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Influence of the bentonite-containing acrylic humectant composite on the soil microflora

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Abstract. Acrylic derivative-based superabsorbents are widely used currently in agriculture as the soil conditioners, plant growth regulators, etc. Their usage has a positive effect on the growth and survival of the plants cultivated in the arid regions. However, the effects of hydrophilic acrylic polymers on the soil microbiocenosis still remain unknown. The influence of the moisture-absorbing acrylic acid-based hydrogels with different proportions of bentonite filler was studied on the soil microbiota. N,N-methylenebisacrylamide was used as a crosslinking agent. Acrylic hydrogels were synthesized by radical polymerization in an aqueous medium at a synthesis temperature of 45 °C during 4 hours. The application of hydrogel of the certain concentrations (1.0, 1.5, and 2.5% wt) into the soil did not cause significant changes in the total abundance of heterotrophic bacteria and the length of the fungal mycelium. The CO_2 emission rates did not change after and during the application of the hydrogel), which indicated the same level of carbon mineralization in the soil with presence of acrylic bentonite-containing hydrogels. The nitrogen fixation rate decreased on the first day after hydrogel application; after 14 days, it was close to the control values. We assume the activity of nitrogen-fixing bacteria has though turned to the normal level.

Key words: acrylic hydrogels, bentonite, soil microflora.

INTRODUCTION

Nowadays, crosslinked hydrophilic acrylic polymers called superabsorbents (SAPs) or hydrogels, which absorb well and retain large amounts of water, are widely used in various fields of agriculture (Magalhaes et al., 2012; Amira & Qados, 2015; Olekhnovich et al., 2015a; Olekhnovich et al., 2015b). Production of water-holding polymer composites for agriculture, ornamental and home gardening is one of the areas of their promising use (Uspenskaya, 1998; Ekebafe et al., 2011).

The use of moisture-absorbing agents based on acrylic derivatives is well-known to have a positive effect on the growth and survival of plants in the soils of arid regions (Hayat & Ali, 2004; Abd El-Rehim, 2006).

However, the effects of hydrophilic acrylic polymers on the soil microbiocenoses are still not entirely clear. For example, hydrogel based on polyacrylamide (PAA) stimulated the growth of *Pseudomonas* bacteria to a small extent (Grula & Huang, 1981;

Grula et al., 1994), which the authors attributed primarily to the additional intake of ammonium nitrogen on the medium during the hydrolysis of the amide groups of the polymer. Later, it has been found that soil microorganisms characterized by amidase activity can utilize PAA as the only source of nitrogen for them (Kay-Shoemake et al., 1998b). Some authors believe that this may be due to the fact that polyacrylamide gradually turns into long-chain polyacrylate, which later decomposes under the influence of physical and / or biological factors (Kay-Shoemake et al., 1998a, 1998b, 2000).

The direct effect of SAPs' content on the soil microflora, i.e. dependence of the number of heterotrophic bacteria on the amount of acrylamide-based hydrogel introduced into the soil, was also studied. Finally, the number of cultivated heterotrophic bacteria increased in the soils treated with PAA and sown with potatoes (1), which was not observed for treated soils with acrylic hydrogel, but sown with beans (2). In the first case, the soils were characterized by significantly higher concentrations of NO_3^- and NH_4^+ than the untreated soils, whereas in the soils that were sown with beans, such a difference was not observed. In addition, PAA-based hydrogel does not adversely affect the viability of soil bacteria in model experiments with pure cultures (Maksimova et al., 2010).

However, the data on the dynamics of heterotrophic bacteria abundance, the length of the mycelium, as well as carbon dioxide emissions from the soil, nitrogen fixation and denitrification, are very scarce. That is why our study targets on the extremely important issue of the effect of acrylic hydrogels on the soil microbial communities.

MATERIALS AND METHODS

Soil sampling and soil parameters. The sampling was carried out in the Chekhovskii District of the Moscow Oblast (Russia), 500 meters from the Simferopol Highway, in the field. The soil was acidic sod-podzolic, medium-grained. Samples were taken from a square plot of 1 ha on a grid with a side of 50 meters from a depth of 5–15 cm in the form of a monolithic cube of 3,000 cm^3 volume. The soil samples were mixed together to obtain a combined sample of a total weight of 21 kg, the sub-samples from this one were then used for the study. Samples of the soil were not subjected to additional processing.

***Hydrogel synthesis*.** The hydrogels (HG) based on sodium acrylate and moisture-absorbing bentonite-containing composites based on HG (the mass fraction of bentonite was 0.5% wt and 1.0% wt) have been tested. HG was synthesized by radical polymerization in an aqueous medium at a synthesis temperature of 45 °C for 4 hours. N,N-methylenebisacrylamide was used as a crosslinking agent. Ammonium persulfate-tetraethylethylenediamine was used as the initiating system.

***Experimental scheme*.** The experiment was carried out in the 2-L glass vials, each contained 1 kg of soil. The vials were exposed in the laboratory for 14 days at natural air humidity (80%), illumination cycle of 12/12 L/D, and air temperature range of 25–27 °C. Moisture-absorbing materials were introduced into the soil with thorough mixing. Then the soil was moistened up to 60% of the field capacity.

***Assessing bacteria abundance and mycelium length*.** The aqueous soil suspension in the ratio of 1:9 (10 g of soil per 90 g of water) was treated in an ultrasonic bath for 3 min. After this pre-treatment, the soil suspension was transferred to a 100 mL measuring cylinder for 2 min. The 2 mL of suspension from the middle part (at 50-mL mark) were

diluted by 18 mL of water. From this mixture, 0.01 mL of the secondary suspension was applied to a glass slide by micropipette. The sampled was evenly distributed on an area of ∅4 cm^2 and air-dried in a drying chamber at a temperature of 27 °C for 5 hours. Then the slide was heated lightly on the flame of a gas burner. The slide was then dyed with acridine orange (bacteria, 4 min) or calcofluor-white, CFW (fungal mycelium, 15 min). Direct microscopy of soil suspension under a fluorescent microscope Axioscop 2 plus (Zeiss, Germany), oil immersion objectives of 40× and 100× magnification, have been performed to count the total bacteria abundance and the mycelium length in the samples according to standard methods (Rodríguez-Urra et al., 2009; Sandle, 2016). In total, 30 slides were prepared with a total number of fields of view equal to 300 in order to fit the significance level of 0.05 and a relative error of 10%.

The number of bacteria per 1 gram of cells was calculated by the formula:

$$M = \frac{4 \cdot K \cdot N}{p} \cdot 10^{10} \quad (1)$$

where K is the average number of cells in the field of view, N is the dilution rate, p is the area of the field of view.

Measuring of nitrogen fixation and denitrification rates. A 5-g sample of soil was cleaned to remove all the foreign inclusions and placed in a 15-mL vials each. The 6 mL of an aqueous solution of glucose (2.5 mg of glucose per 1 g of soil) and potassium nitrate (0.3 mg of potassium nitrate per 1 g of soil) have been added in each vial. The vials were closed with a rubber stopper and washed with argon for 30 s. Then, 1.5 mL of acetylene was injected in each vial. The vials were shaken for 60 s and incubated upside down for 24 hours at 28 °C. After incubation, 1 mL of the gas mixture was taken with a syringe from each vial. The nitrogen fixation and denitrification rates were determined on a gas chromatograph with a flame ionization detector 'Kristall-2000' (Russia).

Measuring of carbon dioxide emission. The 5 g of the root-free soil were sifted through a 1-mm sieve and placed in a 15-mL vials each. The standard aqueous solution of glucose (2% from the mass of absolutely dry soil) was added, the soil was then moistened with sterile water to a moisture content of 80% of the full water capacity. The soil was then stirred until homogeneous. The vials were closed with a cotton stopper and incubated for 24 hours at 28 °C. Then the vials were re-closed with a rubber stopper, 0.5 mL of acetylene was injected into the vials. After 1-hour incubation, 1 mL of the gas mixture was taken with a syringe from each vial.

The chamber static method was used to determine the rate of CO_2 emissions. An insulator (height 20 cm, diameter 15 cm) was cut into the soil to a depth of 10 cm. A gas sample was taken from the top of the insulator with a syringe immediately after installation, at 10 and 20 minutes of exposure. Samples were transferred to pre-vacuumed 15-mL vials. The emission of carbon dioxide was determined on a chromatograph 'Khromatograf 3700/4' (Russia) equipped with a thermal conductivity detector. The obtained concentrations of carbon dioxide in the samples are used to determine the rate of carbon dioxide emission:

$$F = D' \frac{(C_{10} - C_0)}{1 - e^{\frac{-D\tau}{H}}} \quad (2)$$

where $D' = -H \cdot ln\ ln\left(\frac{(C_{10}-C_0)}{(C_{20}-C_{10})}\right)$, τ is 10-min period, C_0, C_{10}, C_{20} are the carbon dioxide concentration at the beginning of experiment, in 10 and 20 minutes after, respectively, H is the height of the isolation layer above the soil surface.

***Statistics*.** In all the experiments and measurements, the number of replications varied from 3 to 30 in regard to the method applied. One-way ANOVA has been used in order to test the effect of HG introduction to the bacteria abundance, mycelium length, carbon dioxide emission, and nitrogen fixation and denitrification rates. The level of significance was set as $p < 0.05$.

RESULTS AND DISCUSSION

Visually, the soil enriched with the polymer moisture sorbent (pure hydrogel and hydrogel + bentonite with a concentration of 0.5% wt and 1.0% wt) became well-structured and had a clearly pronounced grain structure (grains of 3–5-mm diameter), which can be considered as close to the agronomically valuable granular structure. The soil enriched with pure bentonite of the same concentrations without a hydrogel and the control soil (no additives) did not have such a clearly defined structure (Fig. 1).

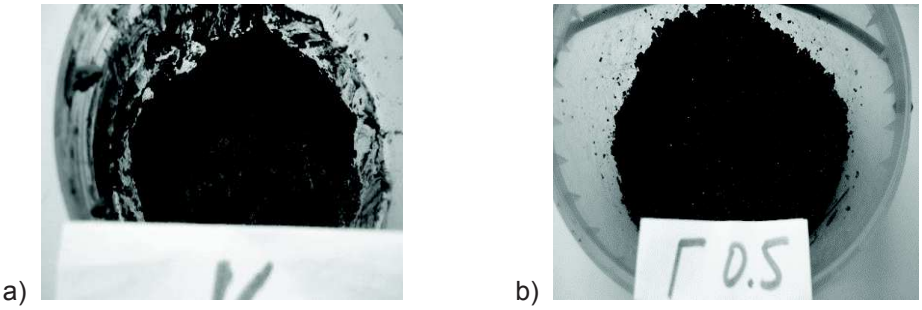

Figure 1. Appearance of the moistened soil: a – control sample; b – hydrogel-applied soil (0.5% wt).

The total bacteria abundance in the studied treatments ranged from 7.05 to 10.52 billion cells per 1 g of soil, which corresponded to the values usually recorded in the cultivated soils of the forest zone (Mamai et al., 2013). The bacteria abundance during the first day was higher in the control and in the treatments containing bentonite with a mass fraction of the filler of 0.5% wt and 1.0% wt (Fig. 2).

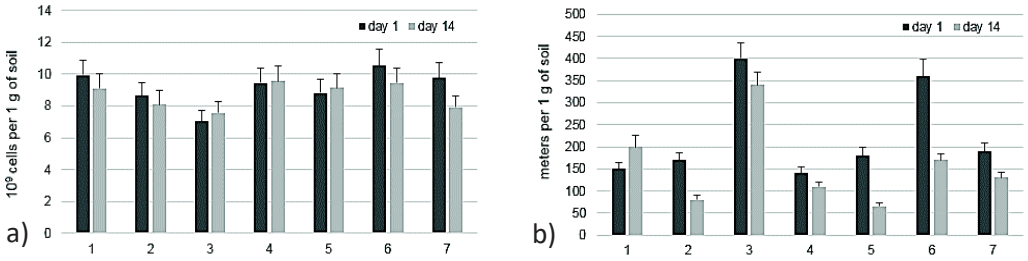

Figure 2. Total bacteria abundance (a) and the total length of mycelium (b) in the soil, M ± *m*, 24 hours and 14 days after the experiment has been started: 1 – control; 2 – hydrogel 0.5% wt; 3 – hydrogel 1.0% wt; 4 – hydrogel + bentonite 0.5% wt; 5 – hydrogel + bentonite 1.0% wt; 6 – bentonite 0.5% wt; 7 – bentonite 1.0% wt.

During the first day, the bacteria abundance compared to control sample has reduced by 30% and less in the soil enriched with HG (1.0% wt) without bentonite. Opposite to our data, an increase in bacteria numbers has been observed earlier in the soils conditioned with superabsorbent polymers (Achtenhagen & Kreuzig, 2011). However, it was later reported that the efficiency of applying the lower rate of hydrogels is better for soil conditioning without adverse effects on plant growth and the beneficial microorganisms of the soil (El-Saied et al., 2016). In addition, a significant increase in the amount of soil bacteria at 1.8 g dm^{-3} superabsorbent dose and no significant influence on actinomycetes and fungi (irrespective of the dose used) has been found (Mikiciuk et al., 2015). Hence, we argue that the effect of SAPs can be adverse and depends, probably, on the certain concentration of agents.

The bacterial cells in the treatments with hydrogel (treatment no. 3, Fig. 2, a) and a hydrogel + bentonite (no. 5, Fig. 2, a) were both single (green glow) and formed the free cell clusters (yellow glow) and the cell clusters in the hydrogel 'capsules' (yellow-green dots in red encirclement) (Fig. 3, a), none of the listed was found in the control (Fig. 3, b).

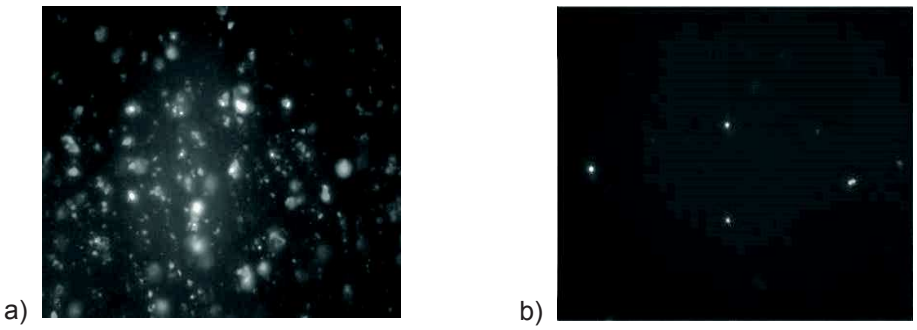

Figure 3. Bacteria in the soil samples after 24 hours the experiment has been started: a – soil with a hydrogel 1.0% wt applied; b – control sample (soil without modifiers). Individual cells give a green glow; clumps of cells, yellow glow; clusters of cells covered with a layer of hydrogel, red glow; 1,000×magnification.

The length of the mycelium varied from 65 to 400 m per 1 g of soil, which corresponds to the values commonly observed in the cultivated soils of the forest zone (Mamai et al., 2013). The application of a hydrogel of various concentrations (treatments nos. 2, 3), as well as a hydrogel + bentonite (no. 5), and pure bentonite of various concentrations (nos. 6, 7) led to an increase in the mycelium length compared to the control in 1.5–2.5 times even within the first 24 hours after the application of soil modifiers (Fig. 2, b).

However, on the 14th day of the experiment, the opposite pattern has been observed (Fig. 2b). Particularly, the application of pure bentonite (treatments nos. 6, 7) and bentonite-containing mixtures (nos. 4, 5) into the soil reduced the length of the mycelium by 1.5–2.0 times in the end of the experiment. In the soil where a pure hydrogel 1% wt has been applied, the length of the mycelium increased by 1.5 times compared with the control. This indicates that the mixtures used did not affect negatively the development of the filamentous fungi in the soil.

Thus, the application of HG, as well as a mixture of HG + bentonite, did not cause a significant reduction in the bacteria abundance both on the first day and after the two weeks of exposition, although a slight decrease in their numbers was recorded in the treatment with 1% wt HG.

The parameters of carbon and nitrogen transformation have been measured on the same dates of the experiment in order to obtain more information about the activity of the soil microflora in the treatments (Fig. 4).

The CO_2 emission rates ('soil respiration') varied from 57 (control) to 96 μg CO_2 g^{-1} day^{-1} (Fig. 4, a), which corresponds to the values usually recorded in the soils of the forest zone (Mamai et al., 2013). At the same time, the application of HG and a mixture of HG + bentonite (treatments nos. 2–5) increased the CO_2 emission rates by 1.5–2.0 times. The application of pure bentonite of various concentrations into the soil had practically no effect on the carbon dioxide emission rate (Fig. 4, a).

In the two weeks after the experiment has started, carbon dioxide emission rate ranged from 26 μg CO_2 g^{-1} day^{-1} (bentonite 0.5% wt) to 41 μg CO_2 g^{-1} day^{-1} (HG + bentonite 1% wt), which corresponded to the range usually recorded in the soils of the forest zone (Mamai et al., 2013). After the two weeks, the application of both HG and bentonite did not have a significant effect on the intensity of CO_2 emission, except for the treatment no. 5 (HG + bentonite 1% wt), where the carbon dioxide emission rate was an average by 10–30% higher comparing with the control and other treatments (Fig. 4, a).

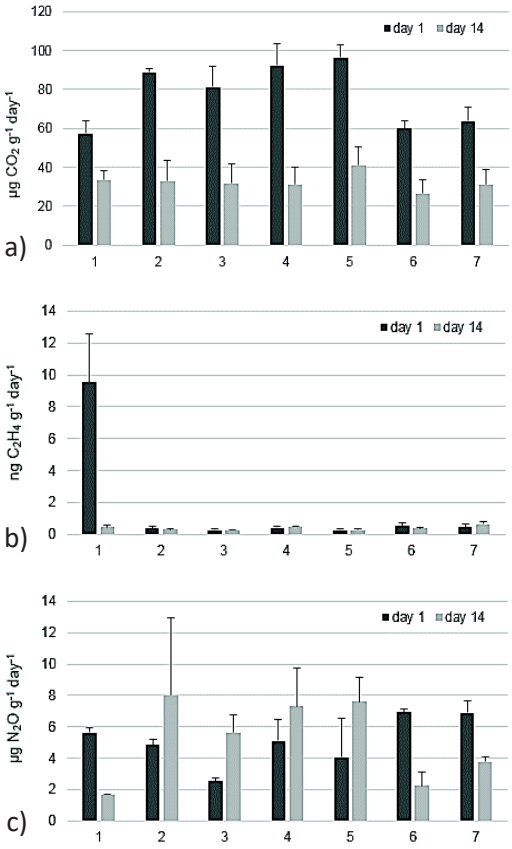

Figure 4. Rates of CO_2 emission (a), nitrogen fixation (b), and denitrification (c) in the soil with composite applied, M ± *m*, 24 hours and 14 days after the experiment has been started: 1 – control; 2 – hydrogel 0.5% wt; 3 – hydrogel 1.0% wt; 4 – hydrogel + bentonite 0.5% wt; 5 – hydrogel + bentonite 1.0% wt; 6 – bentonite 0.5% wt; 7 – bentonite 1.0% wt.

Recently, a positive effect of hydrogels on the nitrate fixation by microflora has been reported (El Saied et al., 2016). Particularly, application of the hydrogels positively affects bio-chemical properties of the soil, including slightly decreasing soil pH, increasing cation exchange capacity of the soil indicating improvement in activating chemical reactions in the soil, increasing organic matter, organic carbon, and total nitrogen percent in the soil. A narrower C/N ratio of treated soils is linked to the increase in organic nitrogen surpassed that in organic carbon (El Saied et al., 2016). Authors

conclude on the mineralization of nitrogen compounds and hence the possibility to save and provide available forms of N to growing plants, increasing available N, P and K in treated soil, and improving biological activity of the soil expressed as total count of bacteria and counts of *Azotobacter* sp., phosphate dissolving bacteria, fungi and actinomycetes in the soil as well as the activity of both dehydrogenase and phosphatase.

In our study, absolute nitrogen fixation rate was highest in the control sample (9.56 ng C_2H_4 g^{-1} day^{-1}), it decreased significantly in all the other treatments; in addition, the decrease was highest in the samples where HG was applied in 1% wt concentration (Fig. 4, b: treatments nos. 3 and 5). This may indicate the oppressed state of bacteria in these treatments, since the process of nitrogen fixation in soils is carried out only by bacteria (Mamai et al., 2013). This result can also be considered as a negative consequence of both HG and bentonite application within the studied period of 14 days. However, this fact requires additional verification and the final conclusion can be made only after the measuring of the nitrogen fixation rates for a longer period.

After two weeks of the experiment, the absolute nitrogen-fixing activity was highest in the three treatments, somehow distinct by the fertilizer parameters: pure bentonite (treatment no. 7), HG + bentonite 0.5% wt (no. 4), and control (Fig. 4b). In the treatments HG and HG + bentonite, the activity of nitrogen fixation was significantly reduced, and this decrease was highest in the treatments HG + bentonite 1 wt% (nos. 3 and 5). However, the decrease was not as significant as on the first day of the experiment, which indicates minimizing of the negative effect of HG on bacteria after two weeks of the experiment.

The denitrification activity in the studied samples varied from 2.52 to 6.93 μg N_2O g^{-1} day^{-1}, it was lower in the treatment, where the HG concentration was 1% wt (Fig. 4, c). The application of bentonite slightly increased the denitrification activity compared to the control. The decrease of the denitrification activity in the HG-containing treatments should be considered as a positive effect, since an increase in denitrification activity indicates the removal of nitrogen from the soil (Ishii et al., 2011).

After two weeks of the experiment, the denitrification activity varied from 1.67 μg N_2O g^{-1} day^{-1} (control) to 8.03 μg N_2O g^{-1} day^{-1} (HG 0.5% wt). Although no specific analyses were performed to determine the abundance of particular groups of microorganisms (i.e. nitrate-assimilating and nitrogen-fixing bacteria), an increase in denitrification activity when introducing HG and a mixture of HG + bentonite indicates the development of nitrate-assimilating bacteria and fungi in the soil, which have amidase activity, i.e. capable of cleaving the amide group from the PAA polymer chain and transforming it into N_2O (Kay-Shoemake et al., 1998b). The application of bentonite somewhat reduced the denitrification activity.

Hence, on the 14th day of the experiment, no significant decrease in CO_2 emissions has been observed in the HG-treatments, the activity of nitrogen fixation has increased in the HG-treatments and was close to that in the control, and denitrification activity in HG and HG + bentonite treatments has increased (Fig. 4). We argue this can indicate the development of bacteria and fungi characterized by amidase activity, however, a targeted research is necessary to prove or decline this.

CONCLUSIONS

The application of hydrogel into the soil at the tested concentrations did not cause significant changes in the total abundance of native bacteria and the length of the mycelium. The CO_2 emission rates did not change significantly, which indicates the absence of any hydrogel effect on the processes of carbon transformation in the soil. The denitrification rate has increased by 14 days after the hydrogel application, this can be considered as a certain negative impact on the processes of nitrogen transformation in the soil (removal of nitrogen from the soil). The activity of nitrogen fixation during the application of hydrogel decreased on the first day after application, but by 14th day, it has restored to the 'normal' values (close to the control); this evidences on the restoration of the activity of nitrogen-fixing bacteria and thus the normal functioning of the soil microbial complex.

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