The Influence of Lipids on the Natural Biodiversity of Cultivated Aboriginal Microflora of Urban Soils [†]

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[†] Presented at the 1st International Electronic Conference on Microbiology, 2–30 November 2020; Available online: https://ecm2020.sciforum.net/

Abstract: Urban soils (urban soils) are subject to significant anthropogenic impact, which affects the physicochemical composition of soils, as well as microbial natural diversity. Anthropogenic pollution of urban soils with lipids, in particular vegetable and mineral oils, can pose a certain danger to the biological balance in the soil ecosystem. For the quantitative determination of the number of heterotrophic microorganisms, the MPA medium was used, for the isolation of lipolytic bacteria, a mineral medium with oil. In the first 2 weeks after the addition of lipids, a decrease was observed in relation to the control of the number of heterotrophic microorganisms in all variants of the experiment. The negative effect of lipid contamination on the native soil microbiota was short-lived, and by the 4th week of the experiment, a sharp increase in heterotrophic microorganisms was noted. 26 strains of bacteria were isolated from the mineral medium with oil. Nine strains were identified that predominated in their numbers over the rest of the bacteria in this environment. Six strains identified belong to the order Enterobacterales. Two strains of the order Pseudomonadales, genus Pseudomonas. One Rhizobium radiobacter strain.

Keywords: bacteria; lipids; urban soils; heterotrophic microorganisms

1. Introduction

Urban soils (urban soils) are subject to significant anthropogenic impact, which affects the physicochemical composition of soils, as well as microbial natural diversity [1]. The ability of the soil to self-purify and maintain ecological balance is largely determined by its dynamic state, since physical, chemical and biological processes continuously occur in it [2, 4]. It is known that the higher the diversity of the biological component, in this case, the soil microbiota, the higher the ecological stability of the soil and its ability to withstand the technogenic load [3]. Under the influence of anthropogenic pollutants, not only a qualitative change in the microflora occurs, but also a change in the quantitative composition of microorganisms. Anthropogenic pollution of urban soils with lipids, in particular vegetable and mineral oils, can pose a certain danger to the biological balance in the soil ecosystem. Microorganisms, releasing lipolytic enzymes, decompose lipids to free fatty acids, glycerol, etc. [5].

2. Methods

To simulate lipid contamination of urban soils, soil samples were taken in the city of Krasnodar. Refined vegetable oil was used as a model lipid contaminant, which was taken at concentrations of 1, 5, 10, 25, and 50% by weight. We used media for the determination of the total number of

heterotrophs - MPA and medium for the isolation of bacteria that decompose lipids of the following composition (g /l): KNO_3 - 4; KH_2PO_4 0.6; Na_2HPO_4 * $12H_2O$ -1.4; $MgSO_4$ * $7H_2O$ - 0.8, agar-agar -18; oil - 10 ml. The isolated strains were identified by the MALDI-TOF method. The experiment lasted 33 weeks.

3. Results and discussion

In the first 2 weeks after the addition of lipids as pollutants, a decrease was observed in relation to the control in the number of heterotrophic microorganisms in all variants of the experiment. Thus, in samples contaminated with 1 and 5% oil, the number of cultivated soil microorganisms decreased by 10 times, in a sample contaminated with 10% oil, the number of heterotrophs decreased by 4 times, and in variants with 25 and 50% oil, the number of heterotrophs decreased by 5 times. ... The negative effect of lipid contamination on the aboriginal soil microbiota was short-lived, and by the 4th week of the experiment, a sharp increase in heterotrophic microorganisms was noted in four of the five experimental samples: 1% oil from $2*10^7$ to $1,1*10^9$ CFU/g, 5% oil from $5,3*10^7$ by $4*10^8$ CFU/g, 10% oil from $1*10^4$ to $2,6*10^5$ CFU/g, 50% oil from $1,3*10^3$ to $1*10^5$ CFU/g. From the seventh to the twenty-seventh week, in four of the five variants of the experiment (with 1, 5, 25, 50% oil), the number of heterotrophic microorganisms reached a plateau and subsequently varied slightly within the order of magnitude, while in the sample with 5 and 50% oil, the amount of heterotrophic bacteria remained practically unchanged (on average $3,95*10^7$ and $2,6*10^3$ CFU/g, respectively) until the end of the experiment. This indicator for a sample contaminated with 50% oil is 2 times less than in the control sample. The high concentration of lipids in this variant promoted a decrease in the amount of oxygen and the development of anaerobic destruction processes, which led to the suppression of the aboriginal aerobic microbial community. In the last week of the experiment, in samples with 1, 10, and 25% oil, a decrease in the number of heterotrophic microorganisms was observed, which is associated with the destruction of the introduced oil and stabilization of the number of the microbial community, followed by the return of the number to the values characteristic of urban soil before the introduction of the lipid pollutant. A gradual decrease in the number of heterotrophic microorganisms in the control sample may be associated with a decrease in the amount of food substrate.

On the medium for bacteria that decompose lipids, $1,6*10^2$ CFU / g was isolated in the control sample. As shown in Figure 1, by the second week in the test samples with 1, 5, 50% oil, an increase in lipolytic bacteria was observed 3; 3,5; 2 times, respectively.
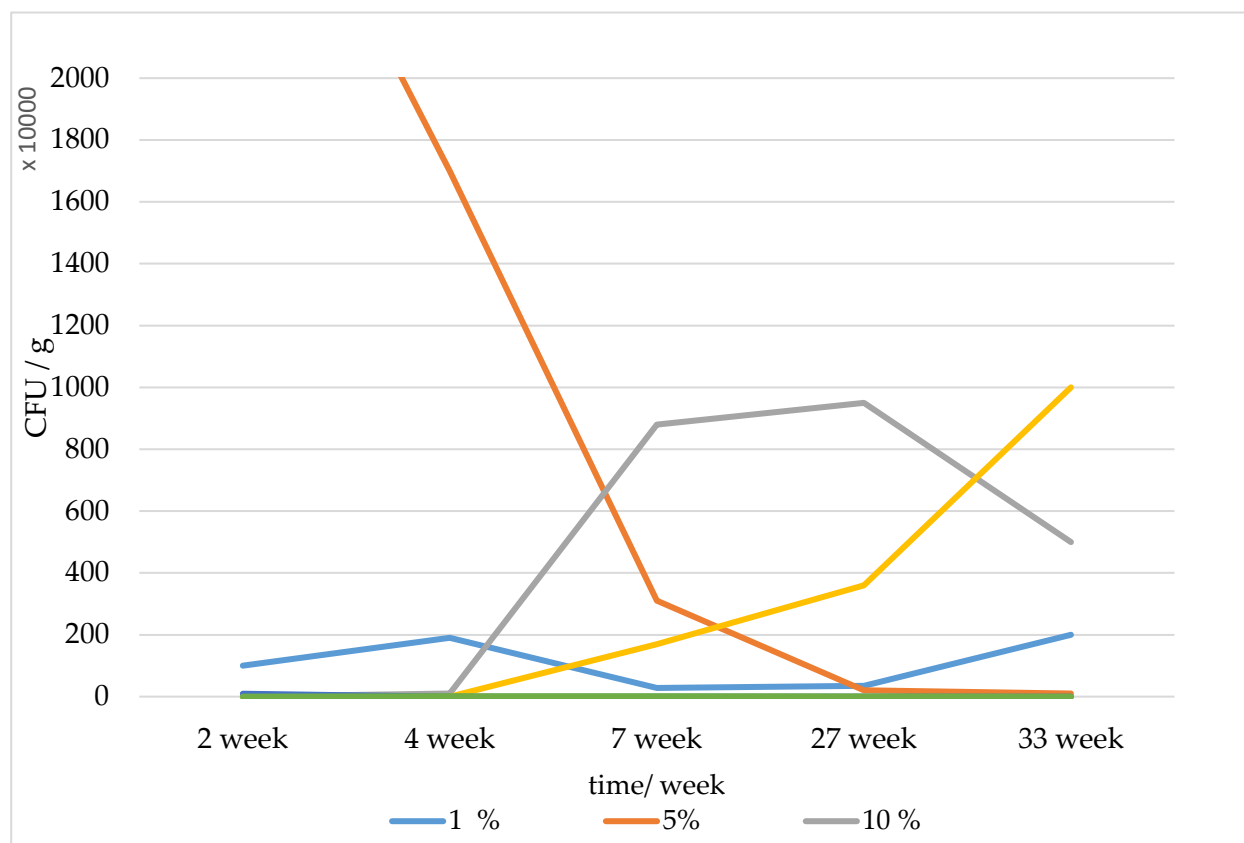
Figure 1. Dynamics of the number of lipolytic bacteria.

On the contrary, in samples with 10 and 25% oil, the number of such bacteria decreased to a minimum value of $8*10^1$ and $6,5*10^1$ CFU/g, respectively. The microbiome of soil contaminated with 50% oil undergoes restructuring due to the development of anaerobic processes of destruction of organic substances, incl. oil, therefore, throughout the experiment, the number of lipid-oxidizing bacteria exceeded the control figures only in the second week ($9,4*10^4$ CFU/g), and then there was a decline in the number of lipolytic bacteria. The peak in the number of lipid-oxidizing bacteria fell on week 2 for a sample with 5% oil - $3*10^7$ CFU/g, on week 4 for samples with 1 % oil and amounted to $1,9*10^6$ CFU/g. At week 27, the sample with 10 % oil is $9,5*10^6$ CFU/g. By the 33rd week of the experiment, only the sample with 25 % oil reached the peak of the maximum number of microorganisms equal to $1*10^7$ CFU/g.

26 strains of bacteria were isolated from the mineral medium with oil, most of them are gram-negative bacilli, mobile or immobile. Nine strains were identified that predominated in their numbers over the rest of the bacteria in this environment. Six strains identified belong to the order Enterobacterales. Two strains of the order Pseudomonadales, genus Pseudomonas. One Rhizobium radiobacter strain.

4. Conclusions

In a model experiment, it was shown that lipid pollution has a significant effect on the natural biodiversity of the indigenous cultivated microflora of urbanozems, changing both the quantitative and qualitative composition of the soil microbiota. A prolonged toxic effect is observed only when the soil is contaminated with 50% oil, in other concentrations the oil is decomposed by soil microorganisms. With model lipid contamination, an increase in bacteria of the order Enterobacterales in the soil is observed, which can lead to negative consequences, therefore, this problem requires further research.

References

1. 1. Usachova, A.N .; Siganova, N.V .; Poleshchuk, O.E. Influence of soil degradation processes on the quantitative composition of bacteria, actinomycetes and fungi. Mater. int. scientific. conf. "Ecology and biology of soils", Russia, 2004, pp. 308-312.
2. 2. Stepanova, LP; Yakovleva, E.V .; Korenkova, E.A .; A. V. Pisareva Agroeconomic assessment of restoration of fertility of anthropogenically disturbed and recultivated gray forest soils. Scientific notes of the Oryol State University; Russia, 2015, Volume 3, pp. 256-261.
3. 3. Yashin, IM; Kogut, L.P .; Prokhorov, I.S .; Vasenev, I.I. The ecological state of soils in the field and forest-park ecosystems of the Moscow metropolis. Agrochemical Bulletin; Russia, 2014, Volume 2, pp. 17-21.
4. 4. Yakovleva, E.V. Environmental assessment of the factors of degradation of gray forest soils and ways of their optimization. abstract of thesis. Candidate of Agricultural Sciences - Oryol: publishing house OGAU, 2006.
5. 5. Ribeiro, B.D. de Castro, A.M .; Coelho M.A.Z .; Freire D.M.G. Production and use of lipases in bioenergy: a review from the feed stocks to biodiesel production. Enzyme research, 2011. Volume 2011. pp. 1-16.