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BIOLOGICAL EFFECTS PRODUCED BY UREA PHOSPHATE IN SOIL

Summary

A pot experiment on eutrophic proper brown soil developed from loamy sand has been conducted in order to determine the effect of urea phosphate on the counts of selected groups of microorganisms, activity of soil enzymes, nitrifying activity of soil and the value of the potential biochemical soil fertility index. The results have demonstrated that urea phosphate stimulated the multiplication of copiotrophic, ammonifying, cellulolytic, Arthrobacter and Pseudomonas bacteria, but inhibited the activity of dehydrogenases, urease, acid and alkaline phosphatase as well as the nitrifying activity of soil. In response to phosphorus added to soil in the form of urea phosphate, values of the indices describing the effect of microorganisms on the rhizosphere have declined.
***Key words**: urea phosphate, enzymes activity, microorganisms numbers, soil fertilization*

BIOLOGICZNE SKUTKI DZIAŁANIA FOSFORANU MOCZNIKA W GLEBIE

Streszczenie

W celu określenia wpływu fosforanu mocznika na liczebność wybranych grup drobnoustrojów, aktywność enzymów glebowych, aktywność nitryfikacyjną gleby oraz na wielkość potencjalnego biochemicznego wskaźnika żyzności gleby wykonano doświadczenie wazonowe na glebie brunatnej eutroficznej typowej wytworzonej z piasku gliniastego. Rośliną uprawną był jęczmień jary. W wyniku badań wykazano, że fosforan mocznika stymulował namnażanie bakterii kopiotroficznych, amonifikacjnych, celulolitycznych, Arthrobacter i Pseudomonas oraz promieniowców i grzybów, a hamował aktywność dehydrogenaz, ureazy, fosfatazy kwaśnej i fosfatazy alkalicznej oraz aktywność nitryfikacyjną. Pod wpływem działania fosforu w postaci fosforanu mocznika zmniejszały się wartości współczynników charakteryzujących efekt ryzosferowy drobnoustrojów. Fosforan mocznika, chociaż korzystnie działa na wzrost i rozwój jęczmienia jarego, to może powodować zakłócenia metabolizmu glebowego, objawiające się obniżeniem potencjalnej żyzności gleby, skorelowanej z aktywnością biochemiczną.
***Słowa kluczowe**: fosforan mocznika, aktywność enzymów, liczebność mikroorganizmów, nawożenie gleby*

1. Introduction

Urea phosphate (UP) has a wide range of applications. For example, it can be used to manufacture fertilizers [4, 10], as a feed additive [7, 23], a fertilizer [9, 12] or a disinfectant [6-8]. Apart from positive effects [5, 22], application of urea phosphate can also reduce amounts of available calcium in soil [12]. Because of H_3PO_4 in its composition, UP causes acidification of the soil environment [7, 12], which may have some influence on soil-borne microorganisms [25] and the activity of soil enzymes [26].

Loss of nitrogen due to the volatilization of ammonia from the hydrolysis of urea included in UP [20] is much smaller than the analogous loss from fertilizer urea [5, 20, 22]. The reason is the inhibitory effect of UP on urease, the enzyme responsible for hydrolysis of urea [5]. Higher amounts of ammonia nitrogen in soil after application of H_3PO_4 in urea coinciding with a smaller pool of nitrate nitrogen was also reported in a study by Ahmed et al. [1].

Typically, plant fertilization causes changes in the microbiological and biochemical properties of soil [14, 15]. Compared to mineral fertilizers, natural and organic fertilizers induce bigger changes, which are usually positive. However, there are reports [19] which demonstrate that nitrogen fertilization in the form of urea and phosphorus nutrition as superphosphate did not affect the total count of bacteria or the number of cellulolytic bacteria, but led to unwanted changes in the structure of bacterial communities.

The purpose of this study has been to determine the effect of urea phosphate on counts of selected groups of microorganisms, activity of soil enzymes, nitrifying activity of soil, yields of spring barley and value of the potential biochemical index of soil fertility.

2. Material and methods

A greenhouse experiment was set up in plastic pots (in 4 replications). The pots were filled with eutrophic proper brown soil developed from loamy sand collected from the arable humic horizon. The soil had the following properties: pH in 1 mol KCl dm^{-3} – 5.60; hydrolytic acidity (HAC) – 13.05 mmol (H^+) kg^{-1}; C_{org} – 5.00 g kg^{-1}; N_{og} – 0.43 g kg^{-1}; (P – 35 mg kg^{-1}) total exchangeable bases (TEB) – 57.06 mmol (+) kg^{-1}; cation exchange capacity (CEC) – 70.11 mmol (+) kg^{-1}; percentage base saturation (V) – 81.39%. Before the experiment was set up, the soil had been mixed with mineral fertilizers and urea phosphate, as specified in the design of the experiment. The same level of fertilization with macro- and micronutrients was applied, which –expressed as pure element - consisted of (in mg kg^{-1} of soil): K - 150 [KCl]; Mg - 20 [$MgSO_4 \cdot 7H_2O$]; Zn – 5 [$ZnCl_2$]; Cu – 5 [$CuSO_4 \cdot 5H_2O$]; Mn – 5 [$MnCl_2 \cdot 4H_2O$]; Mo – 5 [$Na_2MoO_4 \cdot 2H_2O$]; B – 0.33 [H_3BO_3]. Afterwards, urea phosphate [$CO(NH_2)_2 \cdot H_3PO_4$] was added in the doses: 0, 25, 50, 75, 100, 125 and 150 mg P kg^{-1} d.m. of soil. In all the pots, nitrogen was balanced by urea up to the amount of N introduced with the highest dose of urea phosphate.

Before the experiment commenced, both mineral fertilizers and urea phosphate were mixed with a whole batch of soil intended to be placed in a single pot (3 kg of soil). Next, the pots were filled in with the soil, prepared as explained above, and cv. Rabel spring barley was sown. The emerging seedlings were thinned, leaving 12 plants per pot. In order to achieve better understanding of the effect of urea phosphate on the biological life of soils, the trials were performed on soil sown with spring barley and on bare soil.

During the vegetative growth of plants (57 days), the soil moisture was maintained on a constant level of 60% of water capillary capacity. On harvest day, counts of microorganisms and activity of soil enzymes were determined. The scope of microbiological assays included: determinations of counts of copiotrophic bacteria (Cop) on Onta and Hattori medium [17], counts of *Arthrobacter* (Art), *Pseudomonas* (Ps), nitrogen immobilizing (Im), ammonifying (Am) and cellulolityc bacteria (Cel) – on a medium characterized in the paper by Wyszkowska et al. [24]; counts of actinomycetes (ACT) on Kuster and William medium with added nystatin and actidion [18] and fungi (Fun) – on Martin medium [13]. Microorganisms were cultured on Petri plates at the temperature of 28°C. Colony forming units (cfu) were determined with a colony counter.

The scope of biochemical assays consisted of determinations of the activity of dehydrogenases (EC 1.1), urease (EC 3.5.1.5), acid phosphatase (EC 3.1.3.2), alkaline phosphatase (EC 3.1.3.1) and nitrifying activity. Dehydrogenases were tested with the method worked out by Öhlinger [16]; urease was analyzed according to the protocol described by Alef and Nannpieri [2], while acid and alkaline phosphatases were examined as proposed by Alef et al. [3]. Moreover, the nitrifying activity of soil was assayed according to Kandeler [11]. Ammonium sulphate was used as the substrate. Soil was incubated for 21 days at the temperature of 25°C, while the control samples were kept at -20°C. After the incubation, $N\text{-}NH_4$ was determined suing Nessler reagent and the determination of $N\text{-}NO_3$ was aided by phenoldisulphonic acid. Aqueous solution of 1% K_2SO_4 was used for extraction of mineral nitrogen. The extractant to soil ratio was 5 : 1. Extinction was measured at the wavelength of 410 nm. The results were recalculated as a percentage of nitrified nitrogen during 24 hours. Finally, considering the activity of dehydrogenases (Deh), urease (Ure), acid phosphatase (Pac) and alkaline phosphatase (Pal) as well as the percentage of organic carbon (%C), the biochemical index of soil fertility was derived from the formula: BA_{20} = %C (Deh + Ure + Pac + Pal).

The results were processed statistically with Duncan's multiple range test, using two-factorial analysis of variance [21]. Taking into consideration all the three replications in which the microbiological and biochemical assays had been made, Pearson's simple correlation coefficients were calculated between yields of spring barley and the biological activity of soil.

3. Results and discussion

Urea phosphate had a stimulating effect on the multiplication of copiotrophic, ammonifying, cellulolytic, *Arthrobacter* and *Pseudomonas* bacteria as well as actinomycetes and fungi. However, in a vast majority of treatments, urea phosphate inhibited the growth of nitrogen immobilizing bacteria, both in sown and unsown soil. This should be perceived as a favourable development, especially because the populations of saprophytic fungi had not been depressed despite the fact that urea phosphate is often used for disinfection against these microorganisms [6, 7, 8]. Higher coefficients of the correlation between the dose of phosphorus in UP and counts of microorganisms such as copiotrophic, ammonifying and cellulolytic bacteria as well as *Arthrobacter* and fungi were achieved from pots with sown than with unsown soil. In contrast, for *Pseudomonas* bacteria and fungi, the above coefficients were higher in bare soil. The rates of phosphorus from 50 to 75 mg P kg^{-1} d.m. of soil had the most beneficial effect

Table 1. The effect of urea phosphate on numbers of soil microorganisms (cfu kg^{-1} of soil d.m.)

Phosphorus urea dose mg P kg^{-1} of soil	Cop x 10^8	Am x 10^8	Im x 10^8	Cel x 10^7	Art x 10^7	Ps x 10^7	Act x 10^8	Fun x 10^6
Unsown soil								
0	1.577	2.330	3.047	1.649	0.968	1.057	1.577	2.652
25	2.043	3.297	3.262	1.971	1.165	1.219	1.756	3.226
50	3.441	3.763	3.226	2.616	1.953	1.398	1.828	3.477
75	3.871	3.333	2.975	2.796	2.330	1.326	2.258	3.548
100	3.835	3.262	2.796	2.616	2.473	1.487	2.043	3.333
125	3.011	3.047	2.616	2.330	2.151	1.326	1.935	3.262
150	2.939	2.939	2.545	2.473	2.079	1.272	1.864	3.154
average	2.960	3.139	2.924	2.350	1.874	1.298	1.895	3.236
r	0.566	0.144	-0.882	0.605	0.775	0.536	0.511	0.378
Sown soil								
0	9.319	5.771	8.746	3.763	2.760	2.921	3.799	2.688
25	9.964	6.703	8.315	4.194	3.011	3.065	4.695	4.695
50	11.470	7.419	8.172	6.201	3.011	3.029	6.093	5.125
75	11.398	7.276	7.670	6.093	3.871	3.172	4.946	4.982
100	11.720	7.168	7.455	5.484	3.728	3.029	4.516	4.337
125	11.470	6.989	7.348	5.269	3.638	2.903	4.086	4.337
150	10.630	6.662	8.039	4.956	3.198	2.995	4.547	4.091
average	9.319	5.771	8.746	3.763	2.760	2.921	3.799	2.688
r	0.910	0.801	-0.962	0.729	0.911	0.337	0.268	0.658
$LSD_{p=0.05}$*								
a	0.414	0.290	0.362	0.246	0.129	0.141	0.218	0.237
b	0.221	0.155	0.194	0.131	0.069	0.076	0.117	0.127
ab	0.589	0.412	0.516	0.350	0.184	0.201	0.310	0.337

Cop - copiotrophic bacteria, Am – ammonifying bacteria, Im – immobilizing nitrogen bacteria, Cel – cellulolytic bacteria, Art - *Artrobacter*, Ps - *Pseudomonas*, Act – actinomycetes, Fun –fungi.

*LSD for: a - phosphorus dose, b - kind of soil usage;

r – correlation coefficient between phosphorus urea dose and soil microorganisms

Table 2. Effect of urea phosphate on the ratio of microbial counts in sown soil (R) to unsown soil (S)

Phosphorus urea dose mg P kg^{-1} of soil	Cop	Am	Im	Cel	Art	Ps	Act	Fun
	R : S							
0	5.91	2.48	2.87	2.28	2.85	2.76	2.41	1.01
25	4.88	2.03	2.55	2.13	2.58	2.51	2.67	1.46
50	3.33	1.97	2.53	2.37	1.54	2.17	3.33	1.47
75	2.94	2.18	2.58	2.18	1.66	2.39	2.19	1.40
100	3.06	2.20	2.67	2.10	1.51	2.04	2.21	1.30
125	3.81	2.29	2.81	2.26	1.69	2.19	2.11	1.33
150	3.62	2.27	3.16	2.00	1.54	2.35	2.44	1.30
average	3.94	2.20	2.74	2.19	1.91	2.35	2.48	1.32
r*	-0.66	0.05	0.52	-0.52	-0.79	-0.63	-0.39	0.21

explanations as under Table 1

Table 3. The effect of urea phosphate on soil enzymes activity (per kg^{-1} of soil d.m.)

Phosphorus urea dose mg P kg^{-1} of soil	Deh	Ure	Pac	Pal	Nit
	μmol TFF h^{-1}	mmol $N\text{-}NH_4$ h^{-1}	mmol PNP h^{-1}		% N d^{-1}
Unsown soil					
0	5.583	0.480	2.701	0.556	1.252
25	5.775	0.566	2.616	0.590	1.347
50	4.235	0.668	2.599	0.547	1.297
75	4.043	0.480	2.428	0.487	1.293
100	3.561	0.463	2.376	0.479	1.275
125	3.465	0.394	2.394	0.470	1.203
150	3.465	0.360	2.205	0.470	0.995
average	4.304	0.487	2.474	0.514	1.237
r	-0.911	-0.676	-0.966	-0.889	-0.722
Sown soil					
0	13.861	1.200	3.676	0.752	1.964
25	13.572	1.166	3.761	0.786	2.000
50	13.283	1.131	3.676	0.769	1.806
75	13.091	1.114	3.590	0.684	1.793
100	12.995	1.114	2.530	0.667	1.757
125	11.743	1.097	2.445	0.650	1.730
150	10.203	0.960	2.394	0.633	1.653
average	12.678	1.112	3.153	0.706	1.815
r	-0.899	-0.892	-0.898	-0.912	-0.939
$LSD_{p=0.05}$*	a – 0.382; b – 0.209; ab – 0.555	a – 0.029; b – 0.015; ab – 0.041	a – 0.142; b – 0.076; ab – 0.201	a – 0.033; b – 0.018; ab – 0.047	a – 0.034; b – 0.018; ab – 0.048

Deh – dehydrogenases, Ure – Urease, Pac - acid phosphatase, Pal – alkaline phosphatase,
Nit – nitrification activity;
*LSD for: a - phosphorus dose, b - kind of soil usage;
r – correlation coefficient

Table 4. Effect of urea phosphate on the ratio of enzymes activity in sown soil (R) to unsown soil (S)

Phosphorus urea dose mg P kg^{-1} of soil	Deh	Ure	Pac	Pal	Nit
	R : S				
0	2.48	2.50	1.36	1.35	1.57
25	2.35	2.06	1.44	1.33	1.48
50	3.14	1.69	1.41	1.41	1.39
75	3.24	2.32	1.48	1.40	1.39
100	3.65	2.41	1.06	1.39	1.38
125	3.39	2.78	1.02	1.38	1.44
150	2.94	2.67	1.09	1.35	1.66
average	3.03	2.35	1.27	1.37	1.47
r*	0.65	0.55	-0.78	0.18	0.12

* *explanations as under Table 3*

Table 5. Effect of urea phosphate on the value of potential biochemical index of soil fertility (BA_{20})

Phosphorus urea dose mg P kg^{-1} of soil	Unsown soil	Sown soil	Average
0	5.286	10.727	8.006
25	5.447	10.643	8.045
50	4.673	10.333	7.503
75	4.366	10.136	7.251
100	4.077	9.532	6.804
125	3.963	8.833	6.398
150	3.748	7.922	5.835
average	4.508	9.732	7.120
r	-0.959	-0.954	-0.983

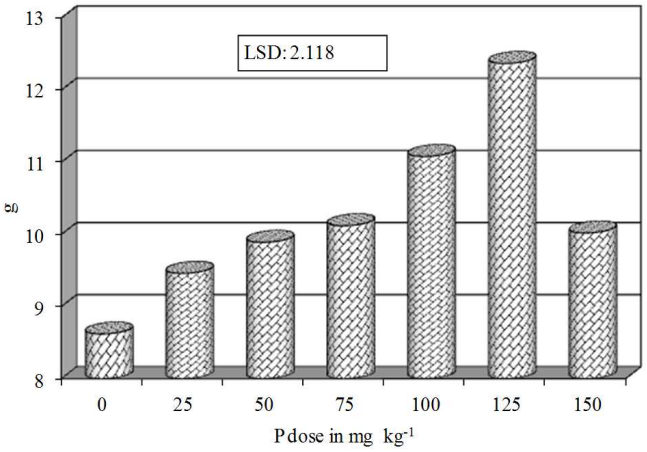

Fig. 1. Effect of urea phosphate on spring barley yield (in g d.m. per pot)

on most microorganisms, which means that phosphorus doses above 75 mg P, although raising microbial counts versus the control, caused their decrease in comparison to treatments fertilized within the range of 50 to 75 mg P kg^{-1} d.m. of soil, which proves that their diversity is altered, an event indicated by Sarathchandr et al. [19].

In view of the above findings, it might appear that in most cases urea phosphate did not have any negative effect on soil microorganisms. However, the data set in table 2 clearly demonstrate that this fertilizer narrowed the R : S ratio, which is an undesirable consequence, most probably caused by soil acidification [7, 12]. The same development might be responsible for the inhibitory effect of phosphorus applied as UP on the activity of dehydrogenases, urease, acid phosphatase, alkaline phosphatase and, in soil sown with spring barley, on the nitrifying activity of soil (tab. 3). Bremner and Dounglas [5] prove that urea phosphate inhibits the activity of urease as well as other enzymes. Despite its almost unquestionably negative influence on the soil enzymatic activity, urea phosphate broadens the ratio of the activity of dehydrogenases in sown versus unsown soil (tab. 4). It did not change the value of an analogous ratio for acid phosphatase and produced variable effects on the R:S ratios for urease, alkaline phosphatase and nitrifying activity.

High correlation coefficients (from -0.954 to -0.959) appeared between the dose of phosphorus and the value of the potential biochemical index of soil fertility (tab. 5). The higher the dose of phosphorus, the lower the BA value, which may suggest that the yield of barley would be the smallest in pots with 150 mg P kg^{-1} d.m. of soil. However, the response of barley was not correlated with the value of the BA index, as each of the phosphorus doses from 25 to 150 mg kg^{-1} raised spring barley yields, and the highest barley mass was obtained under the effect of 125 mg P kg^{-1} d.m. of soil. This discrepancy could be explained by the nutritional requirements of barley plants in respect of nitrogen, as the control treatment was not fertilized with phosphorus.

4. Conclusions

1. Urea phosphate stimulated multiplication of copiotrophic, ammonifying, cellulolytic, *Arthrobacter* and *Pseudomonas* bacteria as well as actinomycetes and fungi. On the other hand, it inhibited the activity of dehydrogenases, urease, acid and alkaline phosphatase and the nitrifying activity of soil.
2. In response to the influence of phosphorus introduced to soil as urea phosphate, values of the indices characterizing the effect of microorganisms on the rhizosphere declined.
3. Although urea phosphate had a positive effect on the growth and development of spring barley, it may interfere with the soil metabolism, leading to a worse potential fertility of soil, correlated with its biochemical activity.

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