COMPARATIVE EVALUATION BETWEEN URBOCENOSIS AND AGROCENOSIS ON BASIS CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SOILS

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Abstract

There are presented chemical and microbiological analyses of soils from urbocenosis and agrocenosis from the region of city of Varna. There are determined macro- and microelements and their influence over the qualitative and quantitative content of the soil microorganisms. The soils from the investigated ecosystems are poorly supplied with total nitrogen. The urbogenic soil is well supplied with absorbable forms of phosphorus and potassium, in contrast to the agrogenic soil, which is slightly supplied with phosphorus and averagely supplied with potassium. As per contetn of organic substance the urban soil is classified as slightly humus, and the one from the agrocenosis as averagely humus. The investigated soils are classified from not contaminated up to slightly contaminated with lead, cadmium, copper, zinc, nickel, manganese and iron. The highest percentage share from the total microflora occupy the bacteria, which are not forming spores, which participate most actively in the processes of self-cleaning of the soils from the investigated pollutants. The specified chemical and microbiological indexes may serve as indicators for passing processes in anthropological soils with low content of heavy metals.

Key words: *urbogenic soil, agrogenic soil, macronutrients, heavy metals, microorganisms*

1. INTRODUCTION

The sources of anthropological contamination of the environment with heavy metals include different branches of the industry, transport, the management of the wastes, the fertilization of the soil. The heavy metals from these sources are dispersed in the environment and contaminate it. Many authors report that these toxic elements can cause long-term noxious effects on the soil ecosystems and they influence negatively over the biological processes in the soil (Chen et al., 2005; D'Ascoli et al., 2006; Kunito et al., 2001; Kuperman and Carreiro, 1997; Lorenz et al., 2006; Speir et al., 1999).

The soil is a basic reservoir and a natural environment for the development of the microorganisms in the nature. The increasing anthropological influence in settlement environment more and more renders its permanent impact over the change of the quantitative and qualitative content of the soil microorganisms, as well as over their biological activity. In the city soils is expressed a big variation of the different indexes of the biological activity and the number of the saprotrophic soil microorganisms (Stepanov et al., 2005). Malcheva and Nustorova (2007) established that the bacteria, which are not forming spores participate basically in the processes of self-cleaning of urbogenic soils with high content of heavy metals from the region of Sofia. While the development of the bacilli, actinomycetes and microscopic fungi is inhibited. There is observed a regrouping of the dominating species in the content of the microflora and appearance of coloured bacteria-indicators for contaminated soils.

In the scientific literature there can be found researches for a favourable effect of sediments from waste waters, regarding the structure of the soil, the content of organic substance, production of plants, maintenance of ecological equilibrium in the agrocenoses (Willems et al., 1981; Bidwell and Dowdy, 1983; Owczarzak et al., 1993). On the other hand, the reported presence of toxic heavy metals and pathogenic microorganisms in sediments from waste waters, by Gambus (1999) and Kalisz et al. (1999), is disturbing. The applying of organic materials leads to gradual decrease of pH of the soil (Gondek and Chmiel, 2006), which on its behalf increases the mobility of the heavy metals.

The quantity of the total microflora is not always in direct dependency from the content of heavy metals in the soil, especially at terrain investigations. Most of the authors report the toxicity of the heavy metals comparatively constant (Guzev et al., 1985; Šmejkalová et al., 2003; Wang Yuan-peng et al.; 2007). Through usage of genetic markers for total quantity of microorganisms and denitrifying microorganisms Sobolev and Begonia (2008) established that the lead has noticeable effects over the diversity of the microbiological community in the soil, even at a very low concentration – 1 ppm. According to them, the denitrifying microorganisms adapt themselves towards the increased levels of lead as they are in forms, hardy to the heavy metals, basically thanks to the enzyme nitrite reductase, which catalyses the last steps from the reduction of the nitrates to ammonia.

A series of researches are carried out about the importance of the metal-microbal interactions and the microbal processes of sorption and desorption of metals over the cellular surface of the soil microorganisms, as well as for the very disintegration of the heavy metals by the microbes in the environment (Beveridge, 1976, 1989; Konhauser et al., 1993; Collins et al., 2004). Less investigations are carried out about the influence of low concentrations of heavy metals in a neutral and alkaline environment. While profound investigations are carried out in strongly acid environment and high mobility of the metals, as for example are the conditions at soils contaminated with mine wastes (Collins et al., 2004; Hallberg and Johnson, 2005). One of the methods for elimination of pollutants in soils is the usage of biofilms. They are complex microbal communities and their studying gives a completely different approach in the solving of problems in the industry and health protection, connected with microorganisms. Together with this it becomes possible and the development of new strategies for management and control of biofilms with unfavourable activities, as well as the creation and exploitation of microbal communities with desired characteristics.They participate in the elimination of different organic and inorganic pollutants (Marhova and Kostadinova, 2008).

2. MATERIALS AND METHODS

The purpose of the present work is to be done a comparison between soils from urbo- and agrocenosis through analyzing of chemical and microbiological indexes.

The research was carried out in month June, year 2014. There were analyzed samples of soils from the following objects:

- Urbocenosis – the soil samples are taken from the circular juncture (central stripe) of Primorski Boulevard and Preslav Street in city of Varna. The soil layers from the two horizons are packed along whole depth as there predominates grass vegetation.
- Agrocenosis – the soil profile is done in agroecosystem from maize along the road II-29 Varna – Dobrich at elongation 10 m from the source of contamination. The soil at depth 0÷20 cm is dark grey, fresh, crumbly. The fallow land is with crumbly-grainy structure, averagely sandy-clay composition, strongly pierced by roots, there is observed a slight gradual transition. The soil horizon with depth 20÷40 cm is characterized by a greyish-brown colour of the soil. It is fresh, averagely thick, with lumpy-prismatic texture.

The soil samples were taken only once by hand of two depths 0÷20 cm and 20÷40 cm, at observance of the requirements as per Bulgarian State Standard 17.4.5.01:1985. The preparation of the samples and the mineralization were carried out in compliance with the standard EN ISO 11466:1995. The content of the elements copper, zinc, lead, cadmium, nickel and manganese was specified through atomic-absorption spectrometry as per EN ISO 11047:1998. The analyses about the content of iron were carried out as per methods EN ISO 11885:2009. The active reaction of the soils (pH) was specified in water extract in compliance with Bulgarian State Standard ISO 10390:2011. There were calculated the indefiniteness of pH and heavy metals in compliance with the requirements of Bulgarian State Standard EN ISO/IEC 17025/AC:2006. The quantity of humus is specified as per method of Turin, and of total carbon – through calculation (Donov et al., 1974). The content of ammonium and nitrate nitrogen is reported photometrically with Nitrospectral as per international standard ISO 14255:2002, in compliance with methods of company „Merck". The content of potassium and

phosphorus is specified as per ISO 11263:2002 through a double-lactate method of Egner-Riehm. The ratio of C:N in the soil is calculated as per the methods of Orlov, Grishina, 1981. The moisture of the soil is specified as per weight method through usage of thermostat and drying at temperature 105ºC up to a constant weight.

The samples for microbiological analysis are taken with a sterile instrument, in sterile paper bags. They are transported and investigated the latest up to 48 hours, as up to the moment of the culture they were stored in a refrigerator at 4-10ºC.

The microbiological researches include specifying of the bacteria, which are not forming spores, bacillar microflora, actinomycetes, micromycetes, bacteria, which assimilate mineral nitrogen. They are specified as per the method of thinning out and culture of solid nutrient environments (meso peptone agar – for specifying of bacteria, which is not forming spores and bacilli; starch-ammonium agar – for specifying of actinomycetes and bacteria, absorbing mineral nitrogen; environment of Chapek-Docks – for specifying of micromycetes), cultivation in a thermostat and following reporting of stock-forming units, recalculated to 1 gr. absolutely dry soil.

3. RESULTS AND DISCUSSION

For the purposes of the present analysis is applied the approach for standardization and evaluation as per Regulation № 3, modification and addition State Gazette issue 71/2008. The obtained results for active reaction – pH, content of iron and manganese are compared with data from literature sources (Gyurov, 1990; Koynov and colleagues, 1998; Gorbanov, 2005). The results from the investigation show that the values of Pb, Cu, Zn, Cd and Ni are under their maximum admissible concentrations, but over the before-protective norms for cadmium and at both objects, as well as for lead at the urbogenic soil at depth 0-20 cm (table 1).

Table 1. Content of heavy metals, mg/kg in air-dry soils.

Experimental area	**Depth (cm)**	**pH /H_2O/**	**Pb**	**Cu**	**Zn**	**Cd**	**Ni**	**Mn**	**Fe**
			mg/kg						
		Location	**MAC***	**MAC**	**MAC**	**MAC**	**MAC**		
			200	**300**	**400**	**8**	**100**		
		Arable land in >7,4	**120**	**300**	**400**	**3**	**150**		
Urbotsenoza - Station Square Varna	0-20	7,60± 0,03	55,56± 2,93	25,42± 0,38	73,19± 1,10	1,58± 0,04	13,75± 0,85	166,67± 2,62	1750,00± 2,58
	20-40	7,50± 0,03	36,11± 1,90	19,17± 0,29	55,69± 0,84	1,45± 0,04	10,00± 0,62	90,00± 2,04	1156,25± 2,32
Agrocenoses maize highway Varna - Dobrich	0-20	7,58± 0,03	27,78± 1,46	16,25± 0,25	44,49± 0,67	1,45± 0,04	26,25± 1,62	260,42± 3,08	2656,25± 2,92
	20-40	7,56± 0,03	26,57± 1,32	17,08± 0,26	45,76± 0,69	1,18± 0,03	25,00± 1,54	312,50± 3,56	2343,75± 2,76

*** MAC – Maximum allowable concentration**

Higher values of lead, cadmium, copper and zinc, and lower of manganese and iron are established at the urbogenic soil in comparison with the agrogenic one. The lead is accumulated mainly in A-horizon of the soils. The increasing of the accumulated quantity of lead in the superficial layer soil towards the next soil layer shows the anthropological character of its humus-accumulative accumulation. At the analyzed soils the quantity of the total lead is higher in the superficial soil layers, as in depth it decreases with 1,5 times at the urbocenosis, while at the agrogenic soil the quantity is almost the same and at both depths. The total quantity of copper is also higher at the soil from the urbanized region in comparison with the one at the soil in the agricultural region. The distribution of the element in depth of the soil shows a higher biological accumulation in the upper soil layer at the urbogenic soil, because of the inclination of the copper to chelatoformation. At the agrogenic soil is established almost even vertical distribution along the soil profile. Higher is the content of zinc at the soil from urban environment, decreasing in depth 1,3 times. At the agrogenic soil is observed a slight movement of this heavy metal in depth. The data show increasing of the accumulated cadmium in the superficial layer of the soils in comparison with the soil layer under it, which proves a humus-accumulative behaviour, which is due to an anthropological influence. Again higher values of this element are reported at the urbogenic soil in comparison with the agrogenic one. The manganese on principle is accumulated in the superficial soil layer, since it connects with the organic substance. Higher values of the element are established at the agrogenic soil and at both depths – corresponding to higher content of organic substance, in comparison with the urbogenic soil, at which the content of manganese decreases in depth 1,9 times. Similar to the manganese, the quantity of the iron is higher at the agrogenic soil in comparison with the urbogenic one. In depth it decreases and for both objects – respectively 1,5 times at the urbogenic soil and 1,1 times at the agrogenic soil. For content of iron and manganese in the soil in Regulation №3/2008 there are not regulated norms. The obtained values are within the limits of the indicated in the literature limits for content of iron and manganese in the soils of the country – iron 55 000 mg/kg, manganese – 5 000 mg/kg (Koynov and colleagues,1998; Gorbanov, 2005). As per data of Gyurov (1990) the average content of iron in the soils in Bulgaria is 3,8% or 38 000 mg/kg. At investigations of soils in 87 settlements from the region of municipality of Varna the values of the manganese are of 200-300 mg/kg, as in separate soil samples they reach from 1000 up to 5000 mg/kg. This element has shown higher concentrations at soils with grain crops – 843,3±38,5 mg/kg. At the same investigations are established values of: lead from 4,6 up to 30 mg/kg, copper from 30 up to 60 mg/kg and in 2% over 60 mg/kg, zinc from 34,3 up to 87 mg/kg, nickel from 6,8 up to 33 mg/kg, cobalt from 5,5 up to 17 mg/kg. The values of these elements vary depending on the sown crops (As per data of Kuyumdzhiev and colleagues, quotation by Stoyanov, S., 1999).

By the values of pH is noticed that the soils are with neutral up to very slightly alkaline reaction, because of which there is not necessary the investigation of the mobile forms of the heavy metals.

The results from the investigation of the biogenic elements prove the differences in the soils from the studied regions.(table 2)

Table 2. Content of humus and nutrients in the soil

Experimental area	**Depth (cm)**	**Humus, %**	**Org. C, %**	**Total N mg / kg**	**Total P mg / 100 g**	**Total K mg / 100 g**
Urbotsenoza- Station Square Varna	0-20	1,16	0,67	1,37	9,5	51,22
	20-40	0,94	0,54	3,01	4,39	29,6
Agrocenoses maize highway Varna - Dobrich	0-20	2,08	1,21	3,37	2,06	9,86
	20-40	1,69	0,98	6,15	1,98	9,48

In the arable lands the content of potassium and phosphorus is five times less in comparison with the grassed areas in the urbanized environment in the superficial layer. In soil layer with depth 20-40 cm the quantity of these elements decreases and at both objects, as the content of potassium is two times, and of phosphorus three times higher in the urbogenic soil in comparison with the agrogenic one. These tendencies prove the anthropological character of accumulation of these elements. The values of the organic carbon and nitrogen are higher at the agrogenic soil in comparison with the urbogenic one. The content of organic carbon decreases, and of nitrogen is increased in depth in the soils and of both objects. As per content of humus (Penkov, 1996; Donov, 1976) the urbogenic soil is slight humus, and the agrogenic one – averagely humus.

The results from the microbiological investigations show a different biological condition of the soils in the separate zones (table 3).

Table 3. Qualitative and quantitative composition of the soil microflora

Experimental area	**Depth (cm)**	**Total microflora**	**Non-spore-forming bacteria**	**Bacilli**	**Actinomycetes**	**Micromycetes**	**Bacteria, assimilating mineral nitrogen**	**Coefficient of mineralization**
		Colony-forming units per g of dry soil (c.f.u.x103/g soil), ± Coefficient of variation (CV), in brackets - as percentage of the total microflora (%)						
Urbotsenoza - Station Square Varna	0-20	7463	3290 ± 1.03 (44)	808 ± 2.35 (11)	1673 ± 0.62 (22)	1692 ± 1.33 (23)	12032 ± 0.52	2,94
	20-40	847	728 ± 1.10 (86)	64 ± 6.25 (8)	20 ± 5.00 (2)	35 ± 5.71 (4)	728 ± 2.15	0,92
Agrocenoses maize highway Varna - Dobrich	0-20	9560	8420 ± 0.62 (88.1)	790 ± 1.58 (8.3)	137 ± 5.97 (1.4)	213 ± 2.93 (2.2)	9728 ± 0.53	1,06
	20-40	1955	1822 ± 0.77 (93)	81 ± 5.38 (4)	17 ± 5,88 (1)	35 ± 2.86 (2)	822 ± 1.35	0,43

The biogenity of the soils is considered as a degree of settling with microorganisms – a summary effect from the development of the bacteria, which are not forming sores, bacilli, actinomycetes and micromycetes. From the data in the table is visible that the higher total quantity of microorganisms is established at the agrogenic soil in the upper soil layer, than at the urbogenic soil for the same soil layer. This tendency is determined by the lower content of heavy metals in the soil (Pb, Cd, Cu and Zn) at the agrocenosis in comparison with the urbocenosis. On the other hand, the higher content of microelements in the upper soil layer activates the microorganisms, as a reaction of the created stress conditions for them. The suppressing action of the heavy metals on the microorganisms is established by a number of authors. Wang et al. (2010) specifies the toxicity of the heavy metals on the microorganisms in the following order: Cr > Pb > As > Co > Zn > Cd > Cu.

And for both objects the biogenity of the soils at depth 0-20 cm is higher in comparison with this at depth 20-40 cm, as this drop is higher at the soil from the urbocenosis – 9 times, while at the soils from the agrocenosis – 5 times. The total microflora of the investigated soils is determined basically by the active development of the bacteria, which are not forming spores, which participate in the initial phases of destruction of the organic matter and they realize the processes of self-cleaning of the soils from pollutants. Among them a basic share in microbiocenosis take the bacilli, except for the soil from the urbocenosis at depth 0-20 cm, where the micromycetes and the actinomycetes have higher percentage participation. The highest content of bacteria, which absorb mineral nitrogen at the urbogenic soil at depth 0-20cm determines and the highest speed of the mineralization processes at this soil (coefficient of mineralization – 2,94), while the disintegration of the organics at the agrocenosis from maize passes slower. At the agrogenic soil the mineralization coefficient is lower with 2,8 times at depth 0-20 cm and with 2,1 times at depth 20-40 cm in comparison with the urbogenic one.

4. CONCLUSIONS

1. The investigated soils are classified from not contaminated up to slightly contaminated with lead, cadmium, copper, zinc, nickel, manganese and iron. The values of Pb, Cd, Cu and Zn are higher, and of Ni, Mn and Fe are lower at the soil from the urbocenosis in comparison with that from the agrocenosis. In depth the content of the heavy metals decreases, which correlates with the neutral soil reaction, which supposes low mobility of the microelements.
2. The investigated soils are well reserved with biogenic elements. The quantities of organic carbon and nitrogen are higher at the arable soil in comparison with the soil in city environment. This fact is due to the accumulation of more organic matter at the soil under maize, which is also fertilized, in comparison with the one under poorly developed grass vegetation. The urbogenic soil is with higher content of phosphorus and potassium in comparison with the agrogenic one. Besides, the significant accumulation of these elements in the superficial soil layer at the urbogenic soil is a proof for their anthropological character of accumulation in this soil.
3. The total microflora of the investigated soils is determined basically by the active development of the bacteria, which are not forming spores, which participate in the initial phases of destruction of the organic matter and they realize the processes of self-cleaning of the soils from pollutants. The quantity of the microorganisms in the soils layer at depth 0-20 cm is higher, as well as at the soils from the agrocenosis in comparison with these from the urbocenosis.
4. The reported microbiological indexes can serve as bioindicators for the passing processes in urbogenic and agrogenic soils with low content of heavy metals.

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