxic
4.	Soil sampling at a distance of 25m from the roadbed	12	14	Practically non-toxic
5.	Soil sampling at a distance of 50m from the roadbed	8	9	Practically non-toxic

The accuracy of determining the toxicity of soil by category according to this method is confirmed by additional experiments conducted by another method of biotesting for test crops in accordance with the "Method for measuring seed germination and root length of seedlings of higher plants for determining the toxicity of technogenically polluted soils"[5]. In this case, soft spring wheat (Triticum vulgare L.) variety Novosibirsk 29 and watercress (Lepidium sativum) variety Donskoy were used as test crops. Experiments with these test cultures showed complete identity of the obtained results on toxicity with Colpoda steinii infusoria.

The proposed method for determining the toxicity of soil and technogenic materials used in road construction makes it possible to identify the analyzed technogenic materials in Siberia by bioassay using Colpoda steinii infusoria and determine their toxicity category based on their survival rate in the tested solutions.

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