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Effect of Different Detergent Concentrations on the Soil Microorganisms Number

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Abstract: As poorly bio-degradable substances chemical in nature, detergents may be discharged into watercourses and arable land areas uncontrollably and so have adverse ecological and economic effect on the biological production of such ecosystems. The researches to determine permissible chemical load limits in the environment are believed to be of an outstanding importance.

The paper was aimed at studying the effects of different detergent (Meril, Merima, Krusevac) concentrations, introduced into the nutritive medium, on the total number of soil microorganisms, fungi and oligonitrophiles.

The research was performed at the Microbiology Laboratory of the Faculty of Agronomy in Cacak. The medium for total number (Torlak, Belgrade) was used for determining the total number of bacteria, Capek agar was used for soil fungi and Fjodorov agar for oligonitrophiles.

The results obtained show that the lowest possible toxic effect on the microorganism groups studied was produced by the lowest detergent concentration (0.001%), whereas the concentrations of 0.1% and, particularly of 1% solution, inhibited microorganism growth nearly utterly. In this sense, oligonitrophilous bacteria appeared to be the most sensitive and soil fungi the most resistant group of microorganisms.

Key words: detergent, bacteria, fungi, oligonitrophiles.

Introduction

Efficacious use of detergents, for the purpose of displaying their target effects, and appropriate standardisation and definition of indirect effects on the biosphere are associated, among other things, with their basic physical and chemical properties. Essentially, they are poorly bio-degradable two-component pollutants. The first component, belonging to sulfonates, is actually a surface active substance (providing the detergent properties) and the other one, the carrier, is mostly in the form of polyphosphates or other substances with conditioning and softening properties. By their structure and purpose, they can be anionic, most commonly used for laundry washing, cationic – used for dish washing and enzymic - used for eliminating colours, meat and other organic stains. The difference between them is in the form and concentration of active substance (Mitrovic and Piletic, 1976). The active centre of anionic detergents contains soluble sulfates and sulfonates (about 40%), as well as different additives (polyphosphates, surface active agents, enzymes etc.). Cationic detergents are composed of quality NH_4 compounds and different additives. Different substances as polyphosphate substitutes have been used in recent years, for the purpose of reducing water and soil contamination. One of these is nitrolotri-acetic acid (a synthetic aminoacid), which is readily biodegradable (70%) in the environment. Even fewer negative effects on the biosphere are caused by enzymic detergents, since their active component is extracted from natural environment and is represented by an ammonifying group of bacteria with pronounced enzyme production (*Bacillus subtilis*).

Uncontrolled discharge of detergents into water and arable soil may cause reduced biological production of these ecosystems, which can have adverse ecological and economic effects. Soil microorganisms, as a biological soil component, can affect their biodegradation to a certain extent, converting them into less toxic or, often, energetically important sources of nutritive elements (Stojanovic et al., 1990). Basically, increased detergent concentrations or their accumulation into the soil (Goncaruka and Sidorenko, 1986) brings about a rapid reduction in the number of microorganisms, and so an analysis of the limiting values of soil load with these chemical compounds would provide a far more realistic picture in the neochemistry-effluents-soil system.

The aim of this research was to investigate the effect of increasing "Meril" detergent concentrations (0.001%, 0.01%, 0.1% and 1%) on the total number of bacteria, soil fungi and oligonitrophiles.

Material and Methods

The investigations were conducted at the Microbiology Department of the Faculty of Agronomy in Cacak. The microorganism groups analysed were isolated from the smonitza soil type and introduced into appropriate nutritional media at three replications. The trial included the following variants:

- the Control (without detergent);
- 0.001% solution of the detergent ("Meril"- Krusevac);
- 0.01% detergent solution;

- 0.1% detergent solution and
- 1% detergent solution.

The total bacteria number medium ("Torlak", Belgrade) was used for determining the total number of bacteria.

Capek agar was used to determine the total number of soil fungi and Fjodorov agar was used for oligonitrophiles (Djukic and Mandic, 2003).

0.5 ml of appropriate detergent concentration was added into each Petri dish and spread over its surface with gentle rotating motions, following which culturing was performed with each 0.5 ml of soil dilution. 10^{-6} dilution was used to determine the total number of bacteria, and 10^{-5} dilution was used for soil fungi and oligonitrophiles. After incubation lasting two days for the total bacteria number and five days for oligonitropiles and fungi at the temperature of 28^{o}C, the number of grown colonies on the nutritive media was determined and calculated per 1g absolutely dry soil.

Results and Discussion

Based upon the obtained research results it can be concluded that the number of the investigated groups of microorganisms was highly correlated with the concentration of the detergent introduced (Figures 1, 2, 3). Namely, it was observed that the highest concentration of the detergent investigated (1%) caused total inhibition of the development of oligonitrophilous bacteria, which was associated with the well-known characteristic of nitrogen fixers that they can hardly tolerate any even the least amounts of chemical compounds, even those with considerably lower surface potential compared to detergents (Djukic and Mandic, 1999; Mandic and Djukic, 1995). Somewhat lower inhibition was produced by 0.1% to 0.01% concentrations whereas the least depressive effect was exerted by the 0.001% concentration (Figure 1).

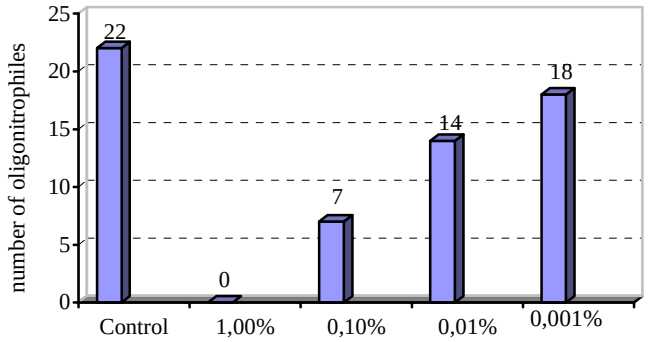

Fig. 1. The Effect of Different "Meril" Detergent Concentrations on the Number of Oligonitrophiles ($10^{5}g^{-1}$ absolutely dry soil)

The detergent investigated brought about, to a smaller degree, a change in the total number of bacteria (Figure 2). As opposed to oligonitrophiles, the lowest detergent concentration (0.001%) did not considerably reduce the total number of bacteria, which was not the case with other three concentrations. Here, too, the 1% detergent concentration produced the highest inhibition of the total bacteria number. The decrease in the number of microorganisms (particularly at higher concentrations) can be due to the ability of detergents for increased liquid wetting (which is also the property of surface active substances), resulting in severe destructive changes in the bacterial cell wall, decomposition of cytoplasmic membrane lipids, protein denaturation, disturbance of the cell division process, increase in the oxidoreduction potential, eventually leading to microorganism cell death (Emtsev and Djukic, 2000).

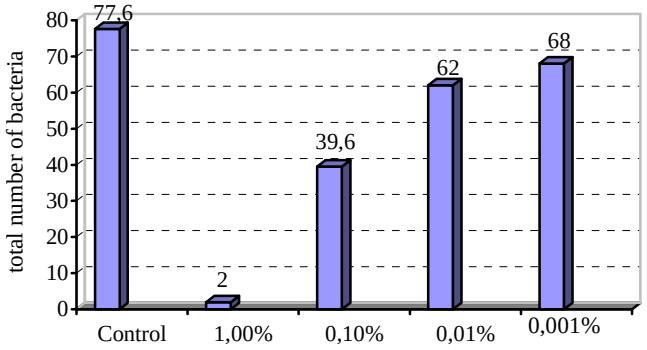

Fig. 2. The Effect of Different "Meril" Detergent Concentrations on the Total Number of Bacteria ($10^6 g^{-1}$ absolutely dry soil)

The applied detergent produced the smallest changes in soil fungi (Figure 3). This particularly referred to low (0.001%) and even ten times higher detergent rates (0.01%) the effect of which was mildly stimulatory or at the control level. This is associated with the well-known characteristic of soil fungi that they have strongly developed enzymic system (Stojanovic et al., 1995) enabling them degradation and intoxication of different xenobiotics in the soil Voets et al., 1974), as well as a pronounced life adaptability even in the presence of increased concentrations of not only detergents, but also pesticides, heavy metals, mineral fertilisers etc. (Umarov, 1980). Similar results can be found in researches by other authors (Djukic and Mandic, 1995) who underlined that polluted irrigation water containing also a significant detergent amount (0.09 mg/l) had exerted the lowest effect on the fungal soil community. However, as in previous cases, high concentrations of this detergent (0.1 and 1%) caused, irrespective of the soil fungi properties mentioned, a substantial fungicidal effect (Figure 3).

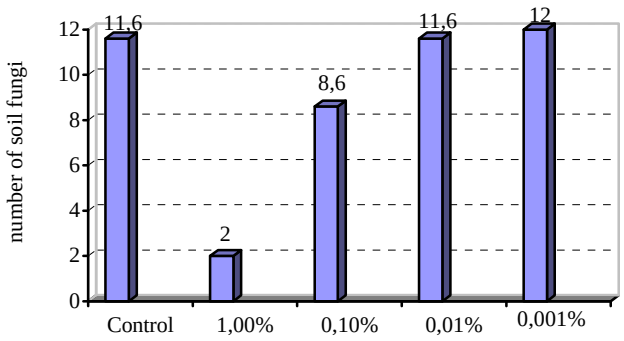

Fig. 3. The Effect of Different "Meril" Detergent Concentrations on the Number of Soil Fungi ($10^5 g^{-1}$ absolutely dry soil)

Conclusion

Based upon the research results on the effect of different "Meril" detergent concentrations on the number of soil microorganisms, the following conclusions can be drawn:

- the total number of bacteria was significantly decreased by introducing all four detergent concentrations (1%, 0.1%, 0.01% and 0.001%) into the nutritive medium, and the toxic effect was reduced when more diluted solutions were introduced;
- the number of soil fungi was reduced by using 1% and 0.1% solutions, whereas the other two concentrations had an insignificant effect;
- a particularly pronounced toxic effect on oligonitrophilous bacteria was produced by the "Meril" detergent, particularly when used at the highest concentration (1%) which completely inhibited the growth of this group of microorganisms;
- the research results indicate that soil microorganisms can be efficaciously used as test organisms in monitoring detergent-contaminated soils.

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UTICAJ RAZLIČITIH KONCENTRACIJA DETERDŽENTA NA BROJNOST ZEMLJIŠNIH MIKROORGANIZAMA

- originalni naučni rad -

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Rezime

Deterdženti, kao biološki slabo razgradive materije hemijske prirode, nekontrolisanim ispuštanjem u vodotoke i obradive zemljišne površine mogu izazvati negativne ekološke i ekonomske posledice, što se odražava na smanjene biološke produkcije ovih ekosistema. Shodno tome potreba za istraživanjima koja definišu granice dozvoljivog opterećenja životne sredine ovim hemijskim jedinjenjima od posebnog su značaja.

Cilj ovog rada je bio da se ispita uticaj različitih koncentracija deterdženta "Merila", unetih u hranljivu podlogu, na ukupnu brojnost zemljišnih mikroorganizama, brojnost gljiva i oligonitrofila.

Istraživanja su obavljena u Laboratoriji Agronomskog fakulteta u Čačku. Za određivanje ukupne brojnosti bakterija korišćena je podloga za ukupan broj (Torlak, Beograd), za zemljišne gljive Čapekov agar, a za oligonitrofile Fjodorov agar.

Dobijeni rezultati pokazuju da je najmanji efekat po ispitivane mikroorganizme imala najniža koncentracija deterdženta (0.001%), dok su koncentracije od 0.1%, a posebno 1% rastvor, gotovo u potpunosti inhibirali razvoj pomenutih grupa mikroorganizama. U tom pogledu najsenzibilnija grupa mikroorganizama su oligonitrofilne bakterije, dok su najotpornije zemljišne gljive.