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Some Changes in the Chemical indexes and Enzymatic Activity of Flooded Soils from the Region of Varna, Bulgaria

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Abstract: *There are studied the changes in some chemical and enzymatic indexes of soils, as a result of a flood in the region of city of Varna. There are established higher values of organic carbon, nitrogen, phosphorus and potassium in the soil samples from floods, in comparison with the controlled region. The cellulase activity is higher also at the soil samples from the flooded terrain. Its activity decreases in depth, more strongly expressed at the soil from the control. And at both studied objects the catalase activity increases in depth of the soil and reaches highest values in the lower soil layer of the control region. The studied changes in the chemical indexes and enzymatic activity in soils can be used as biochemical indicators regarding passing processes after a flood.*

Keywords: *enzymatic activity, flood, soil, macro elements*

1. Introduction

Floods influence the content of oxygen in the soil, on the values of pH – alkalize the soils, increase the values of Mg, decrease the content of chlorides and sulphates [1]. Their frequent repeatedness and bigger duration at one and the same place leads to phosphorus deficiency in the soils [2]. The nutritive substances in the flooded soils are exhausted – there considerably decrease the content of nitrogen, phosphorus and potassium. There is changed the quantity and the forms of some microelements [3]. As a result of the flood there are formed embankment soils with changed physically-chemical and microbiological indexes. Especially the surface embankment layer may contain different harmful substances and pathogenic microorganisms. A number of authors prove a suppressing of the enzymatic activity by toxic elements in the soils [4-16]. Casteel et al. [17] established that the quantity of Cl. perfringens has been significantly higher in flooded agrogenic soils (after a hurricane) in comparison with soils before a hurricane.

The soil enzymes contribute to the general biological activities in the soil, since they catalyze reactions, which are necessary for the dissolving of the organic matter, the circles of nutritive substances, transfer of energy in the eco systems [18-20]. They are the basic mediators of the biological processes, passing in the soil [21], which are concerned by numerous biotic and abiotic factors like the characteristics of the plants and the soil moisture [22].

Chendrayan et al. [23] established that the dehydrogenase activity is increased from 1.25 up to 2.5 times, and the invertase activity decreases at flooded soils in comparison with not flooded soil. The long-term usage of compost at rice-fields at flooded condition helps for the preserving of the organic substance and positive correlation between the content of carbon and the values of the enzymes: dehydrogenase, urease, cellulase, β-glucosidase, fluorescein, diacetate, invertase and amidase [24]. At research of the influence of the water gradients on the enzymatic activities and growth of Carex lasiocarpa on marsh soil, Zhongmei et al. [25] established that with the increase of the level of the water the acid phosphatase, the invertase and urease decrease, while the catalase activity is increased.

In flooded soil the oxygen is exhausted, the soil pores are filled with water and there are created conditions for development of anaerobic microorganisms, as the bacteria, which cause fermentations and methanogenic archaea [26]. The floods increase the activity of anaerobic respiratory enzymes like: pyruvate decarboxylase, alcohol dehydrogenase, lactate dehydrogenase, which can be used like biomarkers at stress-conditions in the soil after a flood [27]. The anaerobic dissolving of the organic matter is less efficient, it passes slower than the aerobic dissolving [28].

The enzymes are acknowledged as more sensitive bioindicators than plants and animals for different damages of the soils [29]. Therefore, their activity can serve as a biomarker for passing changes in the soils, which are due to anthropogenic or natural factor. Despite the significance of the cellulase in the circle of the carbon, most of the researches are engaged only with the production, purifying, characteristic and the immobilization of natural or synthetic adsorbents (and namely cellulose), and not with the activity of the cellulase in natural environments [30].

2. Aim, Objects and Methods

The aim of the present work is to be analyzed the changes at some chemical and enzymatic indexes at flooded soils from city of Varna.

The research is carried out 4 months after a natural calamity, dated 19 June 2014. There are analyzed soil samples from the following objects:

- Side stripe of Narodni buditeli boulevard, which is most affected by the flood, under grass vegetation and courgettes (*Cucurbita pepo* L.);
- Asparuhovo park (conditionally accepted as a control object) – under grass vegetation and blackberry (*Rubus fruticocus* L.).

The gathering of samples is carried out a single time manually from two depths of the soils - 0÷20 cm and 20÷40 cm, in compliance with the requirements of the Bulgarian State Standard 17.4.5.01:1985 [31].

The soil samples are analyzed for content of nitrogen, phosphorus, potassium and pH of the environment.

The content of nitrate and ammonium nitrogen is specified photometrically with Nitrospectral as a result of extraction with solution of calcium chloride ($CaCl_2$) (ISO 14255:2002) [32].

The values of mobile phosphates and absorbable potassium are determined as per standard ISO 11263:2002 [33], through double lactate method of Egner – Reihm. The method is based on the extraction of the movable compounds of phosphorus and potassium with solution of calcium lactate $(CH_3CH.OH.COO)_2Ca$.

The active reaction of the soil (pH) is determined in a water extract, in compliance with Bulgarian State Standard ISO 10390:2011 [34].

The quantity of organic carbon was established through a calculation – as per method of Tyurin [35].

The moisture of the soil is determined as per a weight method through usage of a thermostat and drying at temperature 105^0C up to a permanent weight [35].

The samples for enzymatic analysis are taken by a sterile instrument, in sterile paper bags. They were transported and investigated latest up to 48 hours, as until the moment of the analysis they were preserved in a refrigerator at 4-10°C.

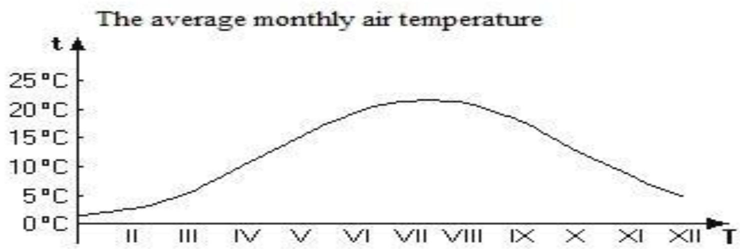
The cellulase activity of the soil is investigated at aerobic (as per two methods) and anaerobic conditions. For reporting of the activity of the enzyme at aerobic conditions in a Petri dish is sprinkled soil with depth around 7 mm, as there is maintained 60 % utmost field moisture capacity. Over the soil are put 3 stripes of sterile filter paper with sizes 10/50 mm. Then follows cultivation in a thermostat at 25°C - 35°C. In 15 days is reported the dissolved area with the help of a net-standard [36]. The culture suspension for the spectrophotometric method of anaerobic decomposition of the cellulase is prepared as 5 g from the substrate set at aerobic conditions as per the described method (the filter leaflets with germinated colonies and part of the soil under the leaflets) is ground in a mortar with 45 ml distilled water (in portions). It is extracted 1 hour and is filtered through a paper filter. For the anaerobic disintegration of the cellulase is prepared nutritive environment of Imshinetski in the following way: mesopeptonic bouillon 500 ml, sterile water-main water – 500 ml, $CaCO_3$ – 2 g, cut stripes of sterile filter paper – 10 g. In a flask is poured high layer of the indicated environment. It is contaminated with the soil. The cultivation is done at 25 °C - 35 °C. After 1-2 weeks is observed formation of foam – an indication for passing of the fermentation, and at the filter papers are observed brown stains and slime. Later the filter papers begin to destroy themselves [37]. From the so prepared culture liquids (set at aerobic and at anaerobic conditions) is taken 1 ml and the cellulase activity is reported spectrophotometrically [38].

The catalase activity of the soil is specified as per manganese-metric method [36].

The obtained agrochemical data are strategically processed and the results are included in a dispersion analysis with calculation of the least significant difference between the variants (LSD). The statistical processing is carried out with the assistance of programme product STATISTICA, version 10. The chemical indexes are statistically processed for average value and standard deviation. The statistical processing of the data for the enzymatic activities includes a calculation of average value and a standard diversion through the usage of the programme Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

The precipitations in the region of Varna are one of the slightest in the country. It rains mostly in June and in November (about 50 l/m^2), and it is driest in August - September, when the precipitations are not more than 30-32 l/m^2 monthly. On figure 1 are presented the annual progress of the perennial average monthly temperatures and average monthly amounts of the precipitations.



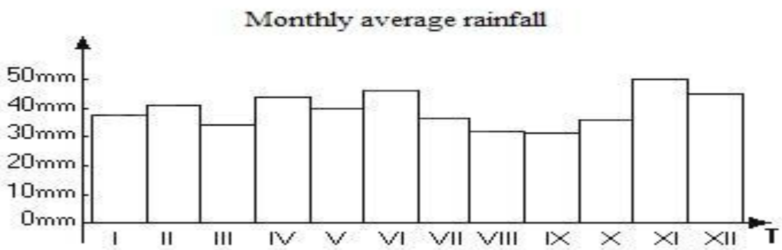


Figure1. *Distribution of temperature and rainfall*

On 20 June 2014 as per data of the National Institute of Meteorology and Hydrology, which have at their disposal 1 pce. synoptic, 5 pces. climatic and 8 pces. precipitation measuring stations in Varna, the precipitations reached quantities of 74 l/m^2.

On figure 2 and 3 are presented the average monthly temperatures and precipitations for the period June – October 2014 for the city of Varna.

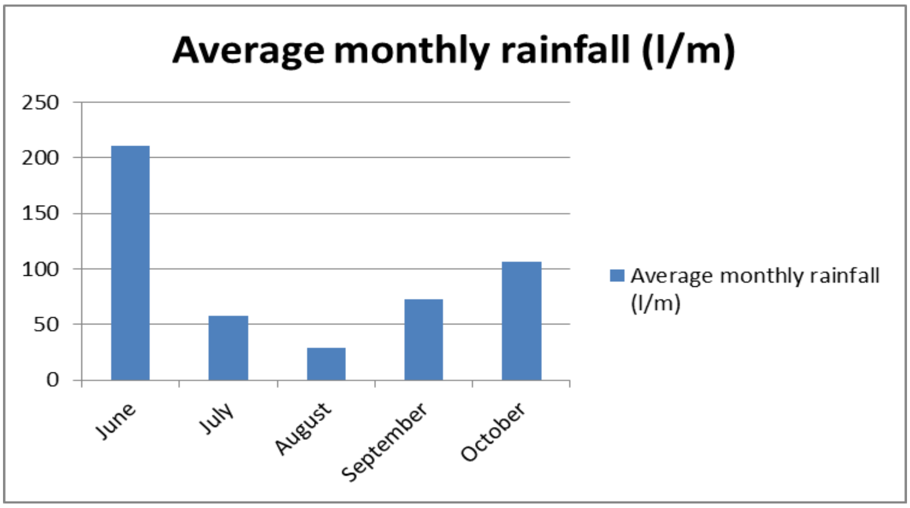


Figure2. *Average monthly amount of the rainfall (l/m) for the period June – October 2014*

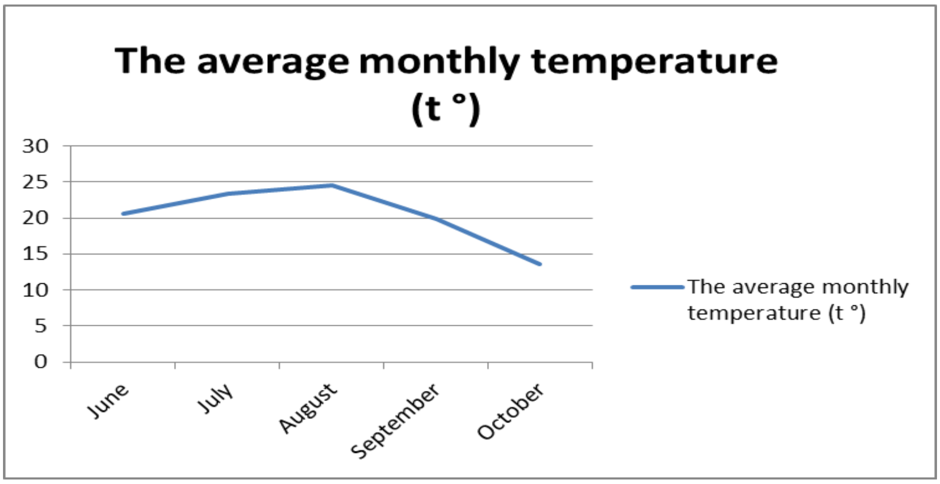


Figure3. *Average monthly temperature (t°) for the period June – October 2014*

In table 1 are put the results for content of nitrogen, phosphorus and potassium. From the data is visible that highest values are established at the samples from Narodni buditeli boulevard, which is most affected by the flood. Highest values for potassium (42.40 mg/100 g) are registered in the soil layer 0-20 cm, and for phosphorus (4.24 mg/100 g) and nitrogen (5.71 mg/kg) – in the layer 20-40 cm. Great part of the nitrogen in flooded soils is lost through leaching and denitrification [39] – it determines its accumulation in depth, and the quantity of the phosphorus is increased at flooded soils, especially with acid reaction [40]. At the samples from the park, conditionally accepted for a control object, the results are lower. The values for total potassium in the layer 0-20 cm are 5 times lower in comparison with the sample from the affected by the flood section. Regarding the total phosphorus and nitrogen the results are lower almost 2 times in the two depths. The releasing of relatively big quantity of ferrous and manganese ions and the increasing of ammonium ions in flooded soils lead to movement of potassium ions from complexes and their releasing in the soil solution. This probably leads to bigger presence of potassium in flooded soils [41].

The storage of the soils with organic carbon is low. Its content with the flooded soil is increased in depth 8 times, because of the fact that this soil is newly formed – it is comprised of risen embankments with different quantity organic matter and there are not clearly distinguished soil layers. At the control soil the quantity of organic substances decreases in depth 14 times – consequently follows the on principle established tendency for decreasing of the humus and the organic carbon in depth of the soil layers.

Some nutritive substances are increased with flooded soils, while others are object of bigger fixation or loss of the soil as a result of floods [41].

Logically the flooded soil in the upper soil layer is with highest percentage content of moisture, as in depth it decreases by 3 %. The lowest is the value of the moisture in the control soil at depth 20-40 cm, and at depth 0-20cm the moisture is higher by 1%. The soil from the park is watered and is with good moisture regime. It is also affected by the flood, but at lower degree. The higher values of the moisture in the soil in side stripe of the boulevard, which is not with a watering regime, are as a result of the fallen high quantity of precipitations, as during the flood, as well as until the moment of taking of samples.

The studied soils are with varying values of the soil reaction – the samples from the flooded region are with a slightly acid up to a neutral reaction, and from the control region – with an expressed acid reaction. The fact that pH of most soils has a tendency to change towards a neutral value after a flood is well known [42, 43].

The obtained agrochemical data are presented in table 1. The results are statistically processed and are included in a dispersion analysis with calculation of the least significant difference between the variants (LSD), but such was not established.

Table1. *Chemical indexes of the investigated soils*

№	Experimental area	Depth, cm	Chemical parameters					
			moisure%	pH	Organic C%	N, mg/kg	P, mg/100 g	K, mg/100g
1	Urbotsenoza - flood soil, Varna	0-20	19.08	6.8	0.18	1.72	3.73	42.70
2	Urbotsenoza - flood soil, Varna	20-40	16.17	6.4	1.50	5.71	4.24	30.96
3	Urbotsenoza - park „Asparuhovo“, Varna	0-20	16.37	3.6	1.62	0.93	1.57	8.18
4	Urbotsenoza - park „Asparuhovo“, Varna	20-40	15.30	3.5	0.12	1.40	1.86	7.39

On figure 4 is presented the dynamics of the cellulase activity of the soils at aerobic conditions and disintegration (reporting of the disintegrated area in % with net standard). The data show that with the strongly flooded soil the cellulase activity is not limited. At this object and in the two soil layers the disintegrated area is more in comparison with the control object. This tendency is established in dynamics for all days of reporting of the cellulase activity and correlates to the higher content of total forms of nitrogen, phosphorus and potassium at the flooded soil. Higher is and the percentage content of moisture at this soil, while the values of pH are almost similar for the soils and from the two objects. The activity of the enzyme decreases in depth – at the flooded soil insignificantly, and at the soil from the park this drop is by around 2 times. This tendency is depending on the significantly higher content of organic carbon in the lower soil layer of the flooded soil and in the upper soil layer of the control object.

The content of the analyzed chemical elements nitrogen, phosphorus and potassium is almost uniformly distributed and in both soil layers and at both objects, with the exception of the nitrogen at the flooded soil, at which its quantity is two times higher at depth 20-40 cm. This nutrient reserve with the investigated macro elements determines high cellulase activity, especially with the flooded soil.

Probably the accumulation of organic remainders from the grass vegetation and blackberries at the control object impedes their mineralization.

The aerobic disintegration of the cellulose is realized by many microorganisms from different systematic groups: bacteria, myxobacteria, actynomycetes, fungi. The most important change in the soil as a result of a flood is the transformation of the root zone of the soil from aerobic environment into anaerobic or almost anaerobic environment, in which lacks oxygen or it is in restricted quantity [44].

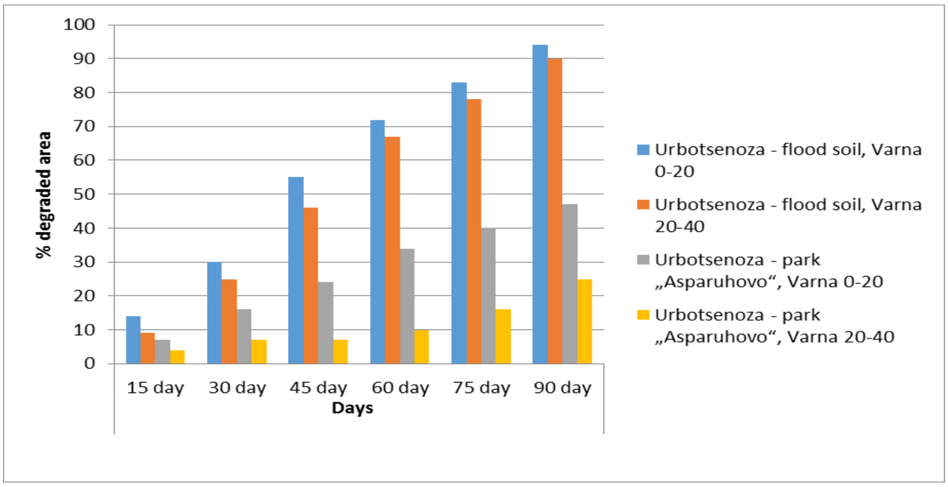


Figure4. *Dynamics of cellulase activity of the soil microorganisms (aerobic conditions)*

In anaerobic environment the disintegration of the organic substances is delayed [40]. However, usually after a flood there is formed an aerobic surface layer with depth up to 20 mm, and under it, in depth of the soil, there prevail anaerobic conditions [45]. This fact to a certain extent explains the higher values of the cellulase activity in the surface layers, although with them the soil is with higher content of moisture. The anaerobic disintegration of the cellulose is realized with formation of methane, hydrogen, carbon dioxide, butyric acid and others. In anaerobic conditions the disintegration of the cellulose is realized by anaerobic bacteria from the genus *Clostridium*, as well as by some other agents of anaerobic cellulose fermentation: mesophilic - *Bacillus cellulosae methanicus, Bacillus cellulosae hydrogenicus*; thermophilic - *Bacillus cellulosae dissolvens*. Their higher quantity at anaerobic conditions to a certain extent shall determine a faster disintegration of the organic matter in the soils.

The spectrometric determination of the cellulase activity of the soils also shows higher activity of the enzyme at the flooded soil, than at the soil from the park (table 2). At flood the air from the soil pores is pushed out and is filled with water. There are created anaerobic conditions. There is established that the cellulase activity is even higher at anaerobic conditions in the flooded soil in comparison with the activity of the enzyme at aerobic conditions. This fact is determined and by the development of higher quantity anaerobic types of microorganisms in flooded soils. The soil enzymes participate in the biological cycles of the elements and they play an important role in the transformation of organic and inorganic compounds.

Table2. *Cellulase activity of the soils at aerobic and anaerobic conditions*

Experimental area	Depth (cm)	Cellulase activity of soils (mg glucose/ml) Mean; SD	
		aerobic conditions	anaerobic conditions

Urbotsenoza - flood soil, Varna	0-20	0.070±0.004	0.085±0.004
Urbotsenoza - flood soil, Varna	20-40	0.056±0.005	0.063±0.006
Urbotsenoza - park „Asparuhovo“, Varna	0-20	0.024±0.006	0.026±0.007
Urbotsenoza - park „Asparuhovo“, Varna	20-40	0.017±0.002	0.017±0.001

Cellulases hydrolyse the cellulose polymer up to smaller oligosaccharides and glucose, as they include three basic types of enzymes: endoglucanases, cellobiohydrolases and β-glucisidases [46-48]. These enzymes can be either free, or more specially in aerobic microorganisms, or grouped in multicomponent enzymatic complex, in anaerobic cellulolytic bacteria [49].

The catalase activity of the soils is shown in the next table 3.

Table3. *Catalase activity of soils*

Experimental area	Depth (cm)	Catalase ml 0_2 / 30 min Mean; SD
Urbotsenoza - flood soil, Varna	0-20	1.88 ± 0.08
Urbotsenoza - flood soil, Varna	20-40	1.98 ± 0.06
Urbotsenoza - park „Asparuhovo“, Varna	0-20	1.70 ± 0.05
Urbotsenoza - park „Asparuhovo“, Varna	20-40	2.12 ± 0.06

According to Zhongmei et al. [25] the catalase activity is increased with increasing of the content of moisture in the soils. At our researches the values of the enzyme are close for the soils from the two objects, which is according to the close values of the humidity. The activity of the enzyme is lowest (1.70 ml 0_2/30 min) at the upper soil layer in the park as in depth it is increased 1 time and it is higher than the one at the soil from the boulevard. At the flooded soil the catalase activity is increased slighter in depth– by 0.10 ml 0_2 / 30 min. This tendency correlates to the higher content of nitrogen and phosphorus in the lower soil layers. Probably the activity of the catalase in depth (in higher degree at the control) is increased and because of the presence of a catalase with vegetative origin [50]. Grozeva and Nustorova [51] established that the activity of the catalase correlates to the content of iron, hygroscopic humidity and in the most of the cases with the organic substance 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 (non-enzymatic catalysts).The catalase activity depends on the content of the researched chemical indexes – total forms of nitrogen, carbon, phosphorus, potassium, pH, moisture of the soils – however, these parameters not always have direct ratio subordination – for example the decrease of the moisture and the potassium in depth does not suppress the catalase activity. Also the values of organic carbon are not in direct ratio subordination with the activity of the enzyme at the control object.

4. Conclusion

1. From the carried out chemical analyses was established that the two researched objects are with slight nutrient reserve of nitrogen, phosphorus and organic carbon.
2. There is established good nutrient reserve of potassium in the soil sample from Narodni buditeli boulevard, which is due to the flood. Average nutrient reserve of potassium is established in the sample from the park, which is also control one.
3. The quantities of nitrogen, phosphorus and potassium are higher in the soil after flood in comparison with the soil from the park. The increase of the quantities of phosphorus and potassium is determined by the increase of construction materials as a result of the destructive power of the flood.
4. The cellulase activity is higher at the flooded soil in comparison with the control one. This tendency correlates to the higher content of nitrogen, phosphorus and potassium at the soil, which is strongly affected by the flood.
5. The activity of the cellulase decreases in depth – this drop is strongly expressed at the soil from the park – around 2 times. This tendency is depending on the significantly higher content of organic carbon in the lower soil layer of the flooded soil and in the upper soil layer of the control object.

6. The catalase activity increases in depth of the soil and at both researched objects as it reaches its highest value in the lower soil layer at the control object. This increase in depth is depending also on the higher content of nitrogen and phosphorus in the lower soil layers at the researched objects.
7. The researched chemical indexes and enzymatic activities may serve as biochemical indicators for passing processes in soils after a flood.

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REFERENCES

[1] Boivin, P., Favre, F., Hammecker, C., Maeght, J.L., Delarivière, J., Poussin, J.C., Wopereis, M.C.S., 2002, Processes driving soil solution chemistry in a flooded rice-cropped vertisol: analysis of long-time monitoring data, Geoderma, 110 (1–2), 87–107.

[2] Sawyer, J., Mallarino, A., Al-Kaisi, M., 2011, Flooded Soil Syndrome. Flood recovery for cropland.http://www.agronext.iastate.edu/soilfertility/info/Flooded%20Soil%20Syndrome%20Fact%20Sheet.pdf.

[3] Snyder, C. S., 2002, Effects of Soil Flooding and Drying on Phosphorus Reactions, NEWS & VIEWS, 1-4.

[4] Effron, D, de la Horra, A.M., Defrieri, R.L., Fontanive, V, Palma, P.M., 2004, Effect of cadmium, copper and lead on different enzyme activities in a native forest soil, Comm. Soil Sci. Plant Anal., 35, 1309–1321.

[5] Khan, S., Cao, Q., Hesham, A.E.L., Xia, Y., He, J., 2007, Soil enzymatic activities and microbial community structure with different application rates of Cd and Pb, J. Environ. Sci., 19, 834–840.

[6] Kahkonen, M.A., Lankinen, P., Hatakka, A., 2008, Hydrolytic and lignolytic enzyme activities in the Pb contaminated soil inoculated with litter-decomposing fungi, Chemosphere, 72, 708–714.

[7] Kizilkaya, R., 2008, Dehydrogenase activity in Lumbricus terrestris casts and surrounding soil affected by addition of different organic wastes and Zn, Bioresour Technol, 99, 946–953.

[8] Kunito, T., Saeki, K., Goto, S., Hayashi, H., Oyaizu, H., Matsumoto, S., 2001, Copper and zinc fractions affecting microorganisms in long-term sludge-amended soils, Bioresour Technol, 79, 135–146.

[9] Lee, I.S., Kim, O.K., Chang, Y.Y. et al., 2002, Heavy metals concentracions and enzymes activities in soil from a contaminated Korean shooting range, Biosci Bioeng, 94 (5), 406-411.

[10] Majer, B.J., Tscherko, D., Paschke, A. et al., 2002, Effects of heavy metals contamination on micronucleus induction in Traes-candia and on microbial enzymes activities: a comparison investigation. Mutation Res, 515, 111-124.

[11] Malley, C., Nair, J., Ho, G., 2006, Impact of heavy metals on enzymatic activity of substrate and composting worms *Eisenia fetida*. Bioresour Technol, 97, 1498–1502.

[12] Oliveira, A, Pampulha M.E., 2006, Effects of long-term heavy metal contamination on soil microbial characteristics, J. Biosci. Bioeng., 102, 157–161.

[13] Perez-de-Mora, A., Burgos, P., Madej ´ on E ´ et al., 2006, Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments, Soil Biol. Biochem., 38, 327–341.

[14] Shen, G., Lu, Y., Zhou, Q., Hang, J., 2005, Interaction of polycyclic aromatic hydrocarbons and heavy metals on soil enzyme, Chemosphere, 61, 1175–1182.

[15] Speir, T.W., Kettles, H.A., Parshotam, A., Searle, P.L., Vlaar, L.N.C., 1999, Simple kinetic approach to determine the toxicity of As[V] to soil biological properties, Soil Biol. Biochem., 31, 705–713.

[16] Wang, Y., Li, Q., Shi, J., Lin, Q., Chen, X., Wu, W., Chen, Y., 2008, Assessment of microbial activity and bacterial community composition in the rhizosphere of a copper accumulator and a non- accumulator, Soil Biol. Biochem., 40, 1167–1177.

[17] Casteel, M., Sobsey, M., Mueller, J., 2006, Fecal contamination of agricultural soils before and after hurricane-associated flooding in North Carolina, Journal of Environmental Science and Health Part A, 41(2), 173-184.

[18] Dick, R P. 1994, Soil enzyme activities as indicators of soil quality. In: Doran J W, Coleman D C, Bezdicek D F, Stewart B A eds. Defining soil quality for a sustainable environment. Madison, WI, USA: American Society of Agronomy, 107–124.

[19] Dick, R P., 1997, Soil enzyme activities as integrative indicators of soil health. In: Pankhurstl C E, Double B M, Gupta V V S R eds. Biological indicators of soil health. Wallingford, UK: CAB International, 121–156.

[20] Yao, X.H., Min, H., LÜ, Z.H., et al., 2006, Influence of acetamiprid on soil enzymatic activities and respiration, European Journal of Soil Biology, 42, 120–126.

[21] Marx, M.C., Wood, M., Jarvis, S.C., 2001, A microplate fluorimetric assay for the study of enzyme diversity in soils, Soil Biology & Biochemistry, 33, 1633–1640.

[22] Guan, S Y., 1986, Soil enzymology and research method. Beijing: Agricultural Press, 274–323.

[23] Chendrayan, K., Adhya, T.K., Sethunathan, N., 1980, Dehydrogenase and invertase activities of flooded soils, Soil Biology and Biochemistry, 12 (3), 271–273.

[24] Nayak, D., Jagadeesh Babu, Y., Adhya, T.K., 2007, Long-term application of compost influences microbial biomass and enzyme activities in a tropical Aeric Endoaquept planted to rice under flooded condition, Soil Biology and Biochemistry, 39 (8), 1897–1906.

[25] Zhongmei, W., Changchun, S., Yuedong, G., Li, W., Jingyu, H., 2008, Effects of water gradients on soil enzyme activity and active organic carbon composition under *Carex lasiocarpa* marsh, Acta Ecologica Sinica, 28(12), 5980-5986.

[26] Liesack, W., Schnell, S., Revsbech, N., 2000, Microbiology of £ooded rice paddies, FEMS Microbiology Reviews, 24, 625-645.

[27] Vantoai, T.T., Fausey, N.R., McDonald Jr, M.B., 1987, Anaerobic metabolism enzymes as markers of flooding stress in maize seeds, Plant and Soil, 102 (1), 33-39.

[28] McDonald, A.J., 2002, Soil Drainage Classification and Hydric Soil Indicators EAS/CSS 260 - Intro Soil Science Cornell University. http://www.css.cornell.edu/courses/260/Lab%20Hydric %20Soils.pdf

[29] Hinojosa, M B, Carreira, J A, Garcła-Rułz, R et al., 2004, Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils, Soil Biol. Biochem., 36, 1559–1568.

[30] Safari-Sinegani, A., Safari-Sinegani, M., 2011, The effects of CaCO3 on adsorption, immobilization and activity of cellulase in a decarbonated soil, Journal of Soil Science and Plant Nutrition, 11 (3), 99-109.

[31] Bulgarian Institute for Standardization 1985, Nature conservation. Soil. General requirements for sampling, BSS 17.4.5.01:1985, Bulgarian Institute for Standardization.

[32] International Organization for Standardization, 2002, Soil quality: Determination of nitrate nitrogen, ammonium nitrogen and total soluble nitrogen in air-dry soils using calcium chloride solution as extractant, ISO 14255:2002, International Organization for Standardization, Geneva.

[33] International Organization for Standardization, 2002, Soil quality: Determination of phosphorus - Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution, ISO 11263:2002, International Organization for Standardization, Geneva.

[34] International Organization for Standardization, 2011, Soil quality: Determination of pH, ISO 10390:2011, International Organization for Standardization, Geneva.

[35] Donov, V., Gencheva, St., Yorova, K., 1974, Manual of Forest Soil, Sofia, 220 p. (In Bulg.).

[36] Khaziev, F., 1976, Enzymatic activity of soils, Nauka, Moscow (In Russ.).

[37] Gushterov, G., Andonov, P., Todorov, Ts., Kominkov, L., Gincheva-Starcheva, M., 1977, Practice in Microbiology with virology, Science and art, 393 p. (In Bulg.).

[38] Gradova, N., Babusenko, E., Gornova, I., 2004, Laboratory practice on general microbiology, DeLi print, 144 p. (In Russ.)

[39] Fageria, N.K., Baligar, V.C., 2005, Enhancing nitrogen use efficiency in crop plants, Advances in Agronomy, 88, 97–185.

[40] Fageria, N.K., Carvalho, G.D., Santos, A.B., Ferreira, E.P.B., Knupp, A.M., 2011, Chemistry of Lowland Rice Soils and Nutrient Availability, Communications in Soil Science and Plant Analysis, 42, 1913–1933.

[41] Patrick Jr., W.H., Mikkelsen, D.S., 1971, Plant nutrient behavior in flooded soil. In Fertilizer technology and use, 2nd ed., ed. R. A. Olson, 187–215. Madison, Wisc.: Soil Science Society of America.

[42] Ponnamperuma, F. N., 1972, The chemistry of submerged soils, Advances in Agronomy, 24, 29–96.

[43] Patrick Jr., W.H., and Reddy, C.N., 1978, Chemical changes in rice soils. In Soils and rice, 361–379. Los Banos, Philippines: IRRI.

[44] Patrick Jr., W.H., Mahapatra, I.C., 1968, Transformation and availability to rice of nitrogen and phosphorus in waterlogged soils. Advances in Agronomy, 20, 323–356.

[45] Bouldin, D. R., 1986, The chemistry and biology of flooded soils in relations to the nitrogen economy in rice fields, Fertilizer Research, 9, 1–14.

[46] Enari, T.M., 1983, Microbial cellulases. In, WM Fogarty. Microbial enzymes and biotechnology, Applied Science, London, 4, 183-223.

[47] Ilmen, M., Saloheimo, A., Onnela, M.L., Penttila, M.E., 1997, Regulation of cellulase gene expression in the filamentous fungus *Trichoderma reesei*, Appl. Environ. Microbiol., 63, 1298-1306.

[48] Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002, Microbial Cellulose Utilization, Fundamentals and Biotechnology, Microb. Mol. Biol. Rev., 66, 506-577.

[49] Bayer, E.A., Shimon, L.J.W., Shoham, Y., Lamed, R., 1998, Cellulosomes structure and ultrastructure, J. Struct. Biol., 124, 221-234.

[50] Malcheva, B., 2008, Catalase activity and some chemical properties of the anthropogenic soils. II Balkan Scientific Conference "Science, Education and Art in the 21st Century", 26-27 September, Bulgaria, Blagoevgrad, 333-339 (In Bulg.).

[51] Grozeva, M., Nustorova, M., 1995, Catalase activity and some chemical properties of soils of the Mediterranean region, Soil Science, Agrochemistry and Ecology, 1-6, 181-182 (In Bulg.).

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