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THE INFLUENCE OF CHEMICAL FERTILIZERS AND MANURE UPON THE PRESENCE OF *CLOSTRIDIUM* AND *AZOTOBACTER* IN THE PEAT SOIL OF MEADOWS*

Investigations were made of the influence of mineral fertilizers and stable manure on the amount of *Clostridium* and *Azotobacter* in the peat soil of a meadow. The results obtained show that *Clostridium* is an organism less sensitive than *Azotobacter* to habitat changes.

The importance of free assimilators of molecular nitrogen has interested, and continues to interest, research workers apart from microbiologists. Meadow soils, as being more humid and rich in organic compounds, from and excellent habitat for assimilators of free nitrogen. *Clostridium* occurs in the turf layer of meadow soils most often within limits of up to 100 thousand per 1.0 g of fresh soil (Zimny 1961). The investigations made by Zimna (1962) showed that this organism occurs in far greater amounts in the soil adhering to root systems than in the soil at greater distances from them. Similar results in relation to cultivated plants were obtained by Strzelczyk (1958b) and others. *Azotobacter* is an organism also occurring in meadow soils, but with less intensity and possessing its own local distribution (Sušina 1949). In addition, free assimilators of molecular nitrogen occur, like other micro-organisms, chiefly in the upper layer of the soil (Zimny 1960, Nepomiluev and Sišov 1962) and others.

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The increasing demand of recent years for permanent meadows has resulted in agrotechnical and land improvement operations being undertaken in meadows and pastures. In the present study the author has decided to investigate the influence of mineral fertilizers and stable manure on the presence of micro-organisms assimilating free nitrogen – *Clostridium* and *Azotobacter*.

STUDY AREA

The investigations were made in a permanent meadow, reclaimed from a peat bog near Otwock. The area is formed by a fen lying in the ancient valley of the Vistula. Until 1959 the area was excessively wet but the land reclamation work carried out has regulated the drainage of the land.

The layers of peat in the lower and middle parts are formed of reed peat, and the upper layer of sedge peat. The depth of the peat layer varies from 1.50 to 1.80 m. The initial vegetation in the study area was an association similar in the composition of its flora to the *Caricetum appropinquatae* association, with a considerable amount of mossy layer – chiefly *Aulacomnium palustre*. The experiments with fertilizers were begun in 1959 by Moraczewski using the random bloc method in five strips and five repeats, on experimental plots of 60 m^2. Preliminary work consisted of removing the mossy layer, leaving the vascular plants with their composition intact. Variations in succession were left to the influence of fertilization. The following combinations were used in the fertilizer experiments: 1) control, 2) K, 3) KP, 4) KPN, 5) KPCa, 6) KPZn, 7) KPMn, 8) KPCu, 9) KPB, 10) KPMo, 11) KPCo, 12) stable manure. The plan and dosage used for each fertilizer are shown in Table I. In 1959 examination was made of the initial state in each of the experimental plots. Fertilizers were used beginning with the Autumn of 1959, giving only stable manure and calcite, the remaining fertilizers being used as from the Spring of 1960. Only KP was used on the plots in 1960, and cobalt as from 1961. Moraczewski (1961) was interested in the nitrogen changes in the soil and plants, and also in cropping. The results of these studies will form a separate subject for publication.

MATERIAL AND INVESTIGATION METHODS

Our investigations were carried out over a period of four years, from 1959 to 1962 inclusively. Average soil samples were taken three times during the vegetation season, with the exception of 1959, in May, July and September. Soil was placed in sterilised glass jars with ground corks and taken to the laboratory of the Institute of Ecology. Microbiological analyses were made immediately after the samples reached the laboratory. The samples were taken from the turf layer only.

Comparison dosage of fertilizers used

Tab. I

Fertilizing	C	K	KP	KPN	KPCa	KPZn	KPMn	KPCu	KPB	KPMo	KPCo	Manure
K_2O	–	120	120	120	120	120	120	120	120	120	120	–
P_2O_5	–	25	25	25	25	25	25	25	25	25	25	–
$(NH_4)_2SO_4$	–	–	–	60	–	–	–	–	–	–	–	–
$CaCO_3$	–	–	–	–	10+	–	–	–	–	–	–	–
$ZnSO_4 \cdot 7H_2O$	–	–	–	–	–	30	–	–	–	–	–	–
$MnCl_2 \cdot 4H_2O$	–	–	–	–	–	–	60	–	–	–	–	–
$CuSO_4$	–	–	–	–	–	–	–	60	–	–	–	–
$Na_2B_4O_7 \cdot 10H_2O$	–	–	–	–	–	–	–	–	30	–	–	–
$Na_2MoO_4 \cdot 2H_2O$	–	–	–	–	–	–	–	–	–	30	–	–
$CoCl_2 \cdot 6H_2O$	–	–	–	–	–	–	–	–	–	–	30	–
Manure 1959	–	–	–	–	–	–	–	–	–	–	–	400+
1960	–	–	–	–	–	–	–	–	–	–	–	300+
1961	–	–	–	–	–	–	–	–	–	–	–	300+

+ portions q/ha

The abundance of *Clostridium* and *Azotobacter* in these soils was then determined. The presence of *Clostridium* was ascertained macroscopically by gassing and microscopically by making preparations stained with Lugol liquid. The amount of *Clostridium* was determined on an agar medium with soil extract and saccharose in tall test tubes by the dilution method. The layer of pure agar isolating the medium and the layer on it of liquid paraffin prevented air from reaching the culture.

The composition of the medium was as follows: 1.0% saccharose, 0.1% K_2HPO_4, 200 ml peat soil extract, 1.5% agar, 800 ml distilled water. The amount of *Azotobacter* was determined in a liquid Beijerinck medium by the dilution method. The results were checked by microscopic examination. The cultures of these micro-organisms were kept in a thermostat at a temperature of 28°C for a period of 6 days.

With regard to the addition of a fairly large amount of soil extract for the elective medium used to determine the abundance of *Clostridium*, I would explain that descriptions of research work have been given in literature of recent years which show that micro-organisms of the genus *Clostridium* develop far better when yeast extract and peptone are added, even in fairly considerable amounts of up to 0.5% of peptone (E m c e v 1961, 1962). In our case the water extract from peat soil contained a fairly large amount of nitrogen compounds and the salts dissolved in it (3.5% of total nitrogen).

RESULTS OF INVESTIGATIONS

a. The effect of fertilizers on the presence of *Clostridium*

The initial state in the soils of all the experimental plots was maintained in 1959 within limits of ten to one hundred thousand organisms of the genus

The influence of mineral fertilizing and natural manure upon the amount of *Clostridium* and *Azotobacter* in peat soil on a meadow (in thousands per 1.0 fresh soil)

Tab. II

Fertilizing combinations	Vegetation season	*Clostridium*				*Azotobacter*			
		1959+	1960	1961	1962	1959+	1960	1961	1962
Control	Spring	–	10	10	10	–	0.01	0.01	1
	Summer	100	10	10	1000	0.01	0.01	0.01	10
	Autumn	–	10	10	1	–	0.01	0.01	1
K	"	–	100	10	100	–	0.01	0.01	1
		10	100	10	100	0.01	0.01	0.01	1
		–	100	10	10	–	0.01	0.1	1
KP	"	–	10	10	100	–	1	0.01	1
		100	1	100	100	0.01	0.01	1	10
		–	100	10	10	–	0.1	1	1
KPN	"	–	10	10	1000	–	0.01	0.1	10
		100	100	100	100	0.01	1	1	1
		–	100	10	100	–	1	1	1
KPCa	"	–	100	100	1000	–	0.01	1	10
		100	10	10	100	0.01	0.1	1	10
		–	100	100	10	–	1	1	1
KPZn	"	–	10	10	100	–	0.1	1	10
		100	10	100	1000	0.01	1	1	10
		–	100	10	10	–	0.1	1	1
KPMn	"	–	100	100	1000	–	0.1	0.01	1
		100	10	100	10	0.01	0.01	1	1
		–	10	10	10	–	0.1	1	1
KPCu	"	–	10	10	10	–	0.1	0.01	1
		100	10	100	10	0.01	0.01	1	1
		–	10	10	10	–	0.1	1	1
KPB	"	–	10	100	10	–	0.1	0.01	1
		100	1	10	100	0.01	0.01	0.1	1
		–	100	10	10	–	0.01	1	1
KPMo	"	–	100	10	100	–	0.1	0.1	10
		10	100	1	100	0.01	0.01	0.1	10
		–	100	10	10	–	0.01	1	10
KPCo	"	–	–	10	1000	–	–	0.1	1
		10	–	100	100	0.01	–	1	100
		–	–	10	10	–	–	1	10
Manure	"	–	10	10	100	–	–	0.01	1
		100	100	100	10	0.01	0.01	0.1	10
		–	100	10	100	–	0.01	1	10

+ starting point without fertilizing

Clostridium (Tab. II). The control plot exhibited a tendency to a decrease in the amount of anaerobic assimilators of free nitrogen. The influence of macro -elements on the presence of *Clostridium* was manifested differently. In the first year of fertilization by potassium this fertilizer had an encouraging effect, probably on account of the insufficiency of this component in the soil (0.08%), while the following year a decrease in the amount of anaerobic assimilators of free nitrogen is observable followed by an increase in 1962. Phosphorus at first exerts an unfavourable influence, but in the following years there are no symptoms of the influence exerted by this element. There is no distinct manifestation of the influence of nitrogen and calcium on the numbers of micro -organisms of the genus *Clostridium* in peat soil under a permanent meadow with other macro-fertilizers (Tab. II). The experimental plots fertilized with stable manure exhibited a certain stability.

Of the micro-elements, copper, manganese, and boron show that they exert an unfavourable influence on the abundance of anaerobic assimilators of free nitrogen. Molybdenum varies in its effect. Cobalt has a tendency to increase the abundance of *Clostridium* (Tab. II). In general it may be said that the micro-organisms assimilating free nitrogen from the air occurred in the greatest numbers in the third year of fertilization. The most favourable vegetation seasons for the development of *Clostridium* are Spring and Summer.

b. The effect of fertilizers on the presence of *Azotobacter*

The state at the beginning of the fertilizing experiments exhibits a relatively small amount of this micro-organism in the peat soil under a permanent meadow. *Azotobacter* was found only in dilutions of 1:10.

The effect of macro-elements on *Azotobacter* differed. A marked increase in the numbers of *Azotobacter* was found in the plots fertilised with phosphorus, nitrogen and calcium. Potassium did not affect the abundance of *Azotobacter*

Of the micro-elements, chiefly in the second year of fertilization, zinc, manganese, copper and cobalt (Tab. II) were found to have a favourable influence on these micro-organisms. The effect of boron and molybdenum on the abundance of *Azotobacter* must be considered to be neutral.

Stable manure had a favourable effect in the second year of fertilization (Tab. II).

No variations in numbers were found in the control plot until 1961, and in 1962 *Azotobacter* was found in far greater amounts than in the preceding years (Tab. II). *Azotobacter*, like *Clostridium*, occurred in the greatest numbers in the third year of fertilization. Fluctuations in abundance were also found to depend on the vegetation season. The most favourable period for the development of *Azotobacter* proved to be the Summer and Autumn periods. Intensification of the development of bacteria assimilating molecular nitrogen under the oxygen conditions in the Summer and Autumn may be explained by the reduction in the humidity of these soils and better aeration.

DISCUSSION OF RESULTS

The results obtained from the investigations show that fertilization of a permanent meadow growing on peat soil does not have any significant effect on the abundance of *Clostridium*. The experimental plots fertilized showed that a tendency takes place in the soils to stabilisation of the number of anaerobic assimilators of free nitrogen in comparison with the initial state, or in certain cases a slight increase in the number of these microorganisms, particularly in the final year of fertilization. On the plots not fertilized a decrease in numbers of anaerobic assimilaters of molecular nitrogen was found to take place as a result of the rapid degradation of the soil (control plots, Tab. II).

Strzelczyk (1958a) also found, under laboratory conditions, that there was no influence exert by mineral fertilization on the abundance of *Clostridium*. Under the conditions of our experiment, with the existing vegetation cover, it is difficult to descry the exclusive effect of fertilizers on the presence of the micro-organisms examined. The effect of fertilization was evident on the development of the vegetation and on soil structure, for instance, the plot fertilized with stable manure was settled by 80–90% of the papilionaceous plants, such as *Trifolium repens*, *T. pratense*, *Lotus uliginosus*, *L. corniculatus* and *Latyrus pratensis*. In the case of nitrogen fertilization (ammonium sulphate) the vegetation consisted chiefly of grasses of a vivid, intensely green colour. Only traces of vegetation, and that chiefly low-growing, also an absence of grasses and papilionaceous plants were found on the control plots. In addition the structure of the fertilized peat was of a greasy and compact consistency, and the unfertilized peat spongy and pulversing.

Azotobacter is an organism more sensitive to the changes taking place in the habitat, as in many cases it is even used as an indicator of fertilizing needs (Rubenčik 1960). In our case *Azotobacter* reacted very markedly to fertilization with phosphorus, nitrogen and calcium, and also zinc and cobalt, and less intensively to fertilization with potassium, stable manure, molybdenum, boron, copper and manganese (Tab. II).

Certain authors emphasise the interdependence existing between the development of *Azotobacter* and *Clostridium* (Rubenčik 1960). Under the conditions in our experiments the ratios of *Clostridium* to *Azotobacter* were as follows: the initial numbers were maintained within limits of 1000 : 1 and 10000 : 1 (Tab. III). This ratio changed very considerably under the influence of fertilizers owing to the increase in the amount of *Azotobacter*, to as much as 1 : 1(Tab. III).

CONCLUSIONS

The results of the fertilizing investigations of the abundance of *Clostridium* and *Azotobacter* in peat soil under permanent meadow in the Biel peat bog near Otwock showed that:

Ratio of *Clostridium* to *Azotobacter* in peat soil as the result of fertilizing the meadow

Tab. III

Fertilizing combinations	Vegetation season	1959+	1960	1961	1962
Control	Spring	–	1000 : 1	1000 : 1	10 : 1
	Summer	10000 : 1	1000 : 1	1000 : 1	100 : 1
	Autumn	–	1000 : 1	1000 : 1	1 : 1
K	"	–	10000 : 1	1000 : 1	100 : 1
		1000 : 1	10000 : 1	1000 : 1	100 : 1
		–	10000 : 1	100 : 1	10 : 1
KP	"	–	10 : 1	1000 : 1	100 : 1
		10000 : 1	100 : 1	100 : 1	10 : 1
		–	1000 : 1	10 : 1	10 : 1
KPN	"	–	1000 : 1	100 : 1	100 : 1
		10000 : 1	100 : 1	100 : 1	100 : 1
		–	100 : 1	10 : 1	100 : 1
KPCa	"	–	10000 : 1	100 : 1	100 : 1
		10000 : 1	100 : 1	10 : 1	10 : 1
		–	100 : 1	100 : 1	10 : 1
KPZn	"	–	100 : 1	10 : 1	10 : 1
		10000 : 1	10 : 1	100 : 1	100 : 1
		–	1000 : 1	10 : 1	10 : 1
KPMn	"	–	1000 : 1	10000 : 1	1000 : 1
		10000 : 1	1000 : 1	100 : 1	10 : 1
		–	100 : 1	10 : 1	10 : 1
KPCu	"	–	100 : 1	1000 : 1	10 : 1
		10000 : 1	1000 : 1	100 : 1	10 : 1
		–	100 : 1	10 : 1	10 : 1
KPB	"	–	100 : 1	10000 : 1	10 : 1
		10000 : 1	100 : 1	100 : 1	100 : 1
		–	10000 : 1	10 : 1	10 : 1
KPMo	"	–	1000 : 1	100 : 1	10 : 1
		1000 : 1	10000 : 1	10 : 1	10 : 1
		–	10000 : 1	10 : 1	1 : 1
KPCo	"	–	–	100 : 1	1000 : 1
		1000 : 1	–	100 : 1	1 : 1
		–	–	10 : 1	1 : 1
Manure	"	–	10000 : 1	1000 : 1	100 : 1
		10000 : 1	10000 : 1	1000 : 1	1 : 1
		–	10000 : 1	10 : 1	10 : 1

+ starting point without fertilizing

1. the abundance of *Clostridium* did not exhibit any significant changes as a result of the use of fertilizers. Variations in numbers were found depending on the vegetation. The most favourable periods were Spring and Summer.

2. *Azotobacter*, as being an organism more sensitive to habitat changes, gave a distinct reaction to mineral fertilizers. The most favourable periods of the development of *Azotobacter* are Summer and Autumn.

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WPŁYW NAWOŻENIA MINERALNEGO I OBORNIKA NA STAN *CLOSTRIDIUM* I AZOTOBAKTERA W GLEBIE TORFOWEJ POD ŁĄKĄ TRWAŁĄ

Streszczenie

Korzystając z doświadczeń nawozowych prowadzonych przez dr R. Moraczewskiego nad wpływem nawożenia na przemiany azotowe i plonowanie łąki trwałej na torfie „Torfowisko Biel", przeprowadzono badania nad wpływem zabiegów nawozowych na stan ilościowy *Clostridium* i azotobaktera. Badania nasze przeprowadzono w latach 1959 do 1962 włącznie, uwzględniając następujące kombinacje nawozowe: 1) kontrola, 2) K, 3) KP, 4) KPN, 5) KPCa, 6) KPZn, 7) KPMn, 8) KPCu, 9) KPB, 10) KPMo, 11) KPCo, 12) obornik. W roku 1959 zbadano na wszystkich poletkach stan wyjściowy. Nawozy były stosowane: jesienią 1959 r. – obornik i wapniak, a wiosną 1960 r. pozostałe – z wyjątkiem kobaltu, który wprowadzono dopiero wiosną 1961 r.

Próbki gleb do analiz mikrobiologicznych pobierano w warunkach antyseptycznych z warstwy darniowej jako próbki średnie; w 1959 roku pobrano jednorazowo, a w następnych latach – w maju, lipcu i wrześniu. W glebach tych każdorazowo określano metodami ogólnie przyjętymi liczebność *Clostridium* i azotobaktera. Wyniki przedstawiono w tabelach II i III.

W wyniku przeprowadzonych badań stwierdzono, że:

1. liczebność *Clostridium* nie wykazywała istotnych zmian pod wpływem nawożenia, natomiast zmiany ilościowe stwierdzono w zależności od sezonu wegetacyjnego. Okresami najbardziej sprzyjającymi w rozwoju *Clostridium* były wiosna i lato.

2. Azotobakter, jako organizm bardziej czuły na zmiany siedliskowe, reagował wyraźnie na nawożenie mineralne. Najbardziej sprzyjającymi okresami rozwoju dla azotobaktera były lato i jesień.

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