

Full Length Research Paper

Control of urban soil's toxicity by evaluation of bacterial community changes

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Since 1990, Almaty City's soils has been considerably polluted by heavy metals, which resulted in its' unhealthy ecological conditions. Almaty soils are light chestnut soils with the following physical and chemical properties: packing, $1.6 \pm 0.5 \text{ g} \cdot \text{cm}^{-3}$; available air space, $36.1 \pm 9.4\%$; salt composition of the soil aqueous extract, $0.12 \pm 0.04\%$; hygroscopicity (water-absorbing quality), 17.2 ± 1.8 , quantity of exchangeable cations, $\text{mg} \cdot \text{eq} \cdot 100 \text{ g soil}^{-1}$ - $59.1 \pm 4.2 \text{ Ca}^{2+}$, $8.4 \pm 0.5 \text{ Mg}^{2+}$, $0.6 \pm 0.1 \text{ K}^+$, $0.3 \pm 0.04 \text{ Na}^+$; humus, $1.6 \pm 0.4\%$; C_{total} , $0.9 \pm 0.2\%$; N_{total} , 0.14 ± 0.02 . The purpose of this research was to determine the degree of soil toxicity by the method of multisubstrate testing (MST) for the integral studies of Almaty city soils' bacterial communities, polluted by heavy metals (HM). This method measures soil bacterial communities' activity in terms of its uptake intensity of 47 different organic substrates. Their stunted condition was determined through a decreased amount of consumed substrates ("N") and urban soils' metabolic activity decay: total biomass ("W"), load upon ecosystem or disturbance "d" coefficient. It was found that: "d" ecoefficient equaled 1.0 for the baseline soil and "d" ratio equaled 0.6 for urban soils. Ecological soil state was assessed using MST method, looking into soil bacterial communities' activity in terms of its uptake intensity of different organic substrates. This method was developed in the last decade of the twentieth century for the purpose of investigation of soil microbial communities' state. Thus, urban soil samples displayed worse uptake intensity than the baseline soil. Hence, the MST method may be used as the original method of evaluation of urban soil toxicity degree.

Key words: Biological control, multisubstrate testing, bacterial communities, heavy metals, urban soils.

INTRODUCTION

Previously, we attempted to determine the toxicity of soil Almaty by means of phyto-object - Perennial Rye lawn grass (Mynbayeva et al., 2012). In the present study, as a test-object for toxicity, we used the soil microbial communities (bacteria, microscopic fungi, yeast, actinobacteria, etc.) as indicators of ecosystem degradation processes. But different microflora species and their interpopulation interactions made this task difficult. It is

also difficult to obtain reliable results on the state of the soil communities only with the study of structural characteristics. Therefore, it is necessary to define the functional (dynamic) characteristics by other methodological techniques. The multisubstrate test (MST) is a known method by using the devices of "Ecologist" or "Biologist" (Garland and Mills, 1991; Gorlenko and Kozhevnikov, 1994; Mills and Bouma, 1997).

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According to the mentioned method, the functional properties of the soil communities are studied by its reaction (direct microscopy) by applying into the soil samples the different artificial substrates, that is, glucose, mannitol, asparagines, citrate, succinate, etc. Further, the method proposed by Yakushev and Byzov (2008), Beleneva et al. (2010), etc. is commonly used in soil and marine microbiology for studying the development of specific active-functioning microbial communities, depending on the applied substrate.

Urban soils' microcenosis initiation by organic substrates till now has not been studied. The purpose of our research was to apply the MST method for the integral studies of Almaty city soils' bacterial communities, polluted by heavy metals (HM), with the use of 47 substrates utilized by these communities.

MATERIALS AND METHODS

To determine the biodiversity and metabolic profiles of urban soils' bacterial communities, soil samples were taken from 5 sectors in 5 replicates of the city along Raiymbek ave. from East to West: No. 1-4- urban soils, No. 5- 25 km from the city (background zonal soil).

The soil samples were collected from each of the 5 sectors during 2005 and 2009 in spring and autumn periods (50 samples) at depth of 0-25 cm (at 5 points) by classical method of soil samples selection. The analysis were performed using an AA-6650 atomic absorption spectrophotometer (Shimadzu Co. Ltd., Japan) equipped with a deuterium lamp which was employed for the determination of heavy metals. All the measurements were based on integrated absorbance. The content of acid-soluble forms of heavy metals was determined after dissolution of soil samples in 1 N HCl, the ratio of soil sample to extra gent was 1:10, exposure time- 60 min.

Multisubstrate testing of microbial communities in Almaty city soils was conducted in a standard way: 2 g of dry-weighed soil was dissolved in the phosphate buffer and centrifuged. Then, 20 ml of supernatant was placed in 200-ml cells of immunological plates for incubation at temperature of 28°C from 12 to 72 h until the coloration of cells may be visually recorded: violet tetrazolium changed to claret color of reduced tripheniltetrazolium. Registration was carried out by using an automated system "Ecologist". The optical density of cell (OD) was measured using a spectrophotometer "Uniplan". Spectrum of consumed substrates (SCS) by the software "Ecologist" was analyzed (Gorlenko and Kozhevnik, 1994).

The bacterial communities with a maximum value "d" are considered as optimal and stable; high values "D" are typical for stable systems with high biodiversity and conversely (Garland et al., 1997). For qualitative identification of system type the cluster analysis (Euclid-Ward) was performed. All laboratory analyzes were done in three replications.

RESULTS

In this research, we conducted a comparative study of bacterial communities' metabolic spectrum of certain urban territory areas by MST method. These areas are constantly and chronically polluted with HM, and unpolluted with HM background soil (used as a control). Content of HM required for discussion of obtained results

is presented in Table 1.

All analyzed HM were detected in Almaty city soils, but their contents varied depending on the place of soil sampling. The content of Cd and Cu in all urban soils samples exceeded the background: the maximum excess was 4.2 and 2.5 times, respectively. The excess of Pb and Zn in urban soils when compared with the background amounted to 2.6 and 1.5 times, respectively.

Thus, urban soil samples were identified as the most toxic due to high HM content. When using the MST method, we received a multidimensional array of data (range of substrate consumption), which was unique functional "portrait" of studied microbial objects in Almaty soil samples. To determine the patterns of change in the microbial communities' functional profiles the MST data were subjected to cluster analysis. It demonstrated that the urban soils' microbial communities (both spring and autumn samples), sharply differed from the background (control) sample regarding to assimilation of individual substrates, generating separate clusters (Figure 1). This pointed out to the long-term preservation of the effect of HM on the soil microbiota. Seasonal trend prevailed over the regional trend, which was especially expressed for urban soils. The least affected by seasonal trend were the control soil samples of suburban soil that displayed a good level of pedobiota's biodiversity. Characterizing the soil samples by their location in the region, using the extensive signs of functional diversity: the number of consumed substrates "N", metabolic work (a function of total biomass "W", ratio of ecosystem stress or disturbance "d"), showed a significant seasonal dynamics of these parameters.

System stability analysis with respect to consumed substrates by the value of "d" coefficient, pointed to significant differences of background soil (d=1.0) from urban soil (d=0.6), which, in turn, indicated significant changes in the original functional integrity and weak recovery in urban soils. The greatest biodiversity had a background soil sample (Table 2).

It was noted that the microbial communities of background zonal soils in spring fell under stress conditions influenced by melting snow and rapid vegetation, while the urban soils had stress in the autumn, which is probably due to depletion of soil resources at the end of the growing season in view of the overall low productivity of urban ecosystems. Soil bacteria communities were more active in utilization of various organic substrates in the background soil; metabolic diversity and activity fell in the urban soil samples with the maximum HM concentrations.

Conclusion

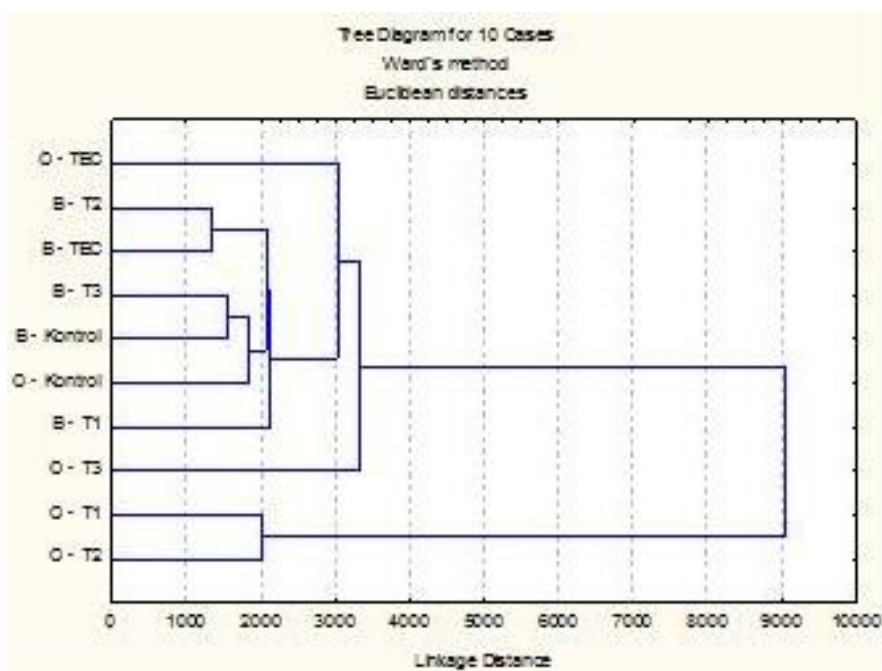
It was shown that Almaty city's soils were significantly polluted with heavy metals (Pb, Cu, Cd and Zn): metals content exceeded MAC. Heavy metals had a major impact on microbial objectives in Almaty city's soil

Table 1. The content of acid-soluble forms of heavy metals in analyzed soil samples by seasons.

The concentration of acid-soluble forms of heavy metals, mg/kg				
Sector	Spring samples (average)			
	Pb	Cd	Cu	Zn
Urban soil	50.3±3.3	0.50±0.04	43.9±2.6	59.8±5.7
Background soil	19.8±1.6	0.16±0.02	21.5±1.9	41.3±4.2
Sector	Autumn samples (average)			
	Pb	Cd	Cu	Zn
Urban soil	35.5±5.2	0.17±0.03	28.7±5.2	44.8±8.7
Background soil	15.8±2.1	0.05±0.02	16.3±2.8	28.4±5.2

Table 2. Characteristics of places selection of Almaty city's soil samples obtained by MST method (Garland et al., 1997).

Sector	Parameter "d"	System description
Urban soils	0.9-0.4	A soil system with depleted resources or system that is under the reversible environmental impact of an upsetting factor
Background soil	0.05	A thriving redundant soil system with a maximum margin of safety

**Figure 1.** Dendrogram of similarities (by ward) of Almaty soil's microbial communities in urban and background soils (MST method). O- autumn soil samples, B- spring soil samples, urban soils- No.1-4 (T1, T2, T3, TEC), background (No.5)- taken outside the city.

samples.

Urban soil's stunted condition was determined through a decreased amount of consumed substrates N and

urban soils' metabolic activity retarded: total biomass W, load upon ecosystem or disturbance d coefficient. For example, disturbance ratio d equaled 1 for the baseline

soil and d ratio equaled 0.6 for urban soils. Coefficient figures received N and W which confirmed low level of functional diversity of soil microbial flora.

The intake of biological substrates was significantly lower in urban soils than in the baseline soil samples, which also gave evidence of the biodegradation of soil in Almaty city. Regularities found have been set both for spring and autumn periods. In springtime stress load (high d) appeared in baseline soil under the influence of snow melting and abrupt vegetation. However, for urban soil stress load appeared in autumn. We connected it with soil depletion at the end of growing season and with overall low urban soil ecosystems productivity. Thus, we established seasonal changes of bacterial biodiversity that prevailed over the spatial parameters. Rural (baseline) soil had good condition characteristics of microbial communities in all the coefficient figures received.

Thus, according to the MST method, the bacterial communities from undisturbed soil oxidized organic substrates are more active than urban soil's communities. These significant differences between soils with different degrees of HM contamination allowed using the MST technique in monitoring of Almaty city soil pollution.

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