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THE INFLUENCE OF ADMIXTURES ACCELERATING THE PINE BARK COMPOSTING PROCESS ON VARIATION IN THE BACTERIOLOGICAL STATE OF COMPOSTS

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Keywords: composting, pine bark, actinomycetes, copiotrophs, oligotrophs, metabolic activity.

Abstract: The focus of the study was on the dynamics of the variation in the population of copiotrophic and oligotrophic bacteria and actinobacteria as well as the level of acid and alkaline phosphatase activities taking place during pine bark composting, depending on the application of different organic admixtures and the Effective Microorganisms microbiological preparation as well as variation in pH values and temperature. Above all, the trend in the variation in the population of microorganisms under analysis and enzymatic activity depended on the type of admixture applied to the composted pine bark. Apart from that, the course of microbiological activity was also influenced by temperature variation, which resulted from the course of the composting process. The results obtained in the experiment proved that the admixture of PGM (plant green matter) to the composted prisms had stimulating influence on the microbiological indexes under analysis.

INTRODUCTION

Rational waste management in Poland is one of the most important social, ecological and economic problems [24].

In recent years in Poland there has been an upsurge of interest in the possibility to include various waste masses of biological, especially plant origin into organic fertilisation. The waste catalogue, which is applicable in Poland upon the Regulation of the Minister of the Environment of 27 September 2001, divides waste into 20 groups, depending on the place of their origin. Among the groups processable organic waste occupies an important position. This group includes waste from agriculture, orchards, forests and wood processing. Composting is an optimal method of waste management. It is a continuous process, which consists in the decomposition of organic substance subjected to biochemical processes and the influence of microorganisms. It is usually defined as the sum of microbiological processes related with the formation of humus.

Apart from the specific substances (fulvic acids, humic acids and humin), humus, which is formed in the process of organic matter mineralisation, contains increased amounts of mineral nitrogen and phosphorus compounds. A considerable amount of the biomass of microorganisms, which is part of the organic fraction of humus, is formed during composting. The studies by Gambuś & Wieczorek [10] and by Czyżyk et al. [7], which prove that composts have higher fertilising value than manure, show the considerable fertilising potential of composts. The admixture or the use of biodegradable waste for composting contributes to higher aeration of the composted mass, facilitates reaching the optimal humidity range of 50–60%, enriches the composted mass with a source of carbon which is accessible to microorganisms and guarantees the optimal C:N ratio.

The subsurface application of composts is the effect of improvement in numerous favourable parameters, i.e. increased microbiological activity, changes in the physical, physiochemical and chemical properties of soils [9, 11, 13, 14, 29]. The authors particularly emphasise better porousness and structure of the soil as well as the stability of soil aggregates. In consequence, this results in favourable water and air conditions of soils. Apart from that, the fertilisation of soil with compost contributes to an increase in the buffer and sorption capacity of soil and simultaneously it decreases soil acidity. The effect of agricultural application of compost is increased fertility of soil, which is expressed with a higher amount of organic carbon, general nitrogen and assimilable macrocomponents. The composts formed from plant waste have low content of metals and good sanitary parameters. This contributes to their wide practical application for agricultural and recultivation purposes.

Due to the diversified tempo of the composting process in different types of plant waste special attention is given to the optimisation of the process, especially when it comes to hardly biodegradable waste, such as bark, for example [26]. However, forest inspectorates see a precious source of compost in them, which is necessary to produce seedlings instead of using peat in light soils.

The aim of the research was to determine the dynamics of variation in the population of selected groups of microorganisms and the level of activity of phosphatases during pine bark composting depending on the application of different organic admixtures and the Effective Microorganisms microbiological preparation as well as variation in the pH value and temperature.

MATERIAL AND METHODS

The experiment was started in 2011 in the village of Świeca, which belongs to Antonin Forest Inspectorate, Wielkopolska Voivodeship. In the local plant nursery compost prisms were made on solid surface soil in an open space. The volume of each prism was 4 m^3 (length – 2.5 m, width – 1 m, height – 0.4 m). The main material for the prisms was pine bark (K1) enriched with the following admixtures: the Effective Microorganisms microbiological preparation manufactured by Greenland company (EM-A) 3 $liter \cdot m^{-3}$ (K2), nitrogen in the form of urea 1.0 $kg\ N \cdot m^{-3}$ (K3), plant green matter PGM 2 Mg (K4), PGM 2 Mg + EM-A 3 $liter \cdot m^{-3}$ (K5), PGM 3.5 Mg (K6), PGM 3.5 Mg + EM-A 3 $liter \cdot m^{-3}$ (K7). The amounts of mineral nitrogen were determined based on a common recommendation found in the literature [18, 25]. The PGM consisted of serradella, buckwheat, vetch and field peas. It contained 23.4% of dry matter and 10.51 $g\ N\ kg^{-1}$ of dry matter, whereas the bark contained 42.23% of dry matter and 3.94 g N kg^{-1} of dry matter. The control item was a pine bark prism without admixtures.

Before the prisms were made, 0.3 kg P_2O_5/m^3 (20% P_2O_5 single superphosphate) and 0.5 kg K_2O in 60% potassium salt had been added to each prism.

Table 1 shows the physiochemical analysis of the basic components of the compost prisms on the day they were made (Tab. 1).

Table 1. The physiochemical properties of the basic components of the composts

Parameter	Unit	Pine bark	Plant Green Matter
Acidity	pH-H_2O	5.02	6.92
Dry mass	%	42.23±1.37	23.40±2.92
Organic matter	%	66.00±0.71	85.00±1.41
Total nitrogen	g kg^{-1} s.m.	3.94±0.88	10.51±0.06
Ash	%	34.00±0.71	15.00±1.41

In addition to the analysis of organic carbon and total nitrogen a quantitative analysis of composts was also performed in starting materials. The data show that at the beginning of the experiment organic C content ranged from 388.3 to 272.9 g·kg^{-1} d.m. of composts. In turn, the total nitrogen content ranged from 7.90 to 3.75 g·kg^{-1} in d.m. The varied content of the two components had an impact on variable ratios of C:N in composts, which ranged from 99.0:1 in the compost of the control bark to 33.5:1 in compost with the mass of plant 3.5 Mg+EM [6].

Compost samples, which were necessary for microbiological analyses, were collected from the prisms at five different periods, depending on the current mean temperature: I – the beginning of the experiment, II – after 5 days, III – after 9 days, IV – after 21 days, V – after 72 days. Mixing prisms using tractor aerator took place in the I period and after taking samples for analysis in the III period. It was detected a clear decrease in temperature in all prisms. Research material in each case was collected as a mean sample from six points from the inside of prisms.

The population of colony forming units (CFU) of copiotrophic and oligotrophic bacteria and actinomycetes was determined on microbiological selective substrates by means of the plate method. The population of copiotrophic bacteria was determined on nutrient broth and the plates were incubated at a temperature of 28°C for 5 days [21]. The population of oligotrophic bacteria was determined on diluted nutrient broth. The plates were incubated at a temperature of 28°C for 21 days [21]. The population of actinobacteria was determined on the Pochon culture medium after seven-day incubation [16]. Apart from that, the spectrophotometric method was applied to determine acid and alkaline phosphatase activities in the collected samples. PNP (4-nitrophenyl phosphate disodium salt hexahydrate) was used as a substrate. The samples were incubated at a temperature of 36°C for one hour, where the wavelength was 400 nm. The activity of the enzyme complex was expressed in μmol PNP g^{-1} of the compost dry matter h^{-1} [28].

Statistica 10 software was used for statistical analyses applied in the experiment [22].

RESULTS AND DISCUSSION

Temperature is one of the basic parameters affecting the population of microorganisms in a composted prism. This factor influences not only the number of microorganisms, but also the succession of consecutive groups of microorganisms, which is reflected with the rate of numerous microbiological processes [5].

The analysis of temperatures measured in the composted prisms revealed that in most of them there were considerable differences between consecutive stages of composting (Fig. 1).

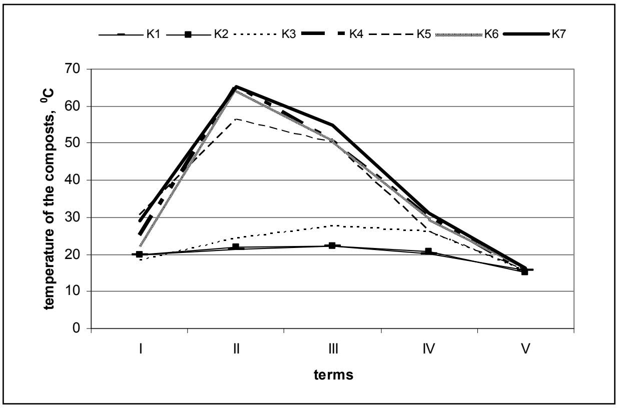

Fig. 1. The changes of temperature during composting

Temperature variation in the volume of organic material subjected to the composting process is a typical phenomenon observed in the process [1, 16, 17]. The findings of microbiological investigations show that variation in the temperature of pine bark composting was most likely the cause of variation in the populations of copiotrophic and oligotrophic bacteria.

This dependence is particularly noticeable in bark and plant green matter (PGM) combinations. Five days after the beginning of the experiment in the prisms enriched with the PGM (K4–K7) there was a distinct increase in the temperature, which reached the value of about 68°C. The period turned out to be the thermophilic stage in the prisms under study. During the period both the populations of copiotrophic and oligotrophic bacteria decreased (Fig. 2 and 3).

Błaszczyk reports that when the composted material reaches the temperature of 45°C, the activity of mesophilic microflora is inhibited. Finally, as a result of protein denaturation the lysis of bacterial cells takes place and only thermoresistant spores remain [4]. This fact may account for the sudden decrease in the populations of the bacteria under analysis. The observed effect refers to the analysis of changes in the number of mesophilic and thermophilic fungi in composting prisms, the results of which are presented by Starzyk et al. [27].

According to De Bertold et al. [8], the increase in the temperature during the composting process causes a considerable decrease in the population of mesophilic bacteria and further development of the compost microflora depends on the access to oxygen.

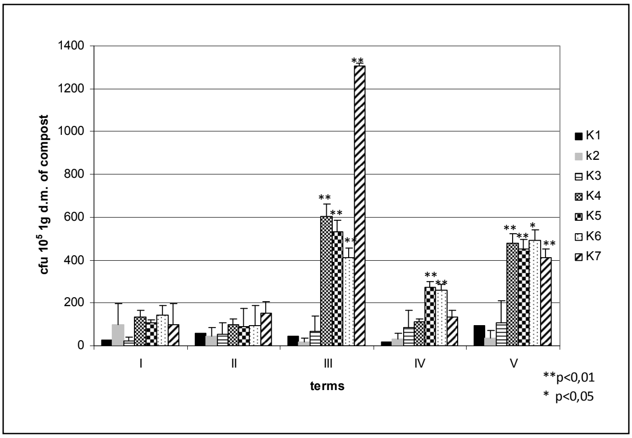

Fig. 2. The number of copiotrophic bacteria in composts

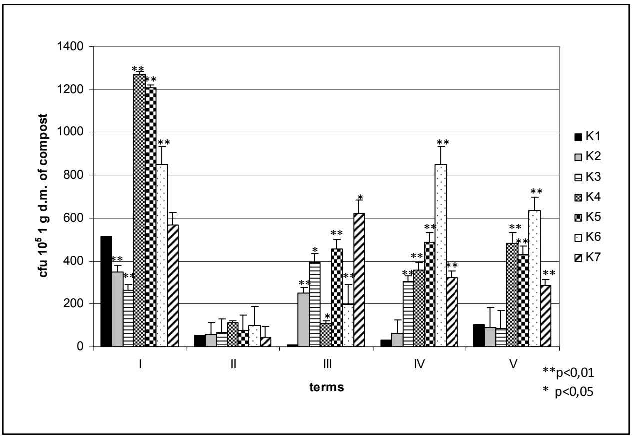

Fig. 3. The number of oligotrophic bacteria in composts

Although during the third period of the experiment the temperature was still within the limit of the thermophilic stage (about 51°C), the gradual decrease in the temperature turned out to be favourable both to the development of copiotrophs and oligotrophs. The results obtained in the experiment correspond with the data published by Starzyk et al. [27], which showed that the population of thermophilic bacteria remained at a high level. However, in comparison with the previous period it is also interesting to observe a significant increase in the population of mesophilic bacteria in the experiment under discussion in all cases of the bark enriched with nitrogen admixtures. The obtained result points to the fact that both the copiotrophs and oligotrophs under investigation may have belonged to the groups of microorganisms with a diversified range of thermal requirements. This effect is not in complete agreement with the data found in the reference books or in the study by Wong [31]. As they prove, only when the temperature in the composted material decreases to 45°C, mesophilic bacteria become predominant again. This situation may be the effect of the appearance of mesophilic forms with a high range of the maximum temperature limit.

In the consecutive periods the observed drop in the temperatures was the effect of a gradual decrease in the population of copiotrophic bacteria, although during the fifth period another increase in the population was observed. On the other hand, until the end of the experiment the population of oligotrophs remained at a similar level to the one observed at the end of the thermophilic stage. This phenomenon may have been caused by the fact that at the time there was a considerable supply of organic matter which had not completely been decomposed yet. It may also have been caused by the metabiosis, which caused the appearance of the products of metabolism of other groups of microorganisms and they may have been the factor activating the group of bacteria under analysis.

The high population of oligotrophic bacteria, which was noted during the first period of the experiment, seems to be interesting. Most likely it confirms other authors' reports on the dominance of abundant mesophilic microflora, which is saprophytic to the plants and waste that make the basis of composts. The predominant genera are: *Lactobacillus, Pediococcus, Streptomyces, Propionibacterium, Pseudomonas* and *Bacillus* [4, 12].

The intensive development of copiotrophs in the environment depends on the organic debris in it. During the decomposition of the debris the population of microorganisms usually increases significantly. During intensive proliferation they decompose both simple and complex carbohydrates, proteins and other compounds from the structure of higher organisms. Their nutritional requirements depend on the concentration of organic components in the growth medium, where the optimal dose is about 1000 mg of soluble carbon per litre [23]. The analysis of variation in the population of copiotrophs, which depended on the applied experimental combination, revealed the favourable influence of PGM admixtures on the increase in the population of the bacteria in the composts. This tendency was observed in all the periods of the experiment. The statistical analysis confirmed highly significant differences between the combinations under investigation and the control item, starting with the third period of sample collection. These results show that PGM proved to be an easily assimilable source of nourishment for the copiotrophs, which most likely contributed to its considerable decomposition in the composted prisms. The low population of the group of microorganisms in question during the first two periods of the experiment can be explained with the need to adapt during the first period and with the excessively high temperature in the prisms during the second period, when the lowest population of copiotrophs was observed in all the combinations during the whole experiment.

Oligotrophs are the microorganisms which can develop in an environment with a low content of organic compounds. The concentration of nutrients which is appropriate for them ranges between 1 and 15 mg of soluble carbon per litre. Their name refers to the bacteria that grow on a poor growth medium, with a low concentration of nutrients, only at the beginning of culturing [23]. The bacteria show low variation in their population and activity. Apart from that, they are highly sensitive to amino acids, organic acids, vitamins and inorganic salts [21].

The analysis of variation in the population of oligotrophs in consecutive periods of the experiment revealed that their population tended to grow in the combinations enriched with admixtures, as compared with the control item. It was interesting to observe that similarly to the copiotrophs, the most noticeable increase in the population of oligotrophs was noted in the combinations with the admixture of PGM. This phenomenon is explained with the fact that this group of microorganisms also uses easily assimilable matter in the first place. A particularly significant increase in the population of oligotrophs was noted in the combination with the admixture of 3.5 Mg of PGM during the last two periods of the experiment, when the population was higher than in the control item by 2517% and 522%, respectively. On the other hand, the highest population of oligotrophs was noted in the first period of the experiment in most of the prisms. However, in view of the fact that the period was the day when the experiment started, this result does not point to the process of proliferation of oligotrophs. It only indicates the presence of endogenous groups of these microorganisms in the components used in the structure of the prisms. The authors have never met so far in the literature detailed data on the growth of copiotrophic and oligotrophic bacteria in composts prepared on the basis of the bark. Therefore, the results discussed are the effect of preliminary studies on this topic.

No significant increase in the groups of microorganisms in question was observed in the pine bark composts enriched only with the EM preparation or with urea. Pine bark is a hardly degradable material due to its properties related with its chemical composition. It is strongly saturated with the substances inhibiting the growth of some microorganisms and they are a natural protective barrier of the plant. The microorganisms included in the EM, despite their wide diversity, do not found in the pile of pine bark suitable conditions to adapt to this environment, most likely due to the difficult accessibility decomposed the material and the lack of available nitrogen. The high carbon-nitrogen ratio in the pine bark causes the need to supply it from the outside. It is most likely that the admixture of urea proved to be insufficient against the high content of carbon in the composted material.

Most composts are abundantly settled by actinobacteria. Many of them proliferate intensively at the last stage of prism cooling, degrading the polymerised organic matter, cellulose, hemicelluloses and lignins [4]. In the experiment under analysis the highest increase in the population of actinobacteria was observed during the third period, when the temperatures in the composts were still high (Fig. 4).

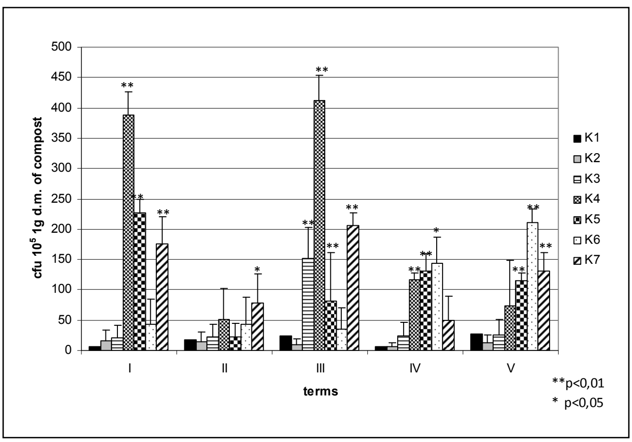

Fig. 4. The number of actinomycetes in composts

McKinley et al. [17] stress that the highest activity of microorganisms during the composting process can be observed at a temperature of about 58°C. Wolna-Maruwka et al. [30] indicate that in the process of composting the largest number of actinomycetes were recorded at 31ºC. Similarly to the groups of microorganisms which were analysed above, the PGM added to the prisms was found to have significantly stimulating effect on the development of the microorganisms under investigation. Especially at the final stages of the process there was a highly significant increase in the population of actinobacteria in the prisms with the admixture of papilionaceous plants, as compared with the control item. However, when only the EM preparation or urea were added to the prisms, they did not cause a significant stimulation of the growth of this group of bacteria.

The population of true bacteria and actinobacteria under analysis must also have been unfavourably influenced by relatively low pH, which averaged between 4.5 and 5.5 (Fig. 5).

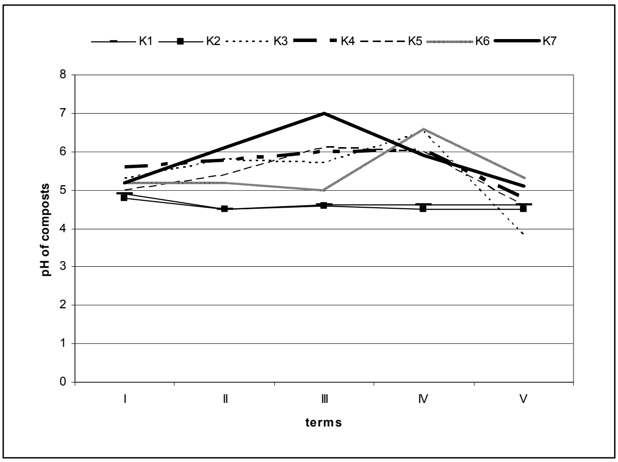

Fig. 5. The changes of pH during composting

Many researchers confirm the fact that phosphatase activity is the most reliable indicator of variation in the microbiological activity in the environment [2, 19, 20]. Phosphatases are in fact quite strongly responsive to the influence of various environmental factors [3]. The analysis of variation in both the acid and alkaline phosphatase activity showed that the lowest activity during the whole experiment was in prisms K1–K3, where the value of the alkaline phosphatase activity was close to 0 (Fig. 6 and 7).

Such a low level of enzymatic activity may have been caused by difficult access to degraded organic matter and unfavourable C/N ratio. Besides, the low pH which persisted in prims K1-K2 during the whole composting process was also an unfavourable factor to the alkaline phosphatase activation.

The highest activity of acid phosphatase was observed in compost K6, especially during the first two periods of sample collection. On the other hand, the bark enriched with the 3.5 Mg PGM + EM-A 3 liter · m^{-3} (K7) turned out to be the most favourable to

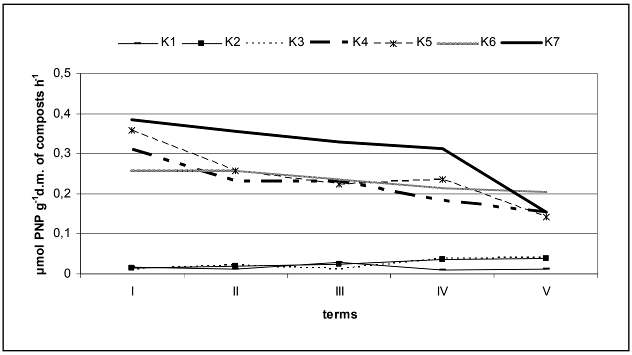

Fig. 6. The changes of alkaline phosphatase activity during composting

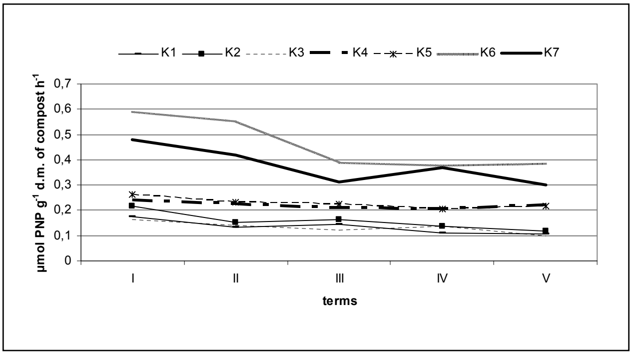

Fig. 7. The changes of acid phosphatase activity during composting

the alkaline phosphatase activation. A high level of the enzyme in compost K7 remained during the four consecutive periods of the experiment. Only during the last, fifth period its activity dropped noticeably. A comparison of variation in the activity of the phosphatases under analysis in the composted prisms revealed that in most cases the admixture of PGM applied to the pine bark caused the activation of both types of phosphatases.

CONCLUSIONS

1. The admixtures in the composted pine bark influenced the increase in the population of copiotrophic and oligotrophic bacteria and actinobacteria in the composted prisms.
2. Temperature variation during the composting process had significant influence on the population of the groups of microorganisms under analysis.
3. The highest population of the bacteria under analysis was noted in the combination of pine bark enriched with 3.5 Mg PGM, which was particularly noticeable at the final stages of the composting process.
4. The admixture of PGM to the composts influenced the activation of both acid and alkaline phosphatases.

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WPŁYW DODATKÓW PRZYSPIESZAJĄCYCH PROCES KOMPOSTOWANIA KORY SOSNOWEJ NA ZMIANY STANU BAKTERIOLOGICZNEGO KOMPOSTÓW

Badano dynamikę zmian liczebności bakterii kopiotroficznych, oligotroficznych oraz promieniowców, a także poziom aktywności fosfataz kwaśnych i alkalicznych, zachodzących podczas kompostowania kory sosnowej, w zależności od zastosowania różnych dodatków organicznych i preparatu mikrobiologicznego Efektywnych Mikroorganizmów oraz zmian wartości pH i temperatury. Kierunek zanotowanych zmian liczebności analizowanych grup drobnoustrojów oraz aktywności enzymatycznej zależne były przede wszystkim od rodzaju zastosowanego dodatku do kompostowanej kory sosnowej. Ponadto wpływ na przebieg aktywności mikrobiologicznej miały również zmiany temperatury, będące efektem przebiegu procesu kompostowania. Na podstawie uzyskanych wyników stwierdzono stymulujący wpływ dodatku ZMR (zielonej masy roślin) do kompostowanych pryzm na analizowane wskaźniki mikrobiologiczne.