

Original Research Article

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Microbiological Characteristic of Cinnamon Pseudopodzolic Soil (Planosol) at Using of Black Sea Deep-Water Sediments (Sapropels)

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The influence of Black Sea deep-water organo-mineral sediments (sapropels) on the quantities of basic for soil fertility trophic groups of microorganisms at cinnamon pseudopodzolic soil (Planosol) was investigated. The study was conducted in conditions of pot experiments with cultivating oriental type tobacco. Sapropels were added in an amount of 30 g / kg dry soil. Soil samples for microbiological analysis from rhizosphere zone of tobacco plants were taken. Totality of microbiological indicators that characterize the general biogenicity in soil environments, mineralization processes and some transformations of nitrogen compounds in the soil have been explored. A positive impact from sapropels upon microflora in cinnamon psevdopodzolic soil (Planosol), resp. at its biological properties was found. The total population density at the microbial community as a whole is increased to 16.47%. The factor - "sapropels" strongly stimulate the quantitative development of the following trophic groups of microorganisms with statistically proven power of impact: at ammonifying (98.95%), at assimilating ammonium and nitrate nitrogen (68.86% and 55.90%), at *Azotobacter* (64.33%) and at Actinomycetes (68.99%). The mineralization processes dominate in the soil microcoenoses, at low degree of oligotrophic, suggesting accumulation of an assimilable by plants nutrients.

Introduction

A key factor for achieving sustainable management of agro-ecosystems is maintain and improve quality of soils, respectively their fertility (Lal, 2009). The content of humus and nutrients for the plants are indicators of soil fertility, but a leading role in the processes of its creation, maintaining and improving there are soil microorganisms. This role is determined by the fundamental activity that they doing in the substances circulation and primary transformation of organic matter flow (Kennedy *et al.*, 2004; Dobrovolskaya *et al.*, 2016). In the specific physicochemical

properties of a soil are formed specific microbial communities, from whose quantitative and functional biodiversity depend intensity of the processes running in the soil (Doran and Zeiss, 2000; Brussard *et al.*, 2007). Microbial communities are dynamic and rapidly adaptable to change. This enables for the impact regulation and management towards sustainability of agro ecosystems (Sparling 1997; Schutter and Dick, 2001; Larkin *et al.*, 2006; Saison *et al.*, 2006; Barrios, 2007; Dobrovolskaya *et al.*, 2015). In this connection rise the demand the

development and implementation of new technological decisions to include organic products of different origins for eco-friendly and sustainable agricultural production (Buyer *et al.*, 2010; Fritz *et al.*, 2012; Lukas S., 2013). Various organo-mineral sediments deposited in water bodies - sapropels are with high potential as an organic fertilizer (Baksiene, 2009; Emeis and Weissert, 2009). They are distinguished by various physical and chemical properties, different contents of organic and mineral substances, as well as specific microflora. The microbiological characteristics of some sapropels from Baltic lakes are characterized by high levels of aerobic heterotrophic bacteria and yeasts, and also the presence of fungi zoospores (Dzynban, 2002; Wurzbacher *et al.*, 2010). Green non-sulfur bacteria (*Chloroflexi*), some archaea (*Srenarchaeota*), facultative anaerobic or aerobic species of *Micrococcus*, *Rhodococcus* and *Agrobacterium* were contained in Mediterranean sediments (Coolen *et al.*, 2002; Süss *et al.*, 2004). An increased numbers in populations of microbial communities and microbial activity in different soil types after applying the lake sapropels as fertilizer was found (Grantina-Levina *et al.*, 2014). Positive results were obtained from the use of deep-sea sediments from the bottom of the Black Sea as agri-ameliorants and bio-stimulators for plants in recent years (Velev *et al.*, 1992; Terziev *et al.*, 2007; Shnyukov *et al.*, 2009; Nikolov and Shaban, 2011; Nikolov, 2011; 2014; Nikolov and Tringovska, 2014; Bozukov and Kochev, 2014). Their impact on soil microflora and direction of mineralization processes in the soil are insufficient explored. Stimulate the development of Nitrogen-Fixing tuberous bacteria in some legumes after used the Black Sea sapropels have been observed (Dimitrov and Sabev, 2005).

The aim of this study is to determine the impact of the deepwater Black Sea sapropels

on the quantities of basic for soil fertility trophic groups of microorganisms at cinnamon pseudopodzolic soil (Planosol).

Materials and Methods

The study was conducted in conditions of potted experiments (capacity of 5 kg), with cultivating oriental type tobacco. Cinnamon pseudopodzolic soil (Planosol), with strongly acidic soil reaction (pH – 4.79) and low humus content (1.97%) it was used (Bozhinova *et al.*, 2015). Sapropels were added in an amount of 30 g / kg dry soil, and the mixture was incubated for 2 months. Containers without sapropels for control it was used. Experience was enshrined in five reps.

Soil samples for microbiological analyses were taken from rhizosphere zone of tobacco plants, at a depth 0-20 cm, 60 days of planting tobacco. The phenological phase of the plants was "active growth". Average soil sample from the five reps was used for analyses. Microbiological analyses were performed by the method of Koch by plating of dilute soil suspensions in relevant for each group of microorganisms culture media, in four repetitions. The quantities of microorganisms were calculated as the most probable number of cells (MPN) per g of absolutely dry soil (a.d.s.) at the confidence level 0.05 the following formula:

$$MPN_{cells} = \frac{(\bar{x} \pm t\sigma_x) \cdot \frac{K}{V}}{D_m}$$

where: \bar{x} is the average number of colonies of all reps; $t = 2$ at $P_{0.95}$; σ_x -mean square deviation; K - dilution from which has been made; V - the volume of the inoculum in ml; D_m -amount of dry matter in g soil (Grudeva *et al.*, 2006).

The following microbiological indicators have been determined:

Number of: Autochthonous microorganisms – on soil extract agar, for ten days were incubated; Oligotrophic microorganisms – on tenfold diluted soil extract agar, for ten days were incubated; Actinomycetes – on starch–ammonium agar, for seven days were incubated; Microscopic fungi – on Chapek agar, for seven days were incubated; Aerobic nitrogen-fixing bacteria of the genus *Azotobacter* – on Ashby agar, for seven days were incubated; Ammonifying microorganisms – on meat – peptone stock agar, for five days were incubated; Mineral nitrogen NH_4^+ assimilating microorganisms – on starch–ammonium agar, for seven days were incubated; Mineral nitrogen NO_3^- assimilating microorganisms – on Chapek-Dox agar, for seven days were incubated.

Structural microbiological indexes have been calculated: Oligotrophic index (OI) – ratio between Oligotrophic microorganisms and Autochthonous microorganisms; Mineralization-immobilization indexes – ratios between assimilating mineral NH_4^+ - N or mineral NO_3^- - N microorganisms and Ammonifying microorganisms.

The data were subjected to a single-factor dispersion analysis. For each trophic group of microorganisms and for overall population density in microbial communities the effect (η^2) of factor "sapropels" and the level of statistical significance (p) according to the Fisher's test (F) have been identified. Statistical evaluation of the specific differences between variants for each trophic group of microorganisms, using the Student's test (t) has been determined (Plochinskiy, 1980).

The relative shares (%) of each of trophic group microorganisms of overall population

density in microbial communities were calculated.

Results and Discussion

The population surveyed by trophic groups of soil microorganisms can be conditionally divided into:

Microorganisms that characterize the general biogenicity in the soil environment and actively participate in the mineralization of organic matter - autochthonous, oligotrophic, soil fungi and actinomycetes

Microorganisms that perform some transformations of nitrogen compounds in the soil - nitrogen-fixing aerobic bacteria from the *Azotobacter*, ammonifying microorganisms, assimilating mineral forms of nitrogen microorganisms.

The results of this study indicated that the presence of sapropels in cinnamon pseudopodzolic soil (Planosol) suppresses the numerical growth of microorganisms of the physiological groups united at ecologically-trophic group associated with general biological state in the soil environment and total mineralization processes, with the exception of soil actinomycetes (Table 1).

The registered reduction of the density at autochthonous microorganisms in without sapropels soil was of the order of 93.48×10^6 /g a. d. s., unproven statistical ($\eta^2 = 0.261$; $F_{\text{exp.}} = 2.48 < F_{\text{tab.90\%}} = 3.59$). Decrease in density at oligotrophic microorganisms was significantly more pronounced - 173.87×10^6 /g a. d. s. The difference with the control was proven at a high confidence level ($p \leq 0.001$).

The force of impact of factor (η^2) was 87.04% ($F_{\text{exp.}} = 47.02 > F_{\text{tab.99\%}} = 12.25$). Reduce of the number with 62.67×10^3 /g a. d.

s. it was recorded and at microscopic fungi with the force of impact of sapropels - 43.65%. Changes at this group microorganisms did confirmed statistically ($F_{\text{exp.}} = 5.42 > F_{\text{tab.90\%}} = 3.59$). Strong stimulating effect on the group of actinomycetes was reported. Their population density has increased more than five times compared to the control, with $13.65 \times 10^6 / \text{g a. d. s.}$. The impact of the factor was 68.99%, at a high level of confidence demonstrated ($p \leq 0.01$; $F_{\text{exp.}} = 15.57 > F_{\text{tab.99\%}} = 12.25$).

Microorganisms of these trophic groups inhabit the soil micro-coenosis from the time of their occurrence (Alexander, 1991). The introduction of sapropels in the soil changes the conditions of life in environments. These changes, together with a relatively brief period of adaptation of microbial communities are the possible reasons for the reported lower density of autochthonous and oligotrophic microorganisms. Regulatory factor for their development is the incoming flow of nutrients. Autochthonous microorganisms are mesotrophic and they are relatively conservative indicator, while oligotrophic microorganisms are active and their population density is higher when the easily degradable organic compounds in the soil environment were depletion (Ishida and Kadota, 1981). Inputting of sapropels due to the specifics of their origin probably initially act as an inhibitor on quantitative development of these two groups microorganisms. According to Nikolov (2014) sapropels are extremely rich in organic carbon - 199.7 g / kg and humus content - 68.5 g / kg. On the other hand received data suggests that this effect was temporary. This is confirmed by low values of oligotrophic index which indicates that the balance between both trophic groups in the microbial communities was maintained (Table 3). The main reason for the reduced density of microscopic fungi that for up growth prefer

acidic living environment (Domsh, 1983) was the amendment in the reaction of the soil. The soil reaction from acidic (pH 4.79) towards slightly alkaline (pH 7.57) in conditions of this experiment has been changed (Bozhinova *et al.*, 2015). Actinomycetes require neutral soil reaction, as well as a high content of nutrients, unlike microscopic fungi (Zenova *et al.*, 2001). The increase in their numbers explains with the change in the soil reaction and the rich flow by organic matter introduced with sapropels. Quantities of actinomycetes in the soil are indicator of its fertility and ecological cleanliness. Their number and activity increases in parallel with increasing the organic matter in whose mineralization participate.

Indicators for the content, availability and the degree of destruction of the organic compounds in the soil are the quantities of autochthonous and oligotrophic microorganisms. Microscopic fungi together with actinomycetes due to their rich enzymatic systems actively decompose complex organic compounds resistant to the activity of most bacteria. The representatives of the four groups of microorganisms not only in mineralization processes, but also for the synthesis of humus in soil are involved (Atlas, 1981; Alexander, 1991). Increase in humus content of 1.97% to 2.38% at soil with sapropels of this experience was registered (Bozhinova *et al.*, 2015).

Inputting into the soil of sapropels stimulates numerical development of all tested groups of microorganisms involved in the various processes of transformations of nitrogen compounds (Table 2).

More than four times the amount of *Azotobacter chroococcum*, the main representative of the group of nitrogen-fixing aerobic bacteria was increased.

Table.1 Changes in the quantities at basic trophic groups of microorganisms in cinnamon pseudopodzolic soil/Planosol/ after introduce of sapropels

| Microbiological indicators – trophic groups microorganisms | Planosol - withoutsapropels /control/ | Planosol + sapropels | Values of the criterion of Student (t _j) | Statistical significance of the differences (± D) |
|--|---|---|--|---|
| | MPN cells ± confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | MPN cells ± confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | significance levels t _{tab.5.0%} = 1.943 t _{tab.1.0%} = 3.143 t _{tab.0.1%} = 5.208 | |
| Autochthonous microorganisms | $737.923 \times 10^6 \pm 27165$ | $644.444 \times 10^6 \pm 25386$ | t _{exp.} = 1.784 < t _{tab.5.0%} | -93.478×10^6 n.s. |
| $GD_{5\%} = 128.239 \times 10^6$; $GD_{1\%} = 194.272 \times 10^6$; $GD_{0.1\%} = 321.292 \times 10^6$ | | | | |
| Oligotrophic microorganisms | $453.502 \times 10^6 \pm 21296$ | $279.630 \times 10^6 \pm 16722$ | t _{exp.} = 7.775 > t _{tab.0.1%} | -173.87×10^6 *** |
| $GD_{5\%} = 54.722 \times 10^6$; $GD_{1\%} = 82.899 \times 10^6$; $GD_{0.1\%} = 133.260 \times 10^6$ | | | | |
| Microscopic fungi | $235.507 \times 10^3 \pm 242.645$ | $172.840 \times 10^3 \pm 207.870$ | t _{exp.} = 2.640 > t _{tab.5.0%} | -62.668×10^3 * |
| $GD_{5\%} = 58.078 \times 10^3$; $GD_{1\%} = 87.983 \times 10^3$; $GD_{0.1\%} = 141.433 \times 10^3$ | | | | |
| Actinomycetes | $3.019 \times 10^6 \pm 868.810$ | $16.667 \times 10^6 \pm 2041.241$ | t _{exp.} = 4.474 > t _{tab.1.0%} | $+13.647 \times 10^6$ ** |
| $GD_{5\%} = 7.464 \times 10^6$; $GD_{1\%} = 11.307 \times 10^6$; $GD_{0.1\%} = 18.176 \times 10^6$ | | | | |

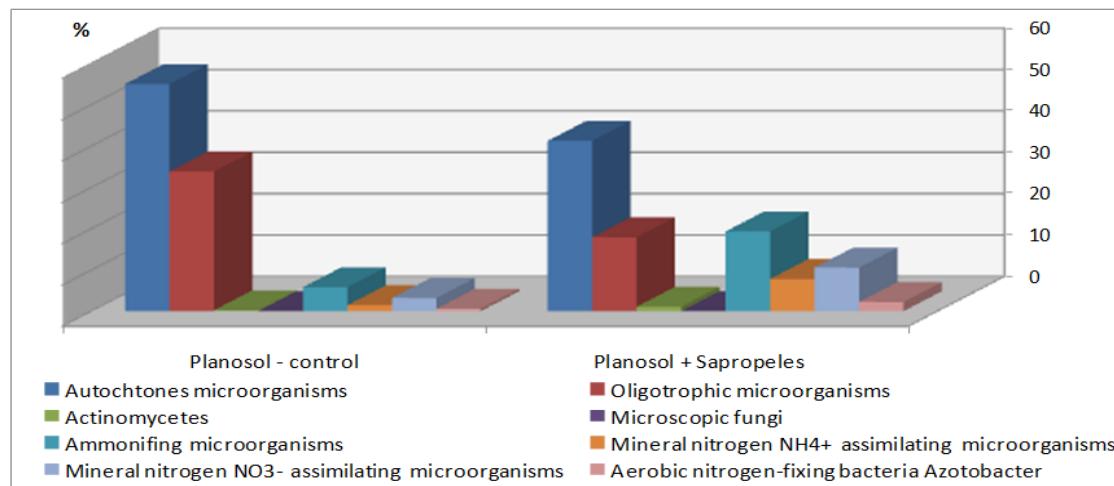
Table.2 Changes in the quantities at basic trophic groups of microorganisms related to transformation of nitrogen compounds in cinnamon pseudopodzolic soil/Planosol/ after introduce of sapropels

| Microbiological indicators – trophic groups microorganisms | Planosol - withoutsapropels /control/ | Planosol + sapropels | Values of the criterion of Student (t _j) | Statistical significance of the differences (± D) |
|---|---|---|--|---|
| | MPN cells ± confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | MPN cells ± confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | significance levels t _{tab.5.0%} = 1.943 t _{tab.1.0%} = 3.143 t _{tab.0.1%} = 5.208 | |
| Aerobic nitrogen-fixing bacteria <i>Azotobacter</i> | $7.850 \times 10^6 \pm 1401$ | $34.568 \times 10^6 \pm 2940$ | t _{exp.} = 4.029 > t _{tab.1.0%} | $+26.718 \times 10^6$ ** |
| $GD_{5\%} = 16.228 \times 10^6$; $GD_{1\%} = 24.585 \times 10^6$; $GD_{0.1\%} = 35.520 \times 10^6$ | | | | |
| Ammonifyingmicroorganisms | $77.899 \times 10^6 \pm 4413$ | $301.852 \times 10^6 \pm 8688$ | t _{exp.} = 29.19 > t _{tab.0.1%} | $+223.95 \times 10^6$ *** |
| $GD_{5\%} = 18.777 \times 10^6$; $GD_{1\%} = 28.446 \times 10^6$; $GD_{0.1\%} = 45.727 \times 10^6$ | | | | |
| Mineral nitrogen NH ₄ ⁺ assimilating microorganisms | $19.324 \times 10^6 \pm 2198$ | $121.605 \times 10^6 \pm 5514$ | t _{exp.} = 4.461 > t _{tab.1.0%} | $+102.281 \times 10^6$ ** |
| $GD_{5\%} = 56.106 \times 10^6$; $GD_{1\%} = 84.996 \times 10^6$; $GD_{0.1\%} = 136.631 \times 10^6$ | | | | |
| Mineral nitrogen NO ₃ ⁻ assimilating microorganisms | $42.874 \times 10^6 \pm 3274$ | $164.815 \times 10^6 \pm 6419.011$ | t _{exp.} = 3.378 > t _{tab.1.0%} | $+121.940 \times 10^6$ ** |
| $GD_{5\%} = 88.341 \times 10^6$; $GD_{1\%} = 133.835 \times 10^6$; $GD_{0.1\%} = 215.139 \times 10^6$ | | | | |

Table.3 Values of structural microbiological indices showing the direction of the mineralization processes in cinnamon pseudopodzolic soil/Planosol/ after introduce of sapropels

| Structural Microbiological indices | Planosol – without sapropels /control/ | Planosol + sapropels | Values of the criterion of Student (t) | Statistical significance of the differences ($\pm D$) |
|--|---|---|--|---|
| | values \pm confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | values \pm confidence interval $\bar{x} \pm \Delta N_{(p \leq 0.95)}$ | significance levels $t_{tab.5.0\%} = 1.943$ $t_{tab.1.0\%} = 3.143$ $t_{tab.0.1\%} = 5.208$ | |
| Oligotrophic index | 0.618 \pm 0.393 | 0.440 \pm 0.332 | $t_{exp}=4.060 > t_{tab.1.0\%}$ | -0.178 ** |
| $GD_{5\%} = 0.107$; $GD_{1\%} = 0.163$; $GD_{0.1\%} = 0.261$ | | | | |
| Mineralization-immobilization index by (NH_4^+) | 0.253 \pm 0.251 | 0.399 \pm 0.316 | $t_{exp}=1.873 < t_{tab.5.0\%}$ | +0.146 n.d. |
| $GD_{5\%} = 0.191$; $GD_{1\%} = 0.289$; $GD_{0.1\%} = 0.465$ | | | | |
| Mineralization-immobilization index by (NO_3^-) | 0.603 \pm 0.388 | 0.544 \pm 0.184 | $t_{exp}=0.256 < t_{tab.5.0\%}$ | -0.059 n.d. |
| $GD_{5\%} = 0.565$; $GD_{1\%} = 0.856$; $GD_{0.1\%} = 1.377$ | | | | |

Fig.1 Relative shares (%) of trophic groups microorganisms of overall population density in microbial communities



The influence of the sapropels had been 64.33% and was proven ($p \leq 0.01$; $F_{exp.} = 12.62 > F_{tab.99\%} = 12.25$). The species is highly sensitive to the conditions of the soil background. The quantity of bacteria is low at low humus content, high acidity and strengthened processes of leaching (Voynova *et al.*, 1983). The presence of

sapropels, changes these qualities at cinnamon pseudopodzolic soil used in the study and create favorable conditions for its development. Strong stimulating effect on population density at the group of ammonifying microorganisms has been recorded. The increase compared to control was 223.95×10^6

/g a. d. s. The force of impact of the factor "sapropel" was 98.95%, statistically significant ($p \leq 0.01$; $F_{\text{exp.}} = 662.47 > F_{\text{tab.99\%}} = 12.25$). The trophic group of ammonifying microorganisms is numerous and diverse by composition that are engaged to the mineralization of nitrogen-containing organic compounds. An increase in the quantities of both groups of mineral nitrogen assimilating microorganisms under the influence of sapropels was observed. An increase at the group of bacteria using NH_4^+ - N for their construction needs was more than six times compared to control - 102.28×10^6 /g a. d. s. The impact has 68.86% effective, proven at high confidence level ($p \leq 0.01$; $F_{\text{exp.}} = 15.48 > F_{\text{tab.99\%}} = 12.25$). The density of bacteria assimilating NO_3^- - N was increased around four times - with 121.94×10^6 /g a. d. s. The changes were reliable, the impact of the factor was 55.90%, at $p \leq 0.01$ ($F_{\text{exp.}} = 8.87 > F_{\text{tab.95\%}} = 5.59$). Stimulation effect on the density in these groups of microorganisms is probably due to the composition of the organic matter in sapropels. According to Velev *et al.*, (1992) it has a multiple character and is genetically heterogeneous. It is characterized by an increased content of slightly-hydrolysable substances (from 12.7 to 31.7%), bitumoides (2.3 to 3%), humic acids and a relatively high content of insoluble organic matter (56.3 to 58.1%). The ratio between the number of ammonifying and mineral nitrogen assimilating microorganisms in the microbial community was not disturbed (Table 3). Index values indicate active processes of mineralization, i.e. increase of nitrogen (NH_4^+ and NO_3^-) ions absorbed by plants.

Population density in the microbial community as a whole has increased by 16.47% under the influence of sapropels. The relative shares of different trophic groups microorganisms in community were changed (Fig. 1). The highest share in the soil without sapropels (control) occupied of autochthonous (54.96%) and oligotrophic microorganisms (33.78%). The other trophic groups had low percentage participation: ammonifying microorganisms had

5.80%; mineral nitrogen assimilating microorganisms had 4.63%; nitrogen-fixing aerobic bacteria *A. chroococcum* had 0.59% and actinomycetes had 0.23%.

The shares of these groups of microorganisms in microbial communities formed after introducing to sapropels greatly were increased: ammonifying microorganisms - 19.30%; mineral nitrogen assimilating microorganisms - 18.34%; *A. chroococcum* - 2.21% and actinomycetes - 1.07%.

In conclusion received results show positive changes at the microflora in cinnamon pseudopodzolic soil (Planosol), respectively its biological properties, under influence the sapropels. Strong stimulating effect on the quantitative development at groups of microorganisms linked with main transformations of nitrogen compounds in the soil have been established. Processes of mineralization are dominating the degree of oligotrophic was low and suggesting accumulation to nutrients assimilable by plants and to increase the humus content. Black Sea deep-water sediments may be used successfully to improve the properties of the cinnamon pseudopodzolic soil (Planosol), nitrogen balance and nutrient regime of soil.

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