CHEMICAL AND ENZYMATIC INDEXES OF URBOGENIC AND AGROGENIC SOILS FROM THE REGION OF MUNICIPALITY OF VARNA

Bojka Z. Malcheva[1], Pavlina N. Atanasova[2], Plamena Y. Panayotova[2], Dragomir P. Dimitrov[2]

[1]Department Soil Science, University of Forestry – Sofia, Bulgaria

[2]Department Plant Production, Technical University – Varna, Bulgaria

Abstract

There are analyzed soils from urbocenosis and agrocenosis from the region of municipality of Varna. There are determined macro- and microelements and their complex impact over the cellulase and the catalase activity 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 poorly supplied with phosphorus and averagely supplied with potassium. As per content of organic substance the urban soil is classified as a slightly humus, and that from the agrocenosis as averagely humus one. There is established that the cellulase activity is lower at the urbogenic soil in comparison with the agrogenic one. It is suppressed by the activity of Pb, Cd, Cu and Zn, but it is not influenced by the summary activity of Ni, Mn and Fe. For the enzyme catalase is marked a contrary tendency – its activity is limited by a higher content of Ni, Mn and Fe and it is not suppressed by Pb, Cd, Cu and Zn. The studied chemical and enzymatic 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, cellulase, catalase*

1. INTRODUCTION

The content of heavy metals in the soils is increased all over the world, as a result of the increase of the industrialization during the last decades. They are considered for one of the biggest sources of pollution of the soil (Huang and Shindo, 2000). Despite that the development of the industry in Bulgaria has decreased, the problem with these toxic elements is still topical, because of the impossibility they to be disintegrated for such short time, for which they have accumulated. In certain regions of the country their content in the soil is highly increased. According to researches (Tassev, 1995; Pushenreiter et al., 2005) in Bulgaria 19 360 hectares are contaminated with heavy metals. They may have long-term noxious influences over the ecosystem of the soil and unfavourable influence over the passing in it biological processes (Lee et al., 2002;. Majer et al., 2002;. Perez-de-Mora et al., 2006). Some heavy metals as cobalt, chromium, nickel, iron, manganese and zinc have basic role in the microbal metabolism, they participate in the enzymatic reactions and regulate the osmotic balance (Hussein et al., 2005). However, even low concentrations of some elements as cadmium, mercury, lead and others are disastrous for the organisms (Roane and Pepper, 2000).

There is proved a strong inhibition of the enzymatic activity by toxic elements in the soils by many researchers (Effron et al., 2004; Khan et al., 2007; Kahkonen et al., 2008; Kizilkaya, 2008; Kunito et al., 2001; Malley et al., 2006; Oliviera and Pampulha, 2006; Shen et al., 2005; Speir et al., 1999; Wang et al., 2008). Wyszkowska et al. (2006) establish that the sensitivity of some enzymes towards heavy metals follows the order: dehydrogenase > urease > alkaline phosphatase > acid phosphatase. The copper can form compounds with the amino acid tryptophan and to inhibit the cellulase activity (Karaca et al., 2010). The catalase activity of contaminated soils with lead and cadmium in a conservatory show that the activity of the enzyme is lower in the samples with heavy metals in comparison with the checking (Sardar et al., 2007).

Zhang et al. (2008) establish that there exists a seasonal difference in the effect of the heavy metals over the soil enzymes – their activity is lower during the spring and summer, rather than during the

autumn. The strong contamination with heavy metals renders a negative influence over the development of the soil microbiocenoses, especially during the spring, because of the poorer vegetation and the poorer storage of nutrient substances (Shilev et al., 2008).

2. MATERIALS AND METHODS

The purpose of the present work is to be analyzed chemical and enzymatic indexes and to be established the influence of low quantities of heavy metals over the microbiological activity of urbo- and agrogenic soils from the region of municipality of Varna.

The research was carried out during month June year 2014. There have been analyzed samples of soils from the following objects:

- Urbocenosis – the soil samples are taken from the circular juncture at Primorski Boulevard and Preslav Street in city of Varna. The soil layers from the two horizons are packed along the whole depth as there predominates grass vegetation.

- Agrocenosis – the soil profile is done in an agroecosystem of maize along the road II-29 Varna – Dobrich at elongation 10 m. from the source of contamination. At depth 0÷20 cm the soil is dark grey, fresh, crumbly. The fallow land is with a 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 in compliance with EN ISO 11885:2009. The active reaction of the soils (pH) was specified in water extract as per methods from Collection methods for hygiene research, National Center of Hygiene, Medical Ecology and Nutrition, volume I/year 1999. 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. The content of potassium and phosphorus is specified as per ISO 11263:2002 through a double-lactate method of Egner-Riehm. The moisture of the soil is specified as per weight method through usage of thermostat and drying at temperature 105 °C 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 analysis they were stored in a refrigerator at 4-10° C.

The cellulase activity of the soil is investigated through a laboratory experiment (Khaziev, 1976). In Petri dishes is sprinkled soil with depth around 7 mm, as there is maintained 60 % Utmost Field Moisture Capacity. On the soil are put 3 tapes sterile filter paper with sizes 10/50 mm. A cultivation in a thermostat at 25°C. In 15 days is reported the disintegrated area with the assistance of a net-reference.

The catalase activity of the soil is specified as per a manganese-metrical method (Khaziev, 1976).

3. RESULTS AND DISCUSSION

The investigated soils 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 poorly supplied with phosphorus and averagely supplied with potassium (table 1) (Ruseva, 2011).

Table 1. Humidity, humus and macronutrients in soil samples

Experimental area	Depth (cm)	Humidity %	Humus %	Org. C %	NH_4-N mg/ kg	NO_3– N mg/ kg	Total N mg/kg	P_2O_5 mg/ 100g	K_2O mg/ 100g
Urbotsenoza-Station Square Varna	0-20	1,48	1,16	0,67	0,78	0,59	1,37	21,73	61,47
	20-40	0,90	0,94	0,54	2,22	0,80	3,01	10,05	35,52
Agrocenose maize highway Varna - Dobrich	0-20	5,40	2,08	1,21	1,79	1,59	3,37	4,72	11,83
	20-40	5,48	1,69	0,98	2,55	3,60	6,15	4,54	11,37

The data from table 1 show that as per the contetn of humus (Penkov, 1996; Donov, 1976) the urbogenic soil is poorly humus, and the agrogenic – averagely humus. The quantity of humus and carbon is higher at the soil from the agrocenosis in comparison with the one from the urbocenosis. The highest is the content of ammonium nitrogen (2,55 mg/kg), nitrate nitrogen (3,60 mg/kg) and summary nitrogen (6,15 mg/kg) in soil horizon 20-40 cm in the investigated agrocenosis along the road Varna – Dobrich. The results show that totally the agrogenic soil is with a higher quantity of total nitrogen in comparison with the urbogenic, which is due to the additional putting in of nitrogen fertilizers. At soils in immediate proximity of roads with heavy automobile traffic, sources of carbon and nitrogen can be also the exhaust gases of the automobiles (Malcheva, 2012). It is established that in depth of the soil profile basically is carried the nitrate form of the nitrogen fertilizer (Gospodinov and Bazitov, 2013; Stoyanova et al., 2010). The data from the analyzes prove bigger content of nitrogen forms in the deeper soil horizon and at both investigated ecosystems.

Regarding the content of phosphorus - its value is the highest for soil layer 0-20 cm from the investigated urboecosystem. As the tendency for bigger quantity of the exchange forms of the phosphorus is preserved and in the second soil layer of the city ecosystem in comparison with the agrocenosis. This tendency proves the anthropological character of accumulation of this chemical element. At investigation of urbogenic soils from the region of Sofia is established that the content of absorbable phosphorus varies from 10 up to over 40 times more at the anthropologically more burdened soils in comparison with this in soil from a city park (Malcheva, 2012).

The enzymes of the soil microorganisms, which catalysed hydrolisis of the phosphoorganic compunds in the soils are referred to the group of phosphohydrolases – phosphatases, phitases and nucleases. The phosphodecomposing activity of the investigated soils is not specified, but we can suppose that it is lower at the agrogenic soil, which is poorly supplied with absorbable phosphorus. Besides, as a result of the high quantities movable potassium is decreased the accessibility of the phosphorus, which is blocked and becomes unusable for the plants and the microorganisms.

In some agricultural regions of Bulgaria there is a satisfying level of potassium in the soils. The potassium salts in the soil increase the osmotic pressure in the soil solution and they damage the young plants (Соок, 1968). The increased osmotic pressure influences and the activity of the soil microorganisms. The last representative soil researches show that the area of the poorly supplied with potassium soils increases from 12% to 22%, of the averagely supplied from 18 to 25%, and the share of the well supplied decreases from 70% to 53% (Nikolova M, 2010). Almost the half of the agricultural lands in Bulgaria need regular putting in of potassium fertilizers for maintaining of the soil

fertility and the productivity. As a proof to these data can be added and the carried out analyzes of the investigated two ecosystems.

The best results from the investigated samples for content of potassium is established at soil layer 0 – 20 cm in the urbogenic soil - 61,47 K_2O mg/100g. The soil from the agrocenosis at depth 0 - 20 cm contains 5 times less than the investigated element in comparison with the soil in city environment. The proportion for the content of K_2O at depth 20 – 40 cm between the urbogenic and the agrogenic soil is 3:1, which proves the preservation of the already established tendencies.

The basic quantity K is located in the minerals and in the organo-mineral complexes. The processes of transformation of minerals from microorganisms are not specific, but in them participate various microorganisms. The reproduction of the microorganisms not only on the surface of the soil particles, but also in the water solution is activated when the soil is enriched with the necessary for it nutrient substances. The joint good content of nutrient elements in the soil renders obvoius influence on the development and the activity of the mictoorganisms.

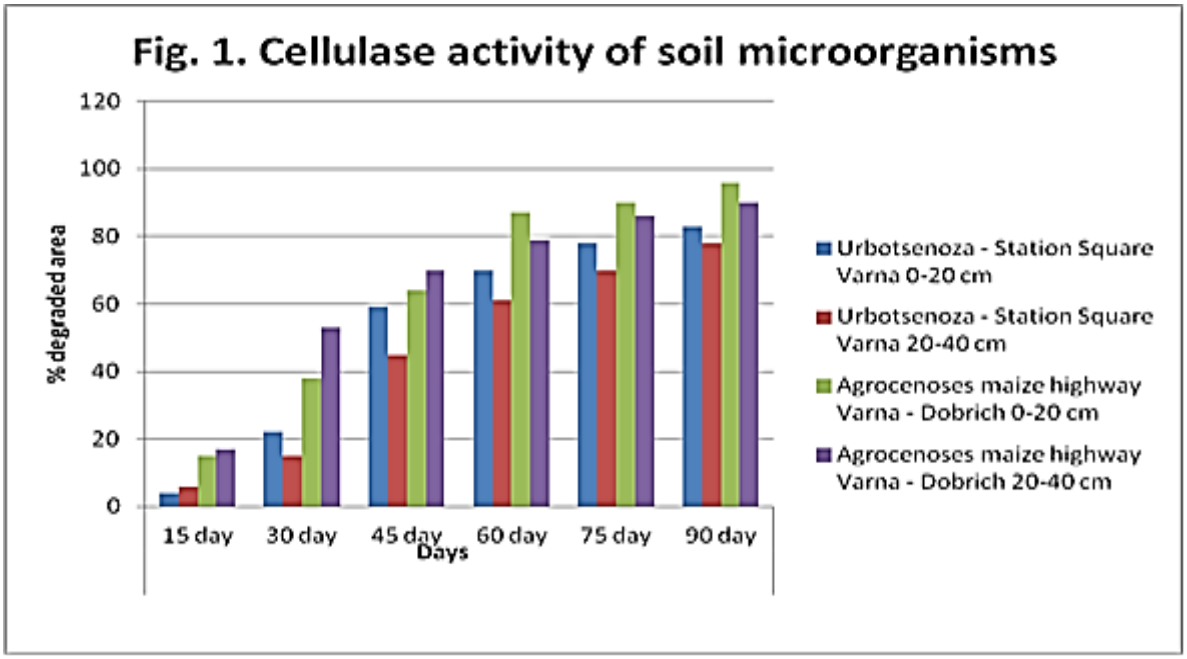
The investigated soils are with low content (under the maximum admissible concentrations) of the specified microelements (Pb, Cd, Cu, Zn, Ni, Mn and Fe). Their influence on the microbiological activity is established through reporting of a polycomponent content, obtained through summing up of the quantities of these elements (table 2).

Table 2. Totally a multiple heavy metal content of soils

Experimental area	**Depth (cm)**	**pH (H_2O)**	**∑ Multiple heavy metal content**		
			(Pb, Cd, Cu, Zn)	**(Ni, Mn, Fe)**	**(Pb, Cd, Cu, Zn, Ni, Mn, Fe)**
			mg/kg		
Urbotsenoza - Station Square Varna	0-20	7,60	155,75	1930,42	2 086,17
	20-40	7,50	112,42	1256,25	1368,67
Agrocenoses maize highway Varna - Dobrich	0-20	7,58	89,97	2942,92	3032,89
	20-40	7,56	90,59	2681,25	2771,84

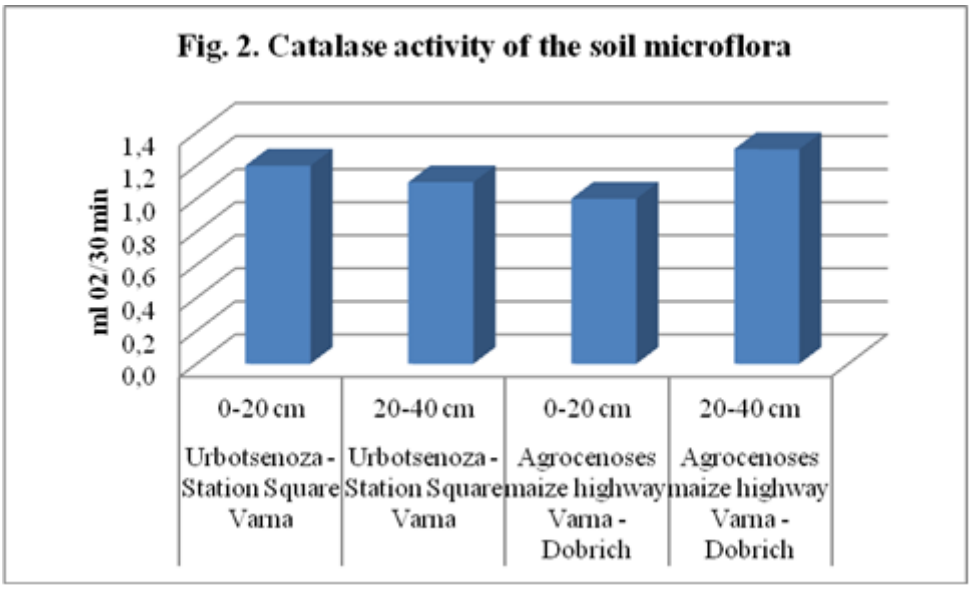
The results show that totally the agrogenic soil is with a higher total quantity of microelements in comparison with the urbogenic soil, which is due to the higher content of Ni, Mn and Fe in the agrogenic soil. The soils from the urbocenosis are with higher content of Pb, Cd, Cu and Zn, as separately, as well as totally. This tendency is determined by the fact that the soil from the urbocenosis is taken in the immediate proximity of a juncture with heavy traffic, and the one from the agrocenosis at elongation 10 m from the source of contamination (road II-29 Varna – Dobrich). The higher content of nickel, manganese and iron in the agrogenic soil can also be due to the usage of fertilizers and preparations, which contain these elements. At depth 0-20 cm this soil contains 1,5 times more total quantity of the investigated heavy metals in comparison with the soil from city environment. In the next soil layer the content of the investigated elements is lower. The soil from the agrocenosis at depth 20-40 cm contains 2 times more totally from the investigated elements in comparison with the urbogenic soil. The reaction of the soils is neutral, because of which the mobility of the heavy metals is slight in depth.

On figure 1 is represented the cellulase activity of the soils.



The cellulase is a basic destructor of cellulose in the soil and in great degree characterizes the direction and the speed of the mineralization processes in it. The decomposition of the cellulose by the microorganisms appears to be a natural destruction process, a section in the circle of the carbon, which provides the retrieval of the fixed in the process of photosynthesis carbon in the atmosphere in the form of CO_2. The favourable hydrothermal regime, the higher content of humus, organic carbon and nitrogen, as well as the lower concentration of potassium salts at the soil from the agrocenosis predetermine a higher cellulase activity at this soil. On the other hand, the worse moisture regime, the lower content of humus, carbon and nitrogen, as well as and the packing and the saltification at the urbogenic soil specify lower activity of this enzyme. The higher content of Pb, Cd, Cu and Zn in the soil of the urbocenosis also suppresses the cellulose activity. These elements limit stronger the cellulase in comparison with Mn, Fe and Ni, which predominate in the agrogenic soil, but the activity of the enzyme is higher. In depth the cellulase activity decreases, but it remains high – 78% disintegrated area at the urbogenic and 90% at the agrogenic soil on the 90-th day of reporting. The high activity of the soil layer at depth 20-40 cm and at both ecosystems correlates basically with the increasing quantity of nitrogen.

The catalase activity of the soils is represented on the next figure 2.



The catalase is a respiratory enzyme, which contains in its prosthetic group iron and disintegrating the hydrogen peroxide, forming in the process of breathing. In some bacteria the quantity of the catalase is around 1% from the dry matter of the cells. The temperature optimum of the enzyme is from 0 up to 10º C. The catalase is not steady at more acid soils (pH≤3). Grozeva and Nustorova (1995) at investigation of the catalase activity and some chemical properties of soils from the region of the Mediterranean Sea established that the activity of the catalase correlates with the content of iron, hygroscopic moisture and in the most of the cases with the humus in the soils. According to them, probably the activity of this enzyme is determined as by the organic part of the soils, as well as by its mineral part (not enzymic catalysts), as according to researches of Kuprevich and Shcherbakova (1966) it reaches up to 14 % from the total soil activity. Kappen (1972) established and residual catalytic activity, which reaches up to 84 % from the initial enzymatic activity.

It is established that the activity of the catalase is the lowest at the agrogenic soil at depth 0-20 cm and the highest at the same soil at depth 20-40 cm. This tendency correlates with highest values of moisture and nitrogen in the lower soil layer at the soil from the agrocenosis in comparison as with the same soil at depth 0-20 cm, as well as with the urbogenic soil and at both investigated depths. Besides the importance of the microorganisms for the catalse activity of the investigated soils, there must be taken into account and the vegetation as a factor for the activity of the catalase with plant origin. Obviously the disintegration of the accumulated organic substance from the falling off of the maize during the years is more impeded in comparison with the disintegration of the grass vegetation. At investigation of catalase of anthopological soils from the region of Sofia was established that the activity of the enzyme depends on the content of total nitrogen, humus, iron and moisture of the soils. However, not always these parameters have direct ratio subordination with the catalase activity. Besides, soils from “Borisov’s garden” park, with lowest coefficient of mineralization, are with lowest catalase activity. Probbably a great part of the organic substance at the soil in the park is preserved and its disintegration is impeded, which leads and to a lower catalase activity (Malcheva, 2008, 2012).

The catalase activity is not suppressed by the activity of lead, cadmium, copper and zinc, as separately, as well as totally. It is higher in the urbogenic soil in comparison with the agrogenic soil at depth 0-20 cm, where the content of nickel, manganese and iron is the highest. In the lower soil layer at the soil from the agrocenosis the total content of Pb, Cd, Cu and Zn is increased slightly, while the one of Ni, Mn and Fe decreases 1,1 times. This fact confirms that the catalase is not suppressed by the total action of lead, cadmium, copper and zinc, but is limited at a higher total quantity of nickel, manganese and iron. The process of disintegration of the organic substance has to be impeded more at the strongly burdened anthropological soils, because of the relatively poor aeration of these soils. However, probably the higher content of some heavy metals does not decrease, and even activates the catalse

activity. A high catalase activity in soils supposes high content of hydrogen peroxide in them, which however is disintegrated with high speed up to water and oxygen. The accumulation of manganese in this case is a secondary process, which is connected with the presence of hydrogen peroxide. Some microorganisms oxidize the bivalent soluble Mn in insoluble four-valent. This process is realized by many unspecific microorganisms from different taxonomic groups – bacteria and fungi, as the process is carried out simultaneously by two organisms (for example association of fungi – Fusarium, Alternaria and etc. with Metallogenium). The mechanism of the interrelations between the symbionts is not completely clarified. Probably in their base lies the ability of Metallogenium to disintegrate H_2O_2.

4. CONCLUSIONS

1. In comparison with the border values the soils in the two 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 poorly supplied with phosphorus and averagely supplied with potassium. As per content of organic substance the urban soil is classified as slightly humus, and this of the agrocenosis as averagely humus.
2. There is considered the total polycomponent influence of two groups of heavy metals – lead, cadmium, copper, zinc and nickel, manganese, iron. The quantity of each of the investigated heavy metals is under the maximum admissible concentration. The first group of elements are with a higher content, as separately, as well as totally in the urbogenic soil. The quantity of the second group of elements is more at the agrogenic soil.
3. The cellulase activity is suppressed by the action of Pb, Cd, Cu and Zn and it is not influenced by the total action of Ni, Mn and Fe. It is higher at the agrogenic soil in comparison with the urbogenic one. In depth (soil layer 20-40 cm) it decreases, but it remains high and at both ecosystems (higher are the values at the agrogenic soil).
4. The catalase contrary to the cellulase is limited by the higher content of Ni, Mn and Fe and it is not suppressed by Pb, Cd, Cu and Zn. The values of the enzyme depend on the variation of the quantity of the investigated microelements – they are higher, as at the urbogenic soil and at both investigated depths, as well as at the agrogenic at depth 20-40 cm.
5. The investigated enzymes can serve as representative biomarkers for passing processes in anthropological soils with low content of heavy metals.

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