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Ecotoxicological assessment of soil using the *Bacillus pumilus* contact test

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ABSTRACT

We estimated the toxicity of soils artificially contaminated with metals and a pesticide using the *Bacillus pumilus* dehydrogenase activity (DHase) inhibition test. We found a masking effect of organic matter while testing the toxicity of soil samples with low content of toxicants. To avoid this effect, uncontaminated soil should be used as a control; in its absence, we recommend using a "substitute" control. We developed a method to estimate the "substitute" control by multiplying the quotient (*So*), which depends on organic matter content (C_{org}) of the soil sample, by the activity of the test culture determined in the presence of water ($Arel_w$). This method does not require uncontaminated soil controls and can be extended to use with other test organisms that are sensitive to C_{org}.

1. Introduction

The chemical pollution of soil is common and is known to decrease soil fertility, deteriorate agricultural products and increase soil toxicity. Biological methods are frequently used to determine soil quality and to test the toxicity of agrochemicals [4,10,11,16].

Biological methods applied to soil analysis can be subdivided into the following two groups: those that estimate the effects of soil toxicants on the biomass or activity of indigenous soil microorganisms (bioindication) and those that estimate the effects of toxicants present in soil samples on the biomass or activity of a laboratory culture of microorganisms (bioassay or bio-testing). Currently, most bio-testing protocols are applied to aquatic, not soil, systems. Microbial bioassays are designed to estimate the toxicity of individual substances or liquid samples [6,12,17]. When testing soil samples, water elutriation is the first step, followed by biotesting on this elutriate. Recently, a contact microbial assay based on the estimation of direct toxicity of the soil sample was proposed [7,13–15]. Each soil testing protocol has particularities associated with interactions between toxicants and the soil matrix [1–3,8]. The organic matter in soil and clay minerals affect the toxicity of individual compounds [5,9,13]; a toxicant can bind with organic matter and clay causing a decrease in bioavailability and, therefore, toxicity. Although the bioavailability of toxicants is one of the most important problems in bio-testing, it is beyond the scope of our work. Organic matter can also affect bio-testing results by stimulating the test-function or test-object used (e.g., growth, respiration activity, dehydrogenase activity (DHase)). To eliminate this masking effect, an additional soil sample, or a control with the same agrochemical properties but free of toxicants, must be tested. Because it is difficult to obtain a toxicant free sample when testing natural soils, we devised a technique to create "substitute" samples to replace for the non-polluted sample controls used in estimating the toxicity of soil contaminants by bio-testing techniques.

2. Materials and methods

2.1. Bacterial test

2.1.1. Bacterial strain and culture condition

We obtained the test strain (*Bacillus pumilus* KM-21) from the Collection of Microbiological Department of Kazan State University (Kazan, Russia). The test strain was previously selected from nine strains of the genera Bacillus (*B. pumilus* KM-21, *Bacillus subtilis* KM-5, *Bacillus circulans* KM-34, *Bacillus thuringiensis* KM-3F, *Bacillus megaterium* KM-16, *Bacillus cereus* KM-22, *Bacillus polymixa* KM-4F, *Bacillus intermedius* KM-13 and *Bacillus mesentericus* KM-6) for a higher level of DHase activity and sensitivity to standard toxic

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