Comparative testing of effect of ammonium phosphates for decontamination of cattle manure

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SUMMARY

Comparative studies of the application of monoammonium and diammonium phosphate for processing of fresh and aged manure litter from dairy cows were carried out with a view to decontamination. For this purpose changes in the quantities of pathogenic test strains of *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, differing in resistance to gentamicin and tetracycline antibiotics, were tracked. They were imported in the materials in quantities by 10^5 CFU/g of their total contents at the beginning of composting. It was found that in all investigated variants even after 2 weeks of the beginning of the experiments the quantities of test bacteria decreased to negligible levels (less than 2 lg). Complete decontamination of the studied composts in terms of pathogenic test bacteria was achieved in period of three weeks at temperature above 20°C. There were no significant differences of the antimicrobial effect between both nitrogen compounds tested (P>0.05), although diammonium phosphate showed slightly better results in this direction. Higher doses (600 ppm) also did not show statistically significant differences compared with smaller concentrations (200 ppm). In fresh and aged cattle manures without chemical treatment complete decontamination with respect to the introduced test strains was achieved for four weeks.

Keywords: cattle manure, decontamination, ammonium phosphates, test bacteria

INTRODUCTION

The effective use of livestock excreta is a global problem. They are mainly utilized for obtaining fertilizer and energy (fuel). Because they contain a wide range of microorganisms, including pathogens, may pose a risk to human health (Bicudo and Goyal, 2003; Heinonen-Tanski et al., 2006). Different

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approaches for decontamination were tested, particularly with use of physical and chemical methods, or combinations thereof. The use of nitrogen and phosphate fertilizers including superphosphates is reliably (Von Raven et al., 1984; Porubcan, 2005). The purpose of such treatment is to reduce or eliminate populations of microorganisms, as it is related primarily with chemical changes. As it is not always achieve good results, if possible also relies on the application of biological methods using microorganisms (biodegradants - microbial consortia of bacteria and fungi) or combinations of *Bacillus* spp. with enzymes (protease, amylase, lipase), with or without chemical additives and others (Porubcan, 2005; Zhang et al., 2005; Sekar et al., 2010).

Besides the classical composting, which is widely used all over the world, it is necessary to search of opportunities for more rapid achievement of decontamination that are simultaneously secure, convenient, economical and environmentally safe. To assess the effect of ammonium phosphates in this respect it is essential the presence of sufficient data on the survival of microorganisms of different groups in fertilizer materials treated with these compounds before composting. There is no evidence in this direction and would be useful to establish the effect of ammonium phosphates at different concentrations on the pathogens in manure, as well as the time of decontamination with their participation.

The purpose of these studies is to follow the survival of pathogenic test microorganisms imported in fresh and aged cow manure litter after treatment with monoammonium and diammonium phosphate in different concentrations in order to assess the opportunities for decontamination and receiving epizootiologically safe end products.

MATERIAL AND METHODS

Cattle manure

Fresh and aged (for 10 days) manure litter from dairy cows were examined.

Chemical compounds

The effect of the following substances was tested: ♦ Monoammonium phosphate (Ammophos) granules from 1 to 5 mm from Agricola - Bulgaria. Nitrogen (N): 12.6%. Content of phosphate (P_2O_5): 52.4%. Moisture: 1.10%. ♦ Diammonium phosphate (DAP) of pellets from 1 to 4 mm from Agricola - Bulgaria. Nitrogen: 18.74%. Content of phosphate (P_2O_5): 46.90%. Moisture: 0.81%. Nitrogen compounds were applied in final concentrations of nitrogen at 200 and 600 ppm.

Microorganisms

Pure cultures of three pathogenic bacterial test strains were used in the investigations: *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis.* They were isolated from animals with chronic infections and were selected by their poly-resistance *in vitro* to Gentamicin and tetracyclines (Tetracycline, Doxycycline and Oxytetracycline). An additional cultivation of these strains on nutrient media with antibiotics from these groups was performed to isolate and use in studies of branches, the most thriving in the presence of high concentrations of these antibiotics.

Nutrient media (Antisel, Sharlau Chemie S. A., Spain).

Selective nutrient media with added together doxycycline (50 μg/ml) and Gentamicin (16 μg/ml) were used for isolation and cultivation of the test bacteria. Eosin Methylene Blue agar for *Proteus vulgaris*, Cetrimide agar for *P. aeruginosa* and Chapman Stone agar for *S. epidermidis* were selected. On these media, other bacteria were not grown, except the test strains selected by the resistance to the antibiotics pointed out. The total number of microorganisms in the studied materials was reported on Mueller Hinton agar without antibiotics. The contents and quantities of *Clostridium perfringens* on selective agar (Biolab Zrt., Budapest), as well as of *E. coli* and *Salmonella enterica* on Eosin Methylene Blue and Salmonella-Shigella agar were also tracked.

Quantitative determination of microorganisms was carried out using the classical method in serial 10 times increasing dilutions of the examined materials in a sterile physiological solution. Cultures on the selected media with and without antibiotics are prepared from these dilutions, three for each medium and dilution. After incubation at 37^{o}C for 24–72 h under aerobic and anaerobic conditions (with Anaerocult® A mini – Merck-Bio Lab, Bulgaria), the mean arithmetical number of the developed colonies was calculated and the colony forming units (CFU) in 1 g of the initial material were determined.

Microscopic studies of microorganisms were carried out under immersion at 1000 x magnification after staining by various classical methods (Gram, Klett for capsules and Mueller for spores) of materials from different cultures on the nutrient media.

Experimental settings were as follows. 1. Fresh manure: 1) untreated control; 2) Treated with 200 ppm Ammophos; 3) Treated with 600 ppm Ammophos. 2. Aged manure: 1) untreated control; 2) Treated with 200 ppm DAP; 3) Treated with 600 ppm DAP.

The samples of fresh and aged manure were distributed in glass containers by 200 g each. By 100 ml of the respective compound in the studied

concentration were added and the mixtures were well homogenized and stored at about 22°C.

After a preliminary determination of the total number of microorganisms and those of the examined groups in the fresh and aged cattle manure, in each were imported test strains, each in quantity 10^5 CFU / g of total material. Samples for quantification of microorganisms were taken weekly from 1 to 6 weeks after the introduction of the test bacteria.

Statistical analysis of results was made using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

RESULTS AND DISCUSSION

Research results of the quantitative changes of microorganisms in fresh cattle manure with and without treatment with monoammonium phosphate are presented in Figures 1-3. In the material was not found content of *Salmonella enterica*.

As can be seen from the graph of the first figure during the first two weeks of composting without treatment with the phosphate, a decrease of total number of microorganisms and coliforms, as well as of *C. perfringens*, was observed. It continued in the third week and in the fourth comes stabilizing of their quantities at levels lower than the original by about 1 lg (tenfold) of coliforms and 2 lg (hundredfold) of total number and that of *C. perfringens*. There was a gradual decrease of the content of the test microorganisms, which was the fastest in *S. epidermidis* and slowest in *P. aeruginosa*.

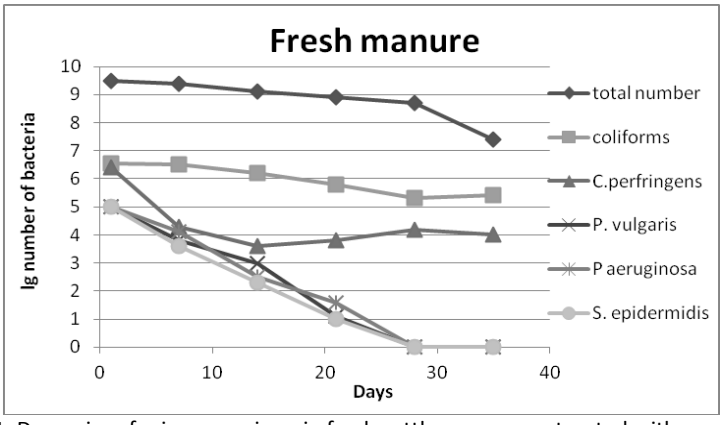

Figure 1. Dynamics of microorganisms in fresh cattle manure, untreated with ammonium phosphates.

Three weeks after adding them they were detected only in small quantities and at the end of the fourth week were no longer set in the

material. This microbial dynamics could be a result of the gradual reduction of moisture in the compost due to evaporation and to competitive relationships between microorganisms. Good aeration affects negatively the development of clostridia.

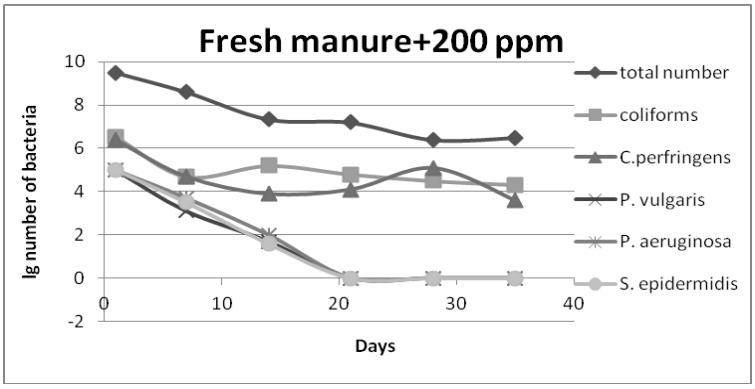

Figure 2. Inactivation of test microorganisms in fresh cattle manure after treatment with monoammonium phosphate in a final concentration of 200 ppm.

The graph in Figure 2 shows that in the treated with 200 ppm monoammonium phosphate fresh cattle manure quantities of test bacteria from the three species had similar dynamics ($P>0.05$). Even seven days after submission to the compost they decreased by about 2 lg. During the second week they were detected in very small quantities, and at the end of the third week no longer were found. In the other monitored groups of bacteria also was a reduction, but it was slower and most significant at the end of the fifth week of research. Then the total number of microorganisms was reduced by almost 3 lg, and that of the coliforms and *C. perfringens* - with 2 lg.

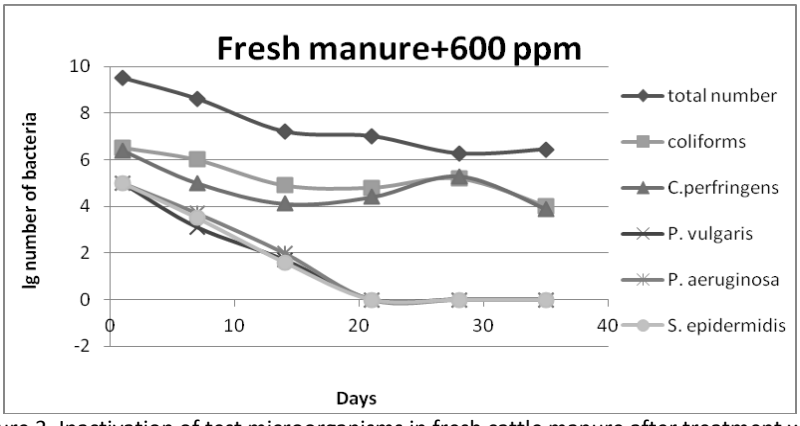

Figure 3. Inactivation of test microorganisms in fresh cattle manure after treatment with monoammonium phosphate in a final concentration of 600 ppm.

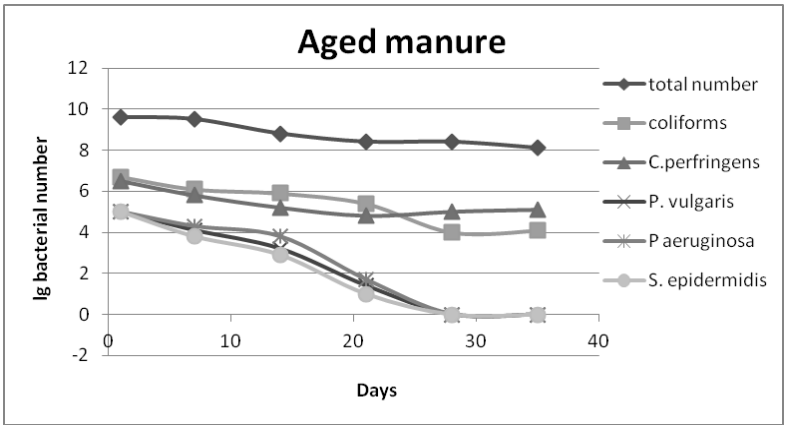

Figure 4. Dynamics of microorganisms in aged cattle manure, untreated with superphosphates.

Similar results were received in fresh compost from cattle manure treated with triple higher concentration of monoammonium phosphate ($P>0.05$), shown in Figure 3. Apparently this chemical treatment in applied concentrations accelerated decontamination compared to conventional composting of cattle manure (Figure 1). This is also evident when comparing these results with those from our previous studies on decontamination of simple composting of cattle manure without chemical treatment (Popova et al., 2009), in which the variations of the total number of microorganisms and of imported test bacteria were similar. The test strains slightly decreased in amounts in the first week and more significantly in the second week of the study. At 21 day they were no longer isolated, also at the later stages of the experiment.

The results of the monitoring of quantitative changes of microorganisms in aged cattle manure in the three experimental variants can be seen from Figures 4-6. In the materials was not found content of *Salmonella enterica*.

The dynamics of the microorganisms in the compost from aged manure, which can be seen in Figure 4, was similar to that in the fresh one. Here, however, the amount of coliforms after one month of composting decreased approximately with 2 lg, while that of *C. perfringens* and the total microbial number - with no more than 1 lg compared to baseline. For the coliforms, which are more demanding to moisture, apparently lower water content in this compost was reflected in a greater degree.

The content of the indicator bacteria also gradually decreased. One week after their introduction in the material they were less by about 1 lg, but after another two weeks their content was minimal. Four weeks after the start of the experiment a complete decontamination of aged compost from cattle manure in terms of the test bacteria was established. It was the fastest for *S.*

epidermidis and slowest for *P. aeruginosa*. At the end of the third week after their adding they were not longer established in the material.

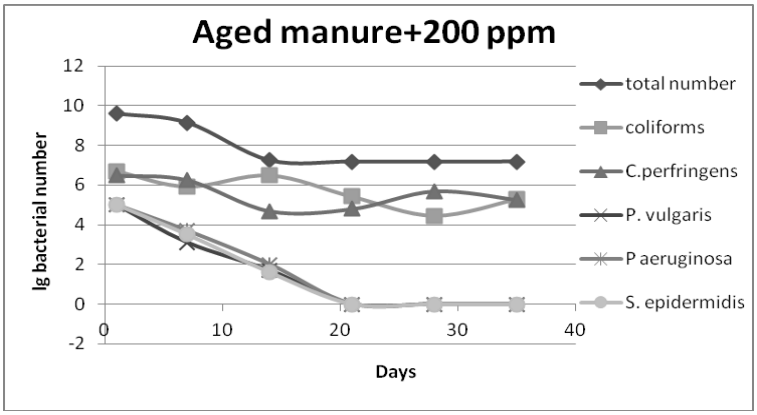

Figure 5. Inactivation of test microorganisms in aged cattle manure after treatment with diammonium phosphate in a final concentration of 200 ppm.

The graph in Figure 5 shows that after treatment with diammonium phosphate in a final concentration of 200 ppm the quantities of test bacteria from the three strains decreased by about 2 lg even seven days after their introduction in the compost. During the second week their reduction was with even about 1.5 lg when settled in small quantities and by the end of the third week disappeared. In the other monitored groups bacteria the reduction was slower. At the end of the fifth week from the beginning of the study the total number of microorganisms was lower by almost 3 lg, and that of the coliforms and *C. perfringens* - with 1 lg. They were obviously less affected by this variation of chemical treatment. This is also evident when comparing the data from their dynamics in the untreated control.

The results of studies of aged compost from cattle manure treated with threefold higher concentration of diammonium phosphate, shown in Figure 6, were alike. The test bacteria were reduced by similar rates. It is noteworthy, however, a significant response of coliforms, which decreased by 1 lg compared to the compost treated with 200 ppm of this phosphate.

The results of the comparative studies show that the processing of fresh and aged cattle manure with mono- and diammonium phosphate result in acceleration of decontamination of composted materials about one week (25% compared to untreated controls). Still in the middle of the three week period the quantities of test bacteria in processed composts decreased from 20 to 30 times and until the end of the third week disappeared. After using the higher end concentrations the results were slightly better. In that respect the effect of

diammonium phosphate was more significant, although the differences with the monoammonium compound were not reliable (P> 0.05).

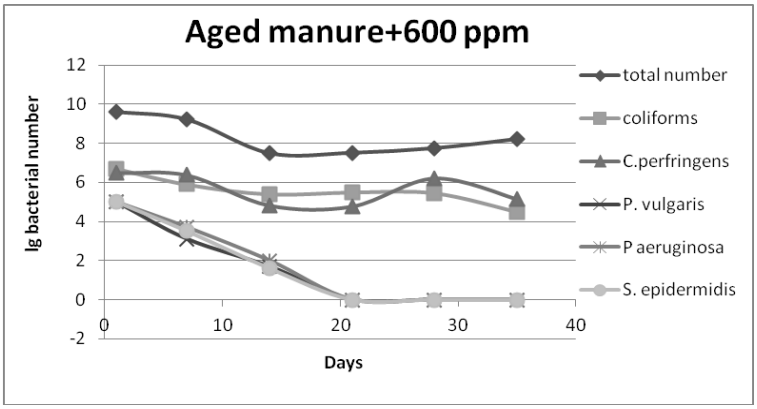

Figure 6. Inactivation of test microorganisms in aged cattle manure after treatment with diammonium phosphate in a final concentration of 600 ppm.

The use of indicator microorganisms to assess the decontamination of fertilizer materials is a brand new approach and in our view it is a convenient and efficient.

The tested quantities of the ammonium phosphates were selected not only in relation on the expected adverse effect on pathogenic microorganisms, but also with a view to achievement of a positive impact on soil fertility and yield of crop production after their application as fertilizer. Higher concentrations would result in faster decontamination, but then there is a risk of loading the soil with large amounts of nitrogen. According to the data of Weil et al. (1977) and Lu et al. (2012) higher nitrogen loading can negatively affect the yield of crops and may has adverse effects on the stagnant waste water, groundwater and soil quality.

CONCLUSIONS

In aerobic composting of fresh and aged cattle manure at temperatures above 20°C full decontamination in respect of imported test strains of *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*, each in quantities 10^5 CFU/g of total fertilizer material, is achieved in four weeks.

The processing of compost with monoammonium phosphate and with diammonium phosphate in a final concentration of 200 ppm and 600 ppm, accelerates decontamination with one week (25% compared to untreated controls). The differences between the two concentration were not reliable (P> 0,05).

In the application of the higher end concentrations of ammonium phosphates (600 ppm) slightly better results are observed. In that respect the effect of diammonium phosphate is more significant, although differences with monoammonium compound are not reliable (P> 0.05).

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