

Some Chemical and Microbiological Indexes at Soils after a Flood in the Region of Varna, Bulgaria

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Abstract: *There are studied the chemical and microbiological indexes of soils 4 months after a flood in the city of Varna. There are established increased values of moisture and pH in the flooded soil in comparison with the conditionally accepted for control soil. The quantities of nitrate nitrogen, absorbable forms of potassium and phosphorus are higher in the soil after a flood, than in the soil from the park. This tendency is determined by the accumulated vegetative remainders and construction materials in the flooded soil. The content of organic carbon is higher in the layer 20-40 cm and lower at depth 0-20 cm at the soil, which is strongly influenced by the calamity, in comparison with the control. The speed of disintegration in these layers, however, is impeded by the accumulated organic substance – the coefficients of mineralization with them are highest. Basic share in the content of the microbocenosis occupy the non-spore forming bacteria, bacilli and bacteria, which assimilate mineral nitrogen, which participate in the initial stages of destruction of the organic substances. Most poorly are presented the actinomycetes and at both objects. The quantity of the anaerobic microorganisms is higher than the aerobic at the flooded soil. The tendency is contrary at the soil from the park. There is not established contamination of the soils with pathogenic types of microorganisms – Escherichia coli and Clostridium perfringens. Regarding the influence of the chemical indexes the interconnection is highest between the content moisture in the soils and the total quantity of microorganisms.*

Keywords: *flood, urbogenic soils, macroelements, microorganisms*

1. INTRODUCTION

According to the soil classification of FAO-UNESCO Soil Map [1] the flooded soils are determined as „Hydric soils“ and they belong to different taxons.

According to some authors the floods lead to alkalization of the soil (pH=15), increase of the values of Mg and decrease the contents of chlorides and sulphates up to very low levels [2]. The flooded rice-fields are one of the basic biogenic sources of methane. After a flood the oxygen in the soil is exhausted and the soil pores are filled with water. There are created conditions for development of anaerobic microorganisms, like the bacteria, which cause fermentations and methanogenic archaea. They prevail in the microbe community, because of which the methane is the end product of the anaerobic disintegration of the organic matter [3]. The releasing of hydrogen sulphide and methane from soils flooded for a prolonged time is due to strong decrease (negative values) of the redox potential of these soils [4]. After prolonged floods of soils is observed a period of phosphorus deficiency, lack of vegetation or slight growth, poorly developed roots. These changes are marked by some authors as a syndrome of the flooded soils [5]. The duration and the depth of the floods influence on the content of oxygen in the soil, the values of pH, the presence of P, as well as on the quantity and forms of some microelements [6].

The risen waters, contaminated with microbe and other pollutants can be detrimental for the plants, as well as for the health of the animals and people. The faecally contaminated waters contain high quantity of coliforms, faecally coliforms, *Escherichia coli*, spores of *Clostridium perfringens*. Casteel et al. [7] established that the levels of the spores of *Cl. perfringens* have been significantly ($P < 0.001$) higher in flooded agrogenic soils (after a hurricane) in comparison with soils before a hurricane. Even

if there is not established contamination with pathogenic microorganisms of soils after floods, as a whole the nutritive substances in these soils begin to exhaust – there significantly decrease the content of nitrogen, phosphorus and potassium. If the affected soil is arable, it is necessary after the flood they to be fertilized with higher doses in order there to be a yield from the cultivated cultures [8].

Each year the arable land on the planet decreases with more than 100 million decares, because of erosion of the soil [9]. One of the detrimental consequences of the floods is the appearance of water erosion. Around 80% of the lands in Bulgaria are put under water erosion in different degree, out of which annually are moved out over 136 million tons of generous soil, there are washed out 2.5 million tons humus and there are lost hundred of thousands mineral nutritive substances [10].

The increase of the floods during the last years on a world scale supposes besides the obligatory preventive events to be carried out and more analyses of the soils after the floods with purpose their recovery and usage of full value in the future.

2. AIM, OBJECTS AND METHODS

The aim of the present work is research of occurring changes of some chemical and microbiological indexes in urbogenic soils after a flood.

There are analyzed soils, which are sample taken from Asparuhovo residential area, city of Varna, 4 months after a natural calamity, dated 19 June 2014. The samples for analysis are taken from a side stripe of Narodni buditeli Boulevard, which was most affected by the flood and from Asparuhovo park (conditionally accepted for a control object, also affected by the flood, but in a smaller degree) (fig. 1). The taking of samples is carried out in two depths 0-20 cm and 20-40 cm in compliance with the requirements of the Bulgarian State Standard 17.4.5.01:1985 [11]. The vegetation at the urbogenic soil after a flood is grass and courgettes (*Cucurbita pepo* L.), and at the one from the park – grass and blackberries (*Rubus fruticosus* L.).



Figure1. Asparuhovo residential area – the objects of taking of samples and catchment regions (flood, dated 19 June 2014)

The soil samples are analyzed for content of movable forms of nitrogen ($\text{NO}_3\text{-N}$; $\text{NH}_4\text{-N}$), phosphates (P_2O_5), absorbable potassium (K_2O), organic carbon, moisture and pH of the environment. The content of nitrate and ammonium nitrogen is specified photometrically with Nitrospectral as a result of an extraction with solution of calcium chloride (CaCl_2) (ISO 14255:2002) [12].

The content of phosphates and absorbable potassium is determined as per standard ISO 11263:2002 [13], through a double lactate method of Egner-Riehm. The method is based on extraction of the movable compounds of the phosphorus and potassium with solution of calcium lactate ($\text{CH}_3\text{CH.OH.COO}$)₂Ca.

The active reaction of the soil (pH) is determined in a water extract, in compliance with Bulgarian State Standard ISO 10390:2011 [14].

The quantity of total carbon is established through a calculation [15].

The moisture of the soil is determined as per weight method through usage of thermostat and drying at temperature 105⁰C up to a permanent weight [15].

The samples for microbiological analysis are taken by a sterile instrument, in sterile paper bags. They are transported and studied latest up to 48 hours, as until the moment of the culture they are preserved in a refrigerator at 4-10⁰C.

The microbiological researches include determining of non-spore forming bacteria, bacillary microflora, actinomycetes, micromycetes, bacteria, assimilating mineral nitrogen. They are determined as per the method of thinning out and cultures (surface – for aerobes and deep – for anaerobes) as there are used the following solid nutritive environments: mesopeptonic agar – for determining of non-spore forming bacteria and bacilli; starch-and-ammonia agar – for determining of actinomycetes and bacteria, assimilating mineral nitrogen; environment of Chapek-Docks – for determining of micromycetes. After a cultivation in a thermostat are reported colony-forming units, recalculated towards 1 gramme absolutely dry soil. For evaluation of the sanitary condition of the soils for finding of allochthonous pathogenic types, indexes for faecal contamination, are carried out: surface cultures on environment of Endo for presence of *Escherichia coli* and deep cultures with usage of TSC agar (tryptose sulphite agar with cycloserine) for presence of *Clostridium perfringens*.

The statistical processing of the data from the microbiological indexes includes a calculation of average value from three repetitions and a coefficient of variation.

The obtained agrochemical data are statistically processed and the results are included in a dispersion analysis with calculation of the least significant difference between the variants (LSD). There are determined correlations between the chemical elements, as well as between them and the general microflora. The statistical processing is realized with the help of programme product STATISTICA, version 10.

3. RESULTS AND DISCUSSION

As per data of the National Institute in Meteorology and Hydrology – Varna the quantity of the fallen precipitations for the day 19-20 June 2014 in Asparuhovo residential area is around 74 l/m² [16]. The meteorological characteristic of the region from the month of the flood up to the month of the sample collecting of the soils is presented in table 1, in which are put the average monthly temperatures and precipitations for the period June – October for city of Varna [17].

Table1. Meteorological characteristics as per elements for city of Varna (June-October 2014)

Month	June	July	August	September	October
The average monthly temperature (t ^o C)	20.6	23.4	24.5	19.8	13.6
Average monthly rainfall (l/m ²)	210.6	58.1	29	73	106.3

In the soil the nutritive elements are found in the form of organic and mineral substances, which together form its total reserves. They, however, do not give notion about the possibility of the soil to satisfy the necessity of the plants of nutritive substances. The determining of the movable (accessible) forms of the nutritive substances is a task of the soil agrochemical diagnostics.

Towards the movable forms in the soil refer the nitrates, nitrites, ammonium ions, phosphates and potassium ions, which can be carried out in soluble condition from the soil solution.

In table 2 are presented the results from the analysis of moisture, pH, organic carbon and accessible forms of nutritive substances in the soil.

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Table 2. Content of moisture, carbon, nitrogen, phosphorus, potassium and values of pH in soils from Asparuhovo residential area

№	Experimental area	Depth, cm	Parameters					
			Moisture %	pH (H ₂ O)	Org.C %	NO ₃ -N, mg/kg	K ₂ O, mg/100g	P ₂ O ₅ mg/100 g
1	Urbotsozoa - flood soil, Varna	0-20	19.08	6.8	0.18	1.72	51.24	8.54
2	Urbotsozoa - flood soil, Varna	20-40	16.17	6.4	1.50	5.71	37.15	9.70
3	Urbotsozoa - park „Asparuhovo“, Varna	0-20	16.37	3.6	1.62	0.93	9.82	3.60
4	Urbotsozoa - park „Asparuhovo“, Varna	20-40	15.30	3.5	0.12	1.40	8.87	4.25

From the carried out analysis is established that the soil samples taken from the flooded boulevard are with high values of the studied elements. There is established poor nutrient reserve of the soil with nitrogen and for both places. At the soil samples from Narodni buditeli boulevard is established content of nitrate nitrogen in the layer 0-20 cm - 1.72 mg/kg. A lower value of nitrogen at the same depth is established in the soil sample from Asparuhovo park (0.93 mg/kg). Higher results are reported in the soil layer 20-40 cm, as in the sample from the boulevard the increase is over 3 times (5.71 mg/kg). In the soil sample from the park in this layer is established content of nitrogen 4 times lower (1.40 mg/kg) than the one at the flooded soil. Moving out of the nitrate form of the nitrogen in the depth of the soils is established and by other authors [18, 19].

Regarding the absorbable potassium there is also noticed a tendency for higher values in the soil samples from Narodni buditeli boulevard. The nutrient reserve of the soil varies from good up to poor and average. A good nutrient reserve is reported in the soil samples from the territory of the boulevard. The highest established value is in the soil layer 0-20 cm (51.24 mg/ 100g), as in depth it decreases 1, 4 times. The soils from the territory of the park are distinguished with lower values. At them is established poor up to average degree of nutrient reserve. In the two studied layers 0-20 cm and 20-40 cm are reported respectively 9.82 mg/100g and 8.87 mg/100g.

From the carried out research is found that the highest content of movable phosphates is established in the samples from the boulevard, and a lower one in the soils on the territory of the park. The nutrient reserve of the soil with the macro element phosphorus is poor up to average. Once again is preserved the tendency for high results of movable phosphates in the soils from Narodni buditeli boulevard (8.54 mg/100 g and 9.70 mg/100 g). The soil samples from the territory of the park distinguish with lower values for content of phosphorus (3.60 mg/100 g and 4.25 mg/100 g). These tendencies are proven and at anthropogenic character of accumulation of phosphorus and potassium in the surface layers of urbogenic soils [20].

The nutrient reserve of the soils with organic carbon is low, but the quantity of organic carbon at the flooded soil is increased in depth, because of the fact that this soil is already comprised of risen embankments with different quantity of organic matter and there are not clearly distinguished soil layers. At the control soil the content of organic substances follows the on principle established tendency for decrease of the humus and the organic carbon in depth of the soil layers. According to Craul [21] and Lorenz et al. [22] the urban soils are a mixture of materials, strongly transformed by the anthropogenic activity, and not soils, formed only by the processes of eroding. We could add that these soils are formed not only by the anthropogenic activity and the processes of eroding, but also by natural calamities like floods and hurricanes.

From the values of pH is noticed that the soils after floods are with a slightly acid up to neutral reaction, and those from the park are with an expressed acid reaction. Consequently the flood has contributed for increase of the values of pH.

The moisture of the soil is highest at the flooded soil in the upper soil layer and lowest in the control soil at depth 20-40 cm. The decrease of the soil moisture in depth is bigger (3%) at the object after a flood, while the soil from the park is 1 %.

In table 3 is presented statistical processing of the data from the chemical indexes.

Table3. Statistical processing of the data

№	Experimental area	Depth, cm	NO ₃ -N, mg/kg		K ₂ O, mg/100g		P ₂ O ₅ , mg/100g	
			\bar{x}	VC	\bar{x}	VC	\bar{x}	VC
1	Urbotsenoza - flood soil, Varna	0-20	1.72 ^{***}	1.51	51.24 ^{***}	0.06	8.54 ^{***}	0.24
2	Urbotsenoza - flood soil, Varna	20-40	5.71 ^{***}	1.58	37.15 ^{***}	0.39	9.70 ^{***}	0.82
3	Urbotsenoza – park „Asparuhovo“, Varna	0-20	0.93 ^{***}	6.45	9.82 ^{***}	0.11	3.60 ^{***}	0.48
4	Urbotsenoza - park „Asparuhovo“, Varna	20-40	1.40 ^{***}	1.42	8.87 ^{***}	1.04	4.25 ^{***}	0.71

*** $p < 0.05$

From the analysis was established that among all variants there are statistically proven differences. Highest variational coefficient regarding the nitrate nitrogen is established in the soil layer 0-20 cm at the control sample (VC= 6.45), and lowest in the sample from the territory of the park in the layer 20-40 cm (VC= 1.42). At the absorbable potassium is observed variation from 0.06 up to 1.04, as highest homogeneity is reported at the sample from the flood in the soil layer 0-20 cm (VC= 0.06) . Regarding the movable phosphates the variation is biggest in the values of the variational coefficients at the analysis of the samples from the flood (VC= 0.24 to VC= 0.82).

The results from the microbiological researches show different biological condition of the soils at the two objects. The total content of aerobic and anaerobic microorganisms as per groups is presented in table 4.

Table4. Qualitative and quantitative composition of the soil micro flora

Experimental area	Depth (cm)	Total microflora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria, assimilating mineral nitrogen	Coefficient of mineralization
Urbotsenoza - flood soil, Varna	0-20	7214.4±0.22	4892.4 ± 0.10 (67.8)	2041.2 ± 0.39 (28.3)	113.4 ± 3.67 (1.6)	167.4 ± 2.03 (2.3)	2980.8 ± 0.26	0.43
	20-40	848.9±0.53	514.6 ± 0.56 (60.6)	48.7 ± 5.86 (5.7)	132.7 ± 5.18 (15.6)	152.9 ± 1.96 (18)	430.1 ± 0.42	0.76
Urbotsenoza - park „Asparuhovo“, Varna	0-20	7198.8±0.02	5829.6 ± 0.05 (81)	806.4 ± 0.44 (11.2)	201.6 ± 1.61 (2.8)	361.2 ± 0.64 (5)	5779.2 ± 0.10	0.87
	20-40	663.9±0.61	584.8 ± 0.83 (88.1)	30.6 ± 4.59 (5.2)	23.8 ± 2.76 (3.6)	24.7 ± 2.00 (3.7)	215.5 ± 0.29	0.35

* *Escherichia coli* и *Clostridium perfringens* not established.

The biogenity of the soils is established as a summary effect from the development of non-spore forming bacteria, bacilli, actinomycetes and micromycetes. The quantity of the general microflora is

higher at the soil after a flood in comparison with the soil from Asparuhovo park, as the difference is bigger for the soil layer at depth 20-40 cm. This tendency is also depending on the higher values of nitrate nitrogen, absorbable forms of potassium and phosphorus, as well as with values of pH close to the neutral ones at the flooded soil. Probably the accumulation of the nitrate nitrogen and the decrease of ammonium nitrogen are due to the high quantity got into plant remainders, brought by the flood and beginning of increase of the processes ammonification and nitrification. These organic substances in the soil become food for the microorganisms, as at the presence of oxygen there are obtained different products – ammonia, nitrogen oxide, nitrites or nitrates. The speed of disintegration of the organic matter, however, is impeded, as at the soil with highest moisture (the flooded soil) at depth 0-20 cm, as well as at the soil layer with lowest moisture – the control soil at depth 20-40 cm. The higher content of organic substances in the soil layer at depth 20-40 cm at the flooded soil, as well as in the surface layer of the control soil, does not impede their disintegration – there are established higher values of the mineralization coefficients in these soil layers. Of course, and the content of oxygen in them is higher in comparison with this at the flooded soil at depth 0-20 cm. The content of potassium and phosphorus is also increased as a result of the flood – accumulation of construction materials in the soil. The potassium salts in the soil increase the osmotic pressure in the soil solution, as this way they damage the young plants and influence the activity of the soil microorganisms [23, 24]. The reproduction of the microorganisms not only at the surface of the soil particles, but also in the water solution is activated, when the soil is enriched with the necessary for it nutritive substances.

The drop in the depth of the general microflora at the flooded soil is 9 times, and at the control soil 11 times. Basic share in the content of the microbocenosis and at both depths, at both soils, take the non-spore forming bacteria, followed by the bacteria, assimilating mineral nitrogen and the bacilli, which participate in the initial phases of destruction of the organic matter and realize the processes of self-purification of the soils from contaminants [25, 26]. This tendency is violated regarding the bacilli for the soil after a flood at depth 20-40 cm. They are activated strongly in the surface layer of the flooded soil and they decrease in depth 42 times. Their drop in depth at the control soil is slighter – 26 times. Similar is and the decrease in the lower soil layer at this soil of the bacteria, which assimilate mineral nitrogen – 27 times, and at the flooded soil – 7 times. The drop in depth at the non-spore forming bacteria is similar and at both studied soils – 10 times. The higher quantity of actinomycetes and micromycetes at the control soil at depth 0-20 cm, as well as at the flooded soil correlates to higher humidity in comparison with the soil layer at depth 20-40 cm at the soil from the park. The high quantity of these groups of microorganism in the surface layer of the control soil correlates and to a favourable pH for their development. A third place in the content of the general microflora of the studied soils occupy the mould fungi, and most poorly presented are the actinomycetes. A significant drop in depth for these two groups microorganisms is established at the soil from the park – the quantity of the micromycetes in the lower soil layer decreases by 15 times, and of the actinomycetes by 8 times. Their distribution in the two studied depths at the soil after a flood is almost uniformly.

And both studied objects are affected by the flood, but the soil from the park, conditionally accepted as a control, is more slightly affected. Because of this fact the statistical processing is carried out jointly for the two objects and the two depths. The quantity of the general microflora is depending mostly on the depth of taking of samples and the moisture of the soil. Between the general microflora and the chemical elements, pH and the region of taking of samples there are not established correlations. The correlations between all chemical indexes and the region are high. The absorbable phosphorus, pH and the moisture are in high dependency on the region and the depth as a general factor. The content of the nitrate nitrogen and the moisture of the soils is depending on the depth of taking of samples. The quantity of the absorbable potassium is depending slightly on the depth, as

well as on the region and the depth as a general factor. Such a low dependency is established and between the content of absorbable phosphorus and the depth, as well as between the quantity of the nitrate nitrogen and the region and the depth as a general index (table 5).

N	Parameters	Area	Depht, cm	Area + Depht, cm	Total microflora
1	K ₂ O	0.96	- 0.21	-0.31	not established
2	NO ₃ -N	- 0.67	0.58	- 0.39	not established
3	P ₂ O ₅	- 0.98	0.17	- 0.80	not established
4	pH	-0.99	not established	-0.91	not established
5	Moisture	0.63	-0.69	-0.71	0.70
6	Depth				-1
7	Area, cm				not established
8	Area + Depth, cm				not established

Table5. Correlation between the chemical indexes, the general microflora, the region and the depth

In the region there are houses without sewerage, but pathogenic type as *Escherichia coli* and *Clostridium perfringens* are not established. On principle *Escherichia coli* are not found in the soils 4-5 months after the contamination. However, the floods cause filling of the pores with water and respectively creation of conditions for development of anaerobic microorganisms. In the studied soils there exist as obligatory, as well as facultative aerobic and anaerobic microorganisms. In the flooded soil the percentage participation of the anaerobic types is bigger in comparison with the share of the aerobic microorganisms. This tendency is contrary for the soil from Asparuhovo park. In depth the quantity of the aerobes is increased at the flooded soil, which correlates to the decrease of the moisture of the soil in the lower soil layer. At the control soil the close values of the moisture in the two soil layers determine and close values of the percentage participation of aerobes and anaerobes for the two depths (fig. 2 ÷fig. 5).

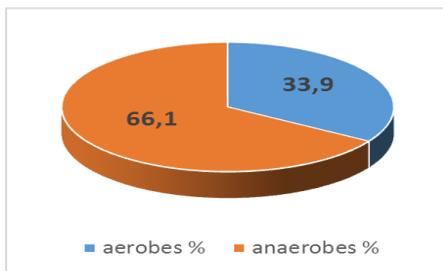


Figure2. Urbotsenzoza – flood soil, Varna 0-20 cm

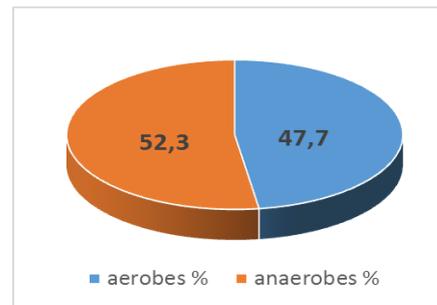


Figure3. Urbotsenzoza – flood soil, Varna 20-40 cm

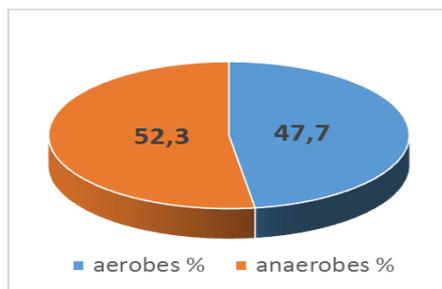


Figure4. Urbotsenzoza – park „Asparuhovo”, Varna 0-20 cm

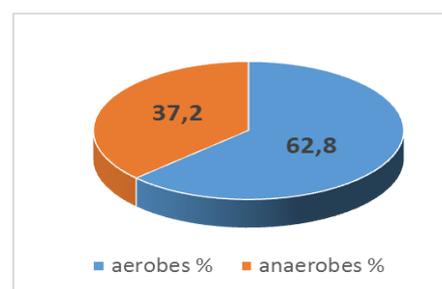


Figure 5. Urbotsenzoza – park „Asparuhovo”, Varna 20-40 cm

4. CONCLUSION

1. The values of the active reaction of the soils are two times higher at the flooded soil (a neutral reaction) in comparison with the control soil (an acid reaction). Consequently the flood has led to increase of the values of pH in the soil.

2. The quantities of nitrogen, potassium and phosphorus are higher in the soil after a flood in comparison with the soil from the park. Regarding the content of nitrogen this tendency correlates to the accumulation of more organic substances for disintegration. The increase of the quantities of potassium and phosphorus is conditioned by the increase of construction materials as a result of the devastating power of the flood.
3. The general micro flora is higher at the flooded soil. This tendency correlates to higher quantity of the studied macro elements in the flooded soil in comparison with the control one. In depth is established decrease of the general quantity of microorganisms, strongly expressed at the control soil. A basic share in the content of microbocenosis occupies the ammonifiers (non-spore forming bacteria and bacilli), as well as the bacteria, assimilating mineral nitrogen at the soils from the two objects. They have a basic role in the processes of mineralization. Most poorly presented are the micromycetes and the actinomycetes. The quantity of the anaerobic microorganisms is higher than the one of the aerobic microbes at the flooded soil in comparison with the control one. This conclusion is based on the creation of anaerobic conditions at flooded soils as a result of the pushing out of the air from the soil pores and their filling with water.
4. The quantity of the general microflora depends most strongly on the depth of taking of samples and the moisture of the soil. High are the correlations and between all chemical indexes.
5. The evaluation of the sanitary condition of the studied soils as per indexes colititer and perfringens titer shows that we can determine them as pure, since there have not been established colonies of *Escherichia coli* and *Clostridium perfringens*.
6. The studied chemical and microbiological indexes can serve as indicators for passing changes in flooded soils and to be used at their following recovery.

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