Katarzyna GRATA 1

ECOLOGICAL EFFECTS OF UREA PHOSPHATE ON SOIL DIAZOTROPHS IN SPRING. PART II*

EKOLOGICZNE SKUTKI DZIAŁANIA FOSFORANU MOCZNIKA NA DIAZOTROFY GLEBOWE W OKRESIE WIOSENNYM. CZ. II*

Abstract: The main of the present study was to evaluation of the influence urea phosphate (UP) on the number and intensity of nitrogen fixing by diazotrophs. Soil samples were taken from the experiment established on brown soil. The examination focused on the following treatment of soils: I – soil, II – soil + urea phosphate, III – soil + manure, IV – soil + manure + urea phosphate. The study results evidenced that urea phosphate limited the total number of diazotrophs in the soil only after 30 days compared to soil and soil with manure. Moreover, theirs number in the presence of urea phosphate was higher in soil with manure than in unmanured soil. Potential ability of nitrogen fixing was stimulated by urea phosphate after 30 and 90 days in both manured and unmanured soil. It shows the lack of correlation between the number of diazotrophs and their N-fixing activity. Biochemical examinations proved that application of urea phosphate increased of mineral nitrogen concentration (especially of N-NH4) in the soil solution.

Keywords: urea phosphate, soil, diazotrophs, N-fixing activity, spring

Atmosphere is one of the biggest sources of nitrogen which contain about 78 % of it. However, this element in gas form is not available to most living organisms. Various diazotrophic Proteobacteria are known for their nitrogen fixing capabilities. Among the aerobic diazotrophs Azotobacter sp., Azotococcus sp., Azotomonas sp., Biejernickia sp. are the major ones. Some facultative anaerobes such as Pseudomonas sp., Bacillus sp., Klebsiella sp.; microaerophilic Azospirillum sp. and some genus of Clostridium sp. posses the ability to reduce atmospheric dinitrogen into ammonia under anaerobic conditions and use it directly for its growth [1–5].

In all these cases nitrogen fixation and the conversion of molecular nitrogen into ammonium is catalyzed by a multimeric enzyme complex-nitrogenase [1, 6–8]. More recent studies have shown that certain environmental factors, such as pH, dissolved oxygen, ammonium concentration, the availability of organic carbon compounds, the

1 Department of Biotechnology and Molecular Biology, University of Opole, ul. kard. B. Kominka 6a, 45–035 Opole, Poland, phone: +48 77 401 60 56, email: kgrata@uni.opole.pl

lack or low content of nitrogen assimilation form, the presence of a sufficient amount of iron, molybdenum, copper and phosphorus, affecting their growth, survival and biochemical activities. [4, 8–12]. Due to the fact that diazotrophs use of soil organic matter, application of natural or organic fertilizer significantly influences their existence and nitrogen fixation process. Particular importance is the large ratio of C/N and the content of mineral forms of nitrogen [13–16]. The aim of this study was to evaluate the effect of urea phosphate (UP) used as a disinfectant of manure on the total number and the nitrogen fixing activity of free-living diazotrophs.

Materials and methods

The research material was brown soil pHKCl 5.5 sampled from the layer of soil from 0–25 cm in the spring period. The experiment was conducted under laboratory conditions in three replications with the following treatments:
- I soil (S),
- II soil + manure (40 Mg · ha⁻¹) (S + M),
- III soil + manure (40 Mg · ha⁻¹) + urea phosphate (4.8 %) (S + M + UP),
- IV soil + urea phosphate (4.8 %) (S + UP).

The soil samples were incubated at 22 °C, kept in constant humidity on the level 60 % of total water capacity and were taken after 7, 30 and 90 days. Microbiological analysis included a determination [17]: the total number of diazotrophs by incubating the cultures on Fenglerowa’s medium at 28 °C for 7 days and potential activity of the fixing nitrogen in the soil by modified Pochon methods (Dn). This method uses the phenomenon of the emergence of ammonia as the first product formed during the process of fixation of nitrogen from the atmosphere. Determination of the amount of diazotrophs was carried out in four replication, and the result are given as average values of replicates per 1 g d.m. soil. Chemical analysis of samples were taken from the average determinations related to pH by potentiometric method and the content of mineral forms of nitrogen (N-NH₄ and N-NO₃) in the soil solution by Grewelinga-Peech method [18]. Statistical calculations were done by the variance method. Significant differences between average were measured according to the Duncan’s multiple range test. Data indexed by the same letter are statistically not significantly different (p < 0.05).

Results and discussion

The results of microbiological tests showed that urea phosphate significantly limited the number of diazotrophs in the soil only after 30 day of study (3.80 log cfu/g d.m. soil) compared with the soil (5.18 log cfu/g d.m. soil) and soil with manure (5.2 log cfu/g d.m. soil) (Fig. 1).

Furthermore, their population in the soil with manure and urea phosphate was 10-fold higher than in soil with urea phosphate, but these differences were not significant. However, after the application of manure, a significant increase in the number of nitrogen-fixing bacteria was observed only after 7 days compared to the
other objects. Taking into consideration the duration of the experiment a gradual increase in population of diazotrophs in all treatments was observed, wherein the most frequently occurred in the 30 day of study (Fig. 1).

![Figure 1](image)

**Fig. 1.** The number of diazotrophs in the soil depending on the of fertilization [cfu/1 g d.m]; small letters significant differences (p < 0.05): S – soil without fertilization; SM – soil + manure; SMUP – soil + manure + urea phosphate; SUP – soil + urea phosphate

Assessment of the intensity of atmospheric nitrogen fixation by diazotrophs showed that in soils with urea phosphate process is the least ran only on 7 day test (significant differences), while the most intense occurred after 30 and 90 days (Fig. 2).

![Figure 2](image)

**Fig. 2.** The potential activity of the fixing nitrogen in the soil depending on the fertilization; explanations are given in Fig. 1

Analyzing the duration of the experiment, it was found that in all examined treatments, this process proceeded the most intense in 90 days, even though the smallest number of diazotrophs at this time was observed. Increase in activity of diazotrophs in fertilized soil along with over time is explained by the gradual decomposition of organic matter in soil. For example, the addition of cellulose, ammonium sulphate increases the production of nitrogenase enzyme. Furthermore, the addition of different carbon sources
(sucrose, starch, mannitol) may promote growth of free-living nitrogen-fixing bacteria [11]. Additional source of nitrogen results in faster development of cellulolytic microorganisms, and consequently causes the stronger growth of microorganisms fixing atmospheric nitrogen [14].

Therefore, taking into account both the number and activity of diazotrophs observed lack of relationship between these parameters. The number of nitrogen fixing bacteria and their activities are not always correlated. This is probably due to the fact that in the process of nitrogen fixation the diazotrophs are involved, that differ in terms of morphological and physiological. Therefore, they can exhibit different yield atmospheric nitrogen fixation process [14, 19].

The results contained in Fig. 3 indicate that the use of urea phosphate resulted in a slight decrease of soil reaction after 7 and 30 days of study, while after 90 days the pH value remained at similar levels in all treatments from 5.21 (SUP) to 5.39 (S).

![Fig. 3. The pH of the soil depending on the fertilization; explanations are given in Fig. 1](image-url)

Biochemical examination on the content of mineral forms of nitrogen showed that the use of urea phosphate increased their content in the soil solution to a considerable degree than farmyard manure. In unfertilized soil samples and fertilized with manure the ammonium nitrogen content did not exceed the value of 7.44 mg/kg d.m soli. However, after application of the urea phosphate, content of this form of nitrogen significantly increased after 7 days, especially in the treatment of soil with urea phosphate (144.77 mg/kg d.m soil). The growing tendency was continued until the end of the study (Fig. 4).

In the soil fertilized only with farmyard manure the content of N-NO₃ was lower throughout the study period compared to the soil unfertilized. Whereas, it was higher in soil with urea phosphate after 30 and 90 days, while in soil with farmyard manure and urea phosphate after 90 days (Fig. 5).

Increasing the content of ammonium ions in soil with urea phosphate can be caused not only by diazotrophs activity, but also, due for instance to an intensive growth of other microorganisms that are active in the transformations of nitrogen (eg ammonificators, nitrificators), or the amount of urea phosphate introduced into the soil [20–23]. Especially important forms of nitrogen for microorganisms and plants are
NH₄⁺ and NO₃⁻ ions, which can be assimilated in them, contribute to their growth [24]. The nitrogen fixing bacteria preferentially take advantage of the ammonium ion. However, it was found that higher concentrations of nitrogen forms acts by inhibiting both the nitrogenase enzyme activity and its synthesis (by inhibition of enzyme glutamine synthetase formation), or just by inhibiting the synthesis of new nitrogenase or else decreasing the number of diazotrophs [4, 8, 25]. Nevertheless, the response of diazotrophs to the presence of these ions is not the same. Some of them such as *Azospirillum* sp., *Azotobacter* sp. immediately respond to inhibition of synthesis of the nitrogenase, while in other representatives this reaction occurs gradually [10, 26]. Thus this process remains in the environment so long as the ammonium ions are present [1, 13, 25]. Moreover, nitrates or amino acids can inhibit the nitrogen fixation, although their influence on diazotrophs is less than that found for the ammonia [10]. In the case of nitrates, it is probably an indirect effect of reducing NO₃⁻ to NH₄⁺. More often, the ammonia can exert inhibitory effects on the enzyme nitrogenase, but not nitrates [26]. However, the effect of nitrogen ions may be weaker in an environment that is the soil. The presence of antagonistic microflora capable of
assimilation the bounded forms of nitrogen, can promote the survival of the diazotrophs population [8]. In addition, fertilization of soil with fertilizer rich in carbohydrates (high ratio of C / N) causes both the number and intensity the process of the nitrogen increases [6].

Conclusions

1. Application of urea phosphate as a manure disinfectant resulted in a slight reduction in the number of diazotrophs only after 30 days, whereas the increase in the intensity of nitrogen fixation in the soil.

2. It had been found a gradual increase the intensity of the nitrogen fixation in all tested treatments, with the highest values were recorded after 90 days study.

3. Urea phosphate influenced to the increase of the content of mineral nitrogen, especially ammonium nitrogen in the soil solution

References

EKOLOGICZNE SKUTKI DZIAŁANIA FOSFORANU MOCZNIKA NA DIAZOTROFY GLEBOWE W OKRESIE WIOSENNYM. CZ. II

Samodzielna Katedra Biotechnologii i Biologii Molekularnej
Uniwersytet Opolski


Słowa kluczowe: fosforan mocznika, gleba, diazotrofy, intensywność wiązania azotu