International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 184-192**
http://www.ijcmas.com

Original Research Article

Decontamination of sewage sludge by treatment with calcium oxide

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A B S T R A C T

Studies were carried out on the effectiveness of application of lime (CaO) for decontamination of fresh sludge from urban wastewater treatment plant, composted at 20,0 ± 1,0°C. CaO was added at various final concentrations: 20%, 23%, 28%, 33%, 38%, and control without CaO. The changes in the quantities of *Escherichia coli*, *Clostridium perfringens*, bacteria of the genera *Staphylococcus*, *Enterococcus*, *Pseudomonas*, Gram-negative aerobic bacteria, fungi, and the total number of microorganisms were traced. Even at 24 hour after application of CaO in all tested concentrations a significant reduction of the microorganisms was established, and *Pseudomonas* sp. disappeared. After one week, the Gram-negative bacteria and *C. perfringens* were killed and the quantity of microorganisms of the other groups was significantly reduced. For four weeks all microorganisms were died in the sludge, containing 38% CaO. In the others only a small amount of microorganisms were survived. The treatment with CaO was particularly suitable to obtain epidemiologically safe sludge for one month or in a shorter period, as it was efficiently and safe for the environment. Sufficient was administration of 28% CaO in order to ensure the destruction of all sanitary indicative microorganisms. The effect was enhanced by increasing the concentration of CaO.

Keywords

Sewage sludge, CaO, Aerobic composting, decontamination

Introduction

Sewage sludge is used in agriculture for decades. Since 1986 their application is subject to the provisions of EU Directive (86/278/EEC). The Directive sets out the requirements regarding the quality of sludge, of soil, in which can be applied and to the crops which can be grown on the treated lands. European Commission adopts the directive for successful and no reports of adverse effects in its annex. To ensure the best results in its implementation, its further improvement is considering (Langenkamp and Part, 2001). It is important to avoid infection risks to consumers (humans and animals) of crops from agricultural areas treated with the outputs from wastewater treatment plants. It is also important the sure removal of the risk of groundwater contamination. Many European countries rely heavily on groundwater for drinking water and for irrigation of crops. Persistent pollutants in groundwater can reach and potentially contaminate surface water. The use of such waters is increasing and because the fact that the amount of water available per capita has fallen by 40% since 1970 One of the reasons is the pollution of land and groundwater resources, especially in highly industrialized areas, which are typical for Central and Eastern Europe.

Applying sewage sludge in agriculture can be a source of biological contamination of soil, water and plants, including with pathogenic microorganisms. Therefore, monitoring and evaluation of these risks are important to the search for environmentally friendly solutions. The recycling of organic waste using the application in agriculture of sewage sludge must not result in adverse effects on product quality, or on the environment (Harshman and Barnette, 2000; Langenkamp and Part, 2001; EPA, 2004).

The aim of this study is tracking the survival of microorganisms from different groups in sludge of urban treatment plant, which were composted by two different methods, in order to assess the possibilities for decontamination and receiving epizootiologically safe end products.

Materials and Methods

Sludge

Fresh sewage sludge from urban waste water treatment plant near to Pernik, obtained in the spring, was investigated. The dry matter content in the sludge was 4.90 ± 0.09%.

Chemical compound

The effect of commercial lime (CaO) in final concentrations 20-38% was tested.

Microbiological studies

Microbiological studies were conducted according to the Ordinance on the terms and manner of utilization of sludge from wastewater treatment through its use in agriculture (Decree № 339 - 14.12.2004). The titers of *E. coli* and *Clostridium perfringens* were established. Additionally were traced amount of bacteria of the genera *Staphylococcus, Enterococcus, Pseudomonas,* Gram-negative aerobic bacteria, fungi, and the total number of microorganisms.

Nutrient media

Elective and selective nutrient media (Sharlau Chemie S. A., Spain) were used for isolation and quantitative determination of the microorganisms from the studied groups and types. The following media have been chosen: Mueller Hinton agar for counting the total number of microorganisms in the examined material, Eosin Methylene Blue agar for *E. coli* and coliforms, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus *Staphylococcus*, Sabouraud agar for fungi, selective medium for enterococci, Salmonella-Shigella agar for *Salmonella enterica* and selective agar for *Clostridium perfringens* (Merck-Bio Lab, Bulgaria).

Quantification

The quantification of the microorganisms was performed by the conventional method in serial tenfold dilutions of the tested materials in a sterile saline solution. Cultures on the selected media were 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

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

Experimental setting

Fresh sludge from urban wastewater treatment plant was composted in laboratory conditions in plastic containers (containers) with holes for aeration in top-side, treated with CaO (quicklime) at different final concentrations in the following versions: № 1-20% № 2-23%; № 3-28% № 4 - № 33% and 5-38%. Control without the addition of CaO also was set. Composting was carried out under aerobic conditions and temperature 20,0 ± 1,0 °C.

The content of CaO in percentage in the samples examined, and the reference to the pH values in them, are presented in Table 1.

Table.1 CaO content and pH values in fresh sludge from urban wastewater treatment plant, mixed with CaO at different final concentrations

Indicator	Material №					
	1	**2**	**3**	**4**	**5**	**Control**
CaO content in %	20	23	28	33	38	0
Value of pH	12,82	12,85	12,89	12,88	12,87	5,53

Samples for determination of the amount of microorganisms were taken from all materials at 24 h, as well as after 1, 2, 3 and 4 weeks from the start of the experiment.

Statistical analysis

All of the experiments were done in triplicate. Statistical analysis of results is made using the classic method of Student-Fisher.

Results and Discussion

One of the most common causes of infections in humans and animals belong to the genera *Staphylococcus*, *Enterococcus*, *Pseudomonas*, Gram-negative aerobic bacteria (including *E. coli* and *Salmonella enterica*), *Clostridium perfringens*, as well as to the fungi. Therefore their tracking in the wastewater and sludge from treatment plants provides more complete information about the effectiveness of their decontamination and environmental safety at their return to nature. *Salmonella enterica* was not isolated from the studied by us sludge from urban wastewater treatment plant. However, it contained *E. coli* and *C.perfringens* in quantities exceeding the requirements set out in our current Ordinance on the manner of utilization of sludge from wastewater treatment through its use in agriculture (Decree № 339 - 14.12.2004). From the sludge were isolated and *Enterococcus* spp. Microorganisms from these groups may cause infections in animals and humans, and materials, containing them, should not be deposited in the soil without prior aerobic or anaerobic processing.

The research results of the composts of fresh sludge at 24th hour after treatment with CaO in various final concentrations are presented in Table 2.

Table.2 Quantities of the traced microorganisms in fresh sludge at 24th hour after treatment with CaO in various final concentrations

Material	**Types of microorganisms – CFU/g**							
	Total number	*Staphylococcus sp.*	*Enterococcus sp.*	*C. perfringens*	Fungi	Coliforms	*E. coli*	*Pseudomonas sp.*
Control	$1,02.10^{10}$* ±0,26**	$4,27.10^{4}$±4,36	$6,80.10^{4}$±0,28	$4,80.10^{3}$ ±1,88	$3,83.10^{5}$ ±1,81	$2,47.10^{4}$± 0,45	$1,07.10^{4}$ ±0,10	$1,25.10^{5}$ ±0,72
20% CaO	$1,67.10^{8}$± 0,47	$9,00.10^{3}$±0,82	$3,20.10^{4}$±0,51	$2,00.10^{2}$ ±0,00	$2,65.10^{4}$ ±1,21	$1,93.10^{3}$± 0,10	$2,00.10^{3}$ ±0,00	0
23% CaO	$1,53.10^{8}$± 0,41	$4,00.10^{3}$±0,71	$4,67.10^{3}$±0,47	0	$2,40.10^{4}$ ±0,74	$2,00.10^{3}$± 0,20	$1,90.10^{3}$ ±0,00	0
28% CaO	$7,48.10^{7}$± 2,69	$1,58.10^{3}$±0,36	$5,17.10^{3}$±3,19	0	$2,50.10^{4}$ ±0,29	0	0	0
33% CaO	$7,10.10^{7}$± 0,10	$2,74.10^{3}$±1,04	$3,87.10^{3}$±0,34	0	$1,17.10^{4}$ ±0,31	0	0	0
38% CaO	$4,50.10^{7}$± 2,24	$1,45.10^{3}$±0,50	$2,50.10^{3}$±0,50	0	$5,60.10^{3}$ ±1,60	0	0	0

** Average. ** Standard deviation.*

As can be seen from the data presented in the table, the bacteria of the genus *Pseudomonas* were died under the effect of all tested concentrations of CaO in the first 24 h after its administration. In the samples treated with 28 and 38% CaO in this period were killed and the others Gram-negative bacteria. In these with the addition of 20 and 23% CaO at 24th hour was established significant reduction of micro-organisms of this group ($P<0.001$). Also, 24 hours after treatment, *C. perfringens* was detected in a small amount ($2,00.10^2 \pm 0,00$) solely in the sludge treated with 20% CaO, but not in those with higher concentrations applied.

Table.3 Quantities of the traced microorganisms in fresh sludge 7 days after treatment with CaO in various final concentrations

Material	**Types of microorganisms – CFU/g**							
	Total number	*Staphylococcus sp.*	*Enterococcus sp.*	*C. perfringens*	Fungi	Coliforms	*E. coli*	*Pseudomonas sp.*
Control	**$8,50.10^{9}$**	**$3,25.10^{4}$**±	**$1,90.10^{4}$**±	**$2,70.10^{3}$**	**$5,33.10^{5}$**	**$2,32.10^{4}$**±0	**$2,00.10^{3}$**	**$7,20.10^{4}$**
	*±1,12**	0,78	0,94	±0,54	±2,87	,61	±0,82	±1,07
20%	**$8,25.10^{7}$**	**$4,27.10^{3}$**±	**$5,25.10^{3}$**±	0	**$2,07.10^{4}$**	0	0	0
CaO	±0,83	0,20	,58		±0,17			
23%	**$6,00.10^{7}$**	**$2,81.10^{3}$**±	**$1,50.10^{3}$**±	0	**$8,05.10^{3}$**	0	0	0
CaO	±4,98	0,35	0,73		±1,34			
28%	**$5,00.10^{7}$**	**$1,33.10^{3}$**±	**$1,05.10^{3}$**±	**$1,00.10^{2}$**	**$6,25.10^{3}$**	0	0	0
CaO	±1,41	0,94	0,27	±0,00	±2,38			
33%	**$5,25.10^{7}$**	**$8,25.10^{2}$**±	**$8,30.10^{2}$**±	0	**$5,75.10^{3}$**	0	0	0
CaO	±2,16	2,86	3,34		±2,59			
38%	**$8,70.10^{6}$**	**$6,36.10^{2}$**±	**$5,92.10^{2}$**±	0	**$3,78.10^{3}$**	0	0	0
CaO	±1,65	2,50	0,22		±1,12			

** Average. ** Standard deviation.*

At 24 hours after treatment of the material with the CaO in all tested concentrations was established a statistically significant reduction in the total number of microorganisms, as well as in that of the cocci and fungi ($P<0.01$ in the sample containing 20% CaO, and $P<0,001$ in the others). With the increase of the applied concentration of CaO the effect increased. The differences in the numbers of microorganisms in the sample with 20% and those with 33% and 38% were reliable ($P<0.001$).

Table.4 Quantities of the traced microorganisms in fresh sludge 14 days after treatment with CaO in various final concentrations

Material	**Types of microorganisms – CFU/g**							
	Total number	*Staphylococcus sp.*	*Enterococcus sp.*	*C. perfringens*	Fungi	Coliforms	*E. coli*	*Pseudomonas sp.*
Control	**$4,27.10^{9}$**	**$2,95.10^{4}$**±	**$3,13.10^{4}$**	**$6,20.10^{2}$**±	**$1,1.10^{6}$**±	**$1,27.10^{4}$**	**$1,33.10^{3}$**	**$6,67.10^{4}$**±
	*±0,20**	0,94	±0,30	0,80	0,14	±0,49	±0,50	1,24
20%	**$1,20.10^{5}$**	**$1,02.10^{3}$**±	**$1,64.10^{3}$**	0	**$1,75.10^{2}$**	0	0	0
CaO	±0,60	0,27	±0,59		±0,83			
23%	**$4,30.10^{4}$**	**$3,00.10^{2}$**±	**$1,13.10^{3}$**	0	**$1,50.10^{2}$**	0	0	0
CaO	±0,71	1,58	±0,41		±0,10			
28%	**$2,90.10^{4}$**	**$2,00.10^{2}$**±	**$1,03.10^{3}$**	0	**$1,40.10^{2}$**	0	0	0
CaO	±0,80	0,71	0,84		±0,10			
33%	**$2,50.10^{3}$**	**$1,33.10^{2}$**±	**$6,50.10^{2}$**	0	**$1,00.10^{2}$**	0	0	0
CaO	±1,20	1,25	±2,30		±0,00			
38%	**$6,75.10^{2}$**	**$2,67.10^{2}$**±	**$2,53.10^{2}$**	0	**$1,00.10^{2}$**	0	0	0
CaO	±0,83	1,88	±1,26		±0,10			

** Average. ** Standard deviation.*

One week after the application of CaO Gram-negative bacteria were killed in the samples with all administered concentrations (Table 3). *C. perfringens* also was not detected in the tested samples except in that with 28% CaO, in which it was found in very small quantity. In highest degree was reduces the amount of the staphylococci, also of the enterococci, but to a lesser extent - of the fungi and the total number of microorganisms ($P<0.001$). The reduction of all groups of microorganisms was most significant in the samples with high concentrations of CaO applied, especially in those with 33 and 38%.

Table.5 Quantities of the traced microorganisms in fresh sludge 21 days after treatment with CaO in various final concentrations

Material	**Types of microorganisms – CFU/g**							
	Total number	*Staphylo-coccus sp.*	*Enteroco ccus sp.*	*C. per-fringens*	Fungi	Coli-forms	*E. coli*	*Pseudomo nas sp.*
Control	**$3,57.10^{9}$***± 0,20**	**$7,30.10^{4}$**± 4,10	**$2,43.10^{4}$** ±1,40	**$2,45.10^{3}$**± 0,90	**$7,50.10^{5}$** ±2,30	**$2,70.10^{4}$** ±5,20	**$1,70.10^{3}$** ±0,50	**$5,07.10^{4}$**± 0,80
20% CaO	**$1,05.10^{5}$**± 0,40	**$6,40.10^{2}$**± 1,20	**$1,05.10^{3}$** ±0,05	0	**$1,40.10^{2}$** ±1,20	0	0	0
23% CaO	**$8,03.10^{4}$**± 0,70	**$1,63.10^{2}$**± 0,50	**$3,75.10^{2}$** ±2,04	0	**$2,87.10^{2}$** ±0,80	0	0	0
28% CaO	**$3,55.10^{4}$**± 0,10	**$1,73.10^{2}$**± 0,20	**$2,57.10^{2}$** 2,00	0	**$1,50.10^{2}$** ±0,50	0	0	0
33% CaO	**$1,17.10^{4}$**± 0,30	**$1,50.10^{2}$**± 0,50	**$3,25.10^{2}$** ±1,48	0	**$1,20.10^{2}$** ±3,30	0	0	0
38% CaO	**$1,03.10^{4}$**± 0,20	**$1,33.10^{2}$**± 1,20	**$2,33.10^{2}$** ±4,78	0	**$1,00.10^{2}$** ±0,80	0	0	0

** Average. ** Standard deviation.*

As seen from the results shown in Table 4, two weeks after the chemical treatment in the tested samples were still established small amounts staphylococci and fungi. The total number of microorganisms was significantly reduced, at highest degree in the test variants containing the highest concentrations of CaO (33 and 38%). The greatest was the quantity of enterococci, but even their number in samples with 33 and 38% CaO was very small. The same trend was observed also in the third week of the study. Data are presented in Table 5.

Four weeks after the application of CaO (Table 6), all microorganisms were killed in the sludge with content of 38% CaO, and in that with 33% of the compound were found only bacilli in a small amount,presented in the column of the total number of microorganisms. They were retained the longest due to the formation of spores, characterized by high resistance to chemical and physical influences. In our opinion, the formation of resistant spores also is a reason for the longest preservation of the fungi in the studied materials. In the presence of 28% CaO very small amount of them was kept viable 4 weeks (only $1,00.10^{2} \pm 0,20$), but concentrations of 33 and 38% destroyed them in this period of time. Single staphylococci and enterococci were survived 4 weeks at presence of 20 and 23% CaO, but at concentrations of 28% or higher, they were killed in this period.

After 4 weeks was also found reduction of the quantity of microorganisms from all studied groups also in the untreated sludge. This at the highest level concerns *E. coli* and coliforms ($P < 0.001$), as well as *C. perfringens* ($P<0.001$). Reduction of the total number of microorganisms was also statistically significant ($P<0.05$), as well as of that of enterococci ($P<0.01$). There was and weak and no reliable increase in the number of fungi, staphylococci and *Pseudomonas* sp. ($P>0.05$). Complete decontamination of the raw sludge, however, did not occur for the one-month study period. This indicates that for the preparation of safe from epidemiological standpoint sludge for one month or for a shorter period, it needs to be subjected to further processing. The treatment with CaO is particularly suitable for this purpose, as it is not dangerous from an environmental perspective and is also efficient. Administration of the compound at a final concentration of 28% is enough in order to ensure the destruction of all sanitary indicative microorganisms within one month. They did not settle in the samples with addition of all tested concentrations of CaO even in the end of the first week after treatment, except the cocci and single *C. perfringens* in one of the samples.

Table.6 Quantities of the traced microorganisms in fresh sludge 28 days after treatment with CaO in various final concentrations

Material	Types of microorganisms – CFU/g							
	Total number	*Staphylococcus sp.*	*Enterococcus sp.*	*C.perfringens*	Fungi	Coliforms	*E. coli*	*Pseudomonas sp.*
Control	**$2,30.10^9$***± 0,62**	**$7,30.10^4$**± 3,60	**$2,20.10^4$** ±0,80	**$6,50.10^2$**± 1,50	**$4,10.10^5$** ±2,10	**$1,30.10^3$** ±0,00	0	**$7,00.10^4$**± 2,90
20% CaO	**$1,10.10^4$**± 0,10	**$1,00.10^2$**± 0,00	**$3,70.10^2$** ±1,20	0	**$5,40.10^3$** ±1,90	0	0	0
23% CaO	**$3,60.10^2$**± 0,30	**$1,00.10^2$**± 0,50	**$1,25.10^2$** ±0,50	0	**$1,80.10^2$** ±0,20	0	0	0
28% CaO	**$3,20.10^2$**± 1,10	0	0	0	**$1,00.10^2$** ±0,20	0	0	0
33% CaO	**$1,80.10^2$**± 0,50	0	0	0	0	0	0	0
38% CaO	0	0	0	0	0	0	0	0

* *Average.* ** *Standard deviation.*

Composting is one of the cheapest and affordable methods for the treatment of organic waste, and therefore is widely used. It must, however, take place under conditions which ensure inactivation of all pathogenic microorganisms. The results of our studies indicate that processing of the fresh sewage sludge with CaO results in a better and more rapid decontamination effect compared to conventional aerobic composting. This is consistent with findings of Popova et al. (2009) who find that complete microbial decontamination of composted poultry litter is achieved in three weeks after treatment with CaO. For this favorable result, except the chemical treatment very important is the low moisture content in the compost from poultry litter, which is much lower than that in the sludge, examined by us. As in our studies and in those of Popova et al. (2009), at ordinary composting of poultry litter and of bovine manure the total number of microorganisms and that of the different groups tested significantly reduce at certain stages, especially in the manure, but in the end of the study is maintained at a certain values. Venglovsky et al. (2005) also establish better sanitation effect of treatment with CaO versus the aerobic mesophilic stabilization.

The results of the present studies indicate that the decontamination of sewage sludge with CaO is directly dependent on the concentration applied and the time of impact. Also of great importance is the good homogenization of the material after addition of the compound in order to penetrate uniformly into the whole volume. The ascertainment of single cells of *C. perfringens* in one of the samples one week after treatment is probably due to the incomplete homogenization of the sludge with CaO. A similar conclusion reach and Popova et al. (2009) in exploring the possibilities for decontamination of animal manures with CaO.

Obviously an important role for the decontamination has the alkaline reaction of the treated with CaO substrates. At such high pH values can survive longer only highly resistant microorganisms such as enterococci, some staphylococci and spores of bacteria and fungi. The differences in pH of the tested substrates with different final concentrations of CaO were very small and hence did not need to be applied concentrations higher than 20-28%. At low pH, such as that of the starting sludge survived not only these resistant organisms, but also some Gram-negative bacteria, especially those of the genus *Pseudomonas*, which can even grow at such values. Perhaps, the high water content of fresh sludge contributed to long-term storage and even multiplication of these microorganisms that are typical inhabitants of the wetlands.

The results of these microbiological studies show that due to content of sanitary indicative microorganisms, some of which were in relatively high values, fresh sewage sludge can not be directly deposited in the nature or used as fertilizer. The results give us reason to recommend microbiological control of each batch of sewage sludge. In establishing sanitary indicative microorganisms (*E. coli*, enterococci, *C. perfringens*) over acceptable values or other pathogenic bacteria such as Salmonella enterica it must be subjected to appropriate processing. This recast can be consists of treatment with CaO, as applied concentration and the exposure time should be selected according to the type and quantity of the identified microorganisms. The use of final concentrations of CaO from 20 to 28% is enough. Higher concentration (33-38%) is justified to apply only at the presence of dangerous microorganisms in large quantities in the sludge such as anthrax bacillus and others.

In conclusions, it is advisable to perform microbiological testing of each batch of sewage sludge. In establishing sanitary indicative microorganisms (*E. coli*, enterococci, *C. perfringens*) over eligible values or other pathogenic bacteria such as *Salmonella enterica*, it should be subjected to processing. Treatment with CaO proves to be very effective method for decontamination of sewage sludge in a relatively short time. The applied concentration of CaO and timing of its impact must be defined according to the types and quantities of the identified microorganisms. Enough is to use final concentrations of CaO from 20 to 28%. Higher quantities (33-38%) must be applied only in the presence of dangerous micro-organisms in large amounts in the sludge.

Acknowledgment

This work is funded by NSF as a result of project implementation FFNIPO-12-01283 "Ecologization of agro-environmental systems and increase their energy efficiency by applying a recast bio organic waste for fertilization, introduction of energy crops and complex use of biomass as an energy source" (Contract DFNI-E01 / 3 of 27.11.2012).

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