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Application of calcium peroxide as an environmentally friendly oxidant to reduce pathogens in organic fertilizers and its impact on phosphorus bioavailability

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Abstract: The article presents the research into hygienizing process of chicken manure using calcium peroxide (CaO_2) as an environmentally friendly biological deactivation agent. The influence of the addition of CaO_2 to chicken manure on the bioavailability of phosphorus was also analyzed. The process of biological deactivation using CaO_2, CaO and $Ca(OH)_2$ agents was analyzed applying the disk diffusion method. To optimize the effect of the hygienizing parameters, (CaO_2 concentration, pH, temperature and time) on the reduction of *Enterobacteriaceae* count the Taguchi method was applied. The content of bioavailable phosphorus was measured with the Egner-Riehm method and determined with spectrophotometry. The reduction in bacterial count followed an increase in the concentration of CaO_2 in a sample. The optimal experimental conditions (CaO_2=10.5 wt.%, pH=9.5, T=40°C, t=180 h) enabled a significant decrease in the *Enterobacteriaceae* count, from 10^7 cfu/g to 10^2 cfu/g. Analysis of the samples with Egner-Riehm method showed that the phosphorus content decreased with the addition of biocide CaO_2: from 26.6 mg/l (for 3.5 wt.%) to 3.5 mg/l (for 10.5 wt.%). These values were slightly higher than the content of phosphorus deactivated with $Ca(OH)_2$ i.e., from 11.25 mg/l (for 3.5 wt.%) to 4.49 mg/l (for 10.5 wt.%). The application of CaO_2 for hygienizing chicken manure enables effective reduction of *Enterobacteriaceae* count to an acceptable level (below 1000 cfu/g). In comparison with the traditional techniques of hygienization, the application of CaO_2 has a positive effect on the recovery of bioavailable phosphorus.

Introduction

The most common practice for managing poultry manure is the soil application (Dróżdż et al. 2020). Due to the high content of valuable nutrients such as nitrogen (N), phosphorus (P) and potassium (K), poultry manure is a natural fertilizer in agriculture which improves the properties of the soil (Bolan et al. 2010).

Poultry manure is also rich in calcium (Ca) and magnesium (Mg), and contains microelements necessary for proper development of plants (Nicholson et al. 1996). The fertilizer value of this biowaste is diversified and depends, among others, on its chemical composition (Wieremiej 2016, Kucharski and Białecka 2019) and the storage method (Yokota et al. 2003). The main factors which determine the fertilizer value include: type of breed, breeding system and the kind of feed.

Phosphorus positively influences the growth and development of plants, its shortage being doubly harmful leading to excessive uptake of other nutrients by plants and weakening their immune system (Tyszkiewicz et al. 2019).

Since the phosphorus deposits around the world are getting depleted, estimated to last for 60–90 years (Steen 1998, Li et al. 2019), the search for other sources of the element continues. One of the options is the use of waste, i.e., sewage sludge or animal manure, which, after processing, can offer an alternative to phosphorus compounds obtained from natural rocks. Chemically, phosphorus is an element available for plants only from the direct vicinity of their roots, the effectiveness of its uptake by plants depending on the pH value of the soil as well as the temperature. The highest amount of its active form, i.e., bioavailable phosphorus, is observed in soils of pH of 5.5–7.0 (Bezak-Mazur and Stoińska 2013). Bioavailable phosphorus occurs in the form of hydrogen phosphate anions ($H_2PO_4^-$, HPO_4^{2-}, respectively), which are directly absorbed by the roots (Syers et al. 2008). At pH below 6, there is a mixture of H_3PO_4 and $H_2PO_3^-$ ions, over pH 6, phosphorus occurs predominantly as $H_2PO_4^{2-}$, and at pH over 9, only orthophosphate (V) ions PO_4^{3-} are formed (Przywara 2006).

Unfortunately, most of the phosphorus in the soil reacts with cations of other elements: Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, and

Al^{3+}, forming poorly soluble compounds, unavailable for plants (Kwaśny et al. 2011). Poultry manure as a fertilizer in agriculture offers benefits due to its significant content of organic matter, which, during composting (Hansen et al. 1989) or land application (Kanwar 2002) transforms over time into humus and significantly improves the fertility of the soil. However, the disadvantage of the composting and fertilizing processes is the release of significant amounts of harmful gases, such as ammonia (NH_3) or hydrogen sulphide (H_2S) (Ghaly and Singh 1991; Baki Unal et al. 2015).

It is estimated that the world production of chickens in poultry farms generates 2.1×10^9 kg NH_3 annually (Abustan et al. 2019), resulting from the breakdown of urea and uric acid in the manure (Ngili 2009). Moreover, fresh feces can emit odors, a product of decomposing organic matter and a source of pathogens, which cause infections both in humans and animals (Ghaly and Alhattab 2013). The literature data (Bicudo and Goyal 2003; Chen and Jiang 2014) indicate clearly that microbial concentrations in chicken manure can reach up to 10^{10} cfu/g. In order to minimize the epidemiological risk and preserve the valuable nutrients, it is necessary to perform proper hygienization of poultry manure processes. One of the simplest methods of hygienizing organic waste is the chemical treatment, i.e., modifying it by applying calcium compounds, such as quicklime (CaO) or hydrated lime ($Ca(OH)_2$). Such waste materials as limestone powder, dolomites, fly ash from brown coal, and dust from lime or cement kiln are applied much more seldom (Bożym 2010). Quicklime is highly reactive and forms $Ca(OH)_2$ in contact with water (Budavari 1996). The reaction (1) is highly exothermic produces a significant amount of heat (Henrickson 2005).

$$CaO + H_2O \rightarrow Ca(OH)_2 + 81.4\ kJ \quad (1)$$

The literature reports different doses of calcium applied for hygienizing or stabilizing organic waste. Heinonen-Tanski et al. (2006) concluded that the concentration of calcium compounds (CaO or $Ca(OH)_2$) of 30 kg/ton is effective for solid manure. However, for complete deactivation of pathogens, parasites and their spores, the dose could be insufficient. Hence, it was assumed that for hygienizing of sewage sludge and organic fertilizers with calcium compounds, the mixture should reach the temperature of 55–70°C and the value of pH of at least 12. Basing on the experimental data, it is assumed that to reach the temperature of 50°C for organic waste containing approximately 36% dry matter (dm), it is necessary to apply 4–5% CaO, i.e., for waste containing 26% dm, it is necessary to add 5–6% CaO (Malej 2000).

After adding CaO to sludge, the strong alkalinization process occurs with ammonia emission and a decrease in the nitrogen content. The lowered content of nitrogen will result in hindering the development of the root system, which is particularly unfavorable in young plants.

Hygienization of organic fertilizers with calcium compounds eliminates pathogens but may also adversely affect the availability of nutrients for plants. In order to eliminate these disadvantages, alternative chemical compounds are suggested for performing effective deactivation of pathogens, a process environmentally friendly and providing phosphorus available to plants. The substance meeting the criteria is calcium peroxide, used commercially as a bleaching agent. In recent years there has been growing interest in percarbonates and metal peroxides, including CaO_2, applied to remove organic and inorganic contaminants from water, sewage and soil (Lu et al. 2017; Thomas et al. 2017; Thomas et al. 2016; Thomas et al. 2015). Metal peroxides, including CaO_2, show multi-directional and non-selective oxidizing properties (biocidal to many microorganisms) towards many organic and inorganic compounds resulting in, e.g., accelerating breakdown of tetracycline an antibiotic present in organic waste (Fu et al. 2018). Moreover, CaO_2 is applied for composting leaves and hay (up to 2 wt.% of the compost weight), altering anaerobic fermentation into aerobic one, as well as for seed pelleting with the agglomeration granulation method (Domaradzki et al. 2012). Additionally, it was demonstrated that CaO_2 increases access of oxygen to the plant roots, thus stimulating the development and germination of seeds. The result, depending on the type and properties of the soil, is visible for 3 to 6 months after applying CaO_2.

The research focuses into optimizing the process of hygienizing chicken manure with CaO_2, an environmentally friendly oxidant, as an alternative hygienizing agent to commonly applied CaO and $Ca(OH)_2$. The aim of the research was to determine the most favorable conditions of chicken manure hygienization with CaO_2. The Taguchi method was applied as a tool for optimizing parameters of the hygienization process. The influence of adding CaO_2 to chicken manure on the bioavailability of phosphorus to plants was also analyzed.

Materials and Methods

Material and chemical reagents

The primary sample was collected from a few prisms of fresh chicken manure in wintertime from a large industrial scale farm for laying hens, located in the Silesia region in Poland (50°11'N, 18.73°'E). The average weight of 1kg laboratory sample was prepared using split method according to PN-R-04006, 2000 through mixing and reducing the primary sample. The sample was then transported to the laboratory and stored at the temperature of 4–5°C until the tests. The raw reference sample of chicken manure used for the experiments was marked with the symbol P0.

As the biological deactivation agents (biocides), industrial quicklime (technical grade, 92.2% CaO, Trzuskawica SA, Poland), calcium peroxide (technical grade, 78.1% CaO_2, Ixper® 75C, Solvay Chemicals International S.A., Belgium) and calcium hydroxide (technical grade, >92% $Ca(OH)_2$, Brenntag Polska Sp. z o.o., Poland) were applied. In the tests, analytical grade chemical reagents and double-distilled water were used.

Analytical Procedures

The moisture content in sample P0 was determined with the weight method through drying to constant weight at the temperature of 105±2°C, according to PN-EN 15934, 2013. The total content of macronutrients (C, N, K, Ca, P and Mg) in the raw sample P0 was determined with the Wavelength-Dispersive X-ray Fluorescence (WDXRF) spectrometry method. X-ray measurements were performed with Rigaku ZSX Primus spectrometer with a rhodium target X-ray tube (max. power 4 kW). The pH-value was measured by the pH meter (CPC-411), with combination electrode IJ44AT (Elmetron, Poland).

Determination of Enterobacteriaceae count

Enterobacteriaceae count in sample P0 and the samples with a biocide was determined according to PN ISO 4832, 2007. A 10±0.01 g representative test sample of the tested material was suspended in 90 ml of a sterile saline (0.85% NaCl with 0.1% peptone), and shaken for 5 minutes. Then further decimal dilutions, from 10^{-1} to 10^{-7}, were prepared. Volume of 1 ml of each dilution was put on two sterile Petri dishes, and covered with 15 ml of the VRBL medium (Violet Red Bile Lactose Agar). The dishes were left to solidify, and incubated at the temperature of 37±1°C for 24±2 hours. After incubation, typical, purple/red colonies of at least 0.5 mm in diameter. were counted. In case of doubts, the colonies were tested with Brilliant Green Bile Lactose Broth. They were sieved into vials with the broth and incubated at the temperature of 37±1°C for 24±2 hours. Finally, the bacteria which produced acid and gas in the Durham tube (at least 1/10 of the volume) were considered to be *Enterobacteriaceae*. The equation (2) was used to evaluate the *Enterobacteriaceae (EC)* count expressed as a number of colony forming units (cfu) per 1 gram of the analyzed chicken manure (Mrozowska 1999):

$$EC = \frac{a}{b} \cdot (10^{-x})^{-1} \tag{2}$$

where:

a – number of colonies
b – volume of plated sample
10^{-x} – dilution coefficient

Phosphorus extraction and spectrophotometry

Chicken manure samples of 5±0.01g dm and 1±0.01g dm (P0) were placed in a 100 cm^3 tight containers. Then, one of three types of biocide, CaO_2, $Ca(OH)_2$ and CaO was added to the samples in concentrations of 3.5 wt.%, 7.0 wt.% or 10.5 wt.%. The samples were mixed for 2 hours with a rotary stirrer and then left for 180 hours for complete biological deactivation. The symbols and characteristics of the analyzed samples are collected in Table 1.

Table 1. Symbols and characteristics of the tested samples

Sample	Type of biocide	Amount of biocide (wt.%)
P1	CaO_2	3.5
P2	CaO_2	7.0
P3	CaO_2	10.5
P4	$Ca(OH)_2$	3.5
P5	$Ca(OH)_2$	7.0
P6	$Ca(OH)_2$	10.5
P7	CaO	3.5
P8	CaO	7.0
P9	CaO	10.5

The determination of phosphorus total in water extracts complies with the leaching procedure for solid waste materials according to standard PN-EN 12457-2, 2004. The ratio of water to solid phase was 10:1 on dry matter. The samples were mixed for 8 hours.

The value of total bioavailable phosphorus was determined with Egner-Riehm method (GOST 26208-91, 1993). The ratio of calcium lactate solution (calcium 2-hydroxypropanoate, $C_6H_{10}CaO_6$) with hydrochloric acid (HCl) to solid phase was 50:1 according to Tyszkiewicz et al. (2019). The samples were stirred for 1.5 hours.

After mixing, the samples were centrifuged (Centrifuge 5810, Eppendorf, Hamburg, Germany), and the obtained extracts were filtrated under pressure using Whatman 0.45 μm filters (GE Healthcare, USA). The content of phosphorus total in the samples was determined with the spectrophotometry, after pressure mineralization ($K_2S_2O_8$, 120°C, 30 min), with quick Nanocolor P45 tests (5–50 mg/l) and Nanocolor 500D spectrophotometer (Macherey-Nagel GmbH & Co. KG, Germany).

Optimization of the parameters for deactivation process

Procedure of the disk diffusion method (DDM)

To initially optimize the process of biological deactivation of pathogens in chicken manure and select the most effective biocide, the modified disk diffusion by the Kirby-Bauer method was used. The DDM is commonly applied to test the sensitivity of microorganisms to medications and antibiotics (Jałowiecki et al. 2016).

In a Petri dish with sterile Tryptic Soy Agar medium (TSA), 0.1 ml of 10^{-2} suspension of the material obtained through diluting 1±0.01 g of chicken manure in 99±0.5 ml solution of physiological sterile saline (0.85% NaCl with 0.1% peptone) was applied. Then, on top of an agar surface, there was placed a 16-mm-diameter sterile paper disk with a biocide suspension of CaO_2, CaO or $Ca(OH)_2$ in the concentrations of 1.5, 2.5, 3.5, 7.0 and 10.5 wt.%. The tests were repeated three times.

The dishes were incubated at the temperature of 20±1°C, for 48±2 hours. After the incubation, the diameter of the inhibition zone around the disk was measured in three points (A-C) as it is shown in Figure 1. The final result was the average of the three measurements, expressed in mm.

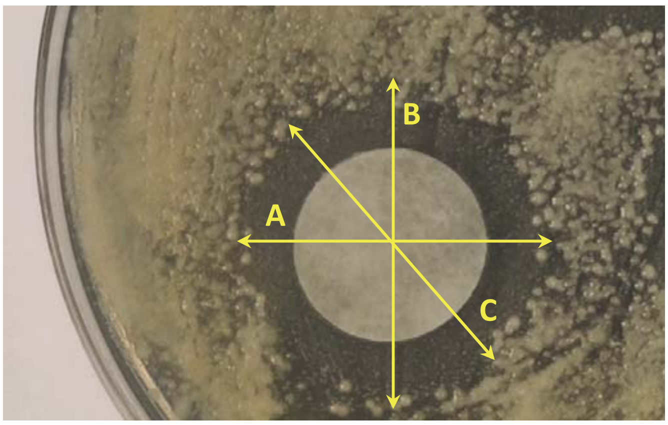

Fig. 1. Measurement of the diameter zone of inhibition around a disk with a biocide agent

Procedure of the Taguchi method (TM)

The application of the Taguchi method and Statistica 13 (Tibco Software Inc., USA) served to optimize the process of biological deactivation of organic fertilizers with CaO_2 (Rahul and Pretesh, 2018). As the criterion of the process effectiveness, the decrease in *Enterobacteriaceae* count, measured on the VRBL medium in the experimental conditions was assumed. We analyzed the influence of four input parameters (CaO_2 concentration, pH, temperature and the time of the deactivation) on the value of *Enterobacteriaceae*, cfu/g. Additionally, the influence of the number of repetitions was studied. Basing on the initial tests, associated with the determination of the zones of inhibition of microorganisms, for further analyses CaO_2 was selected and proper boundary values of the following parameters were assumed: CaO_2 concentration (3.5, 7.0 and 10.5 wt.%), pH (7.5, 9.5 and 10.5), the process temperatures (20, 30 and 40°C) and the times of deactivation (60, 120 and 180 hours). The planned tests consisting of 27 experiments (nine experimental systems in three repetitions) are presented in Table 2.

The 25±0.01 g sample was added to the amount of CaO_2 assumed in the plan of the experiment. Then, the sample was vigorously mixed, and its pH was adjusted to the assumed value with 25% H_2SO_4 (Chempur, Poland). The samples were then stored in an incubator (Zalmed, Poland), at the set temperature for the given time. Then in the tested sample the *Enterobacteriaceae* count was measured as described above. The obtained results of the experiments were analyzed statistically (ANOVA), and with the equation (3) the expected Signal to Noise ratio (S/N) as well as the dependency of the average values of S/N function on the value of input parameters (Rahul and Pretesh, 2018) were determined:

$$\frac{S}{N} = -10\ log[\frac{1}{n}\sum_{i=1}^{n} y_i]^2 \rightarrow min \qquad (3)$$

where:
i – number of measurements,
n – number of measurements for a particular process,
y – measured feature.

The smaller-the-better criterion was applied to minimize the response of unfavorable features of the product (*Enterobacteriaceae* count). It showed that the higher the sum

Table 2. The experimental conditions (factors and levels of an orthogonal array) and results of *Enterobacteriaceae* (EC) cfu/g of chicken manure

Run	Experimental conditions					Experimental results
	Repetition	CaO_2 (wt.%)	pH	Temperature (°C)	Time (h)	EC (cfu/g)
1	1	3.5	7.5	30	60	150 000
2	1	3.5	9.5	30	120	140 000
3	1	3.5	10.5	40	180	110 000
4	1	7.0	7.5	30	180	9 000
5	1	7.0	9.5	40	60	10 500
6	1	7.0	10.5	20	120	11 000
7	1	10.5	7.5	40	120	1 150
8	1	10.5	9.5	20	180	920
9	1	10.5	10.5	30	60	1 700
10	2	3.5	7.5	20	60	151 000
11	2	3.5	9.5	30	120	141 000
12	2	3.5	10.5	40	180	110 000
13	2	7.0	7.5	30	180	9 200
14	2	7.0	9.5	30	60	10 600
15	2	7.0	10.5	20	120	11 100
16	2	10.5	7.5	40	120	1 120
17	2	10.5	9.5	20	180	980
18	2	10.5	10.5	30	60	1 750
19	3	3.5	7.5	20	60	152 000
20	3	3.5	9.5	30	120	143 000
21	3	3.5	10.5	40	180	111 000
22	3	7.0	7.5	30	180	9 300
23	3	7.0	9.5	40	60	10 600
24	3	7.0	10.5	20	120	11 200
25	3	10.5	7.5	40	120	1 160
26	3	10.5	9.5	20	180	940
27	3	10.5	10.5	30	60	1 790

of squares *y* (negative quality of the product), the lower the S/N coefficient.

Results and discussion

Physicochemical and microbiological analysis of organic fertilizer

The determined value of the dry matter in sample P0 was 38.32%, which is compatible with the source value presented in Table 3. According to Wieremiej (2016), the average content of dry matter, calculated on the basis of the review of literature data, is 43.00% in laying hen manure and 64.50% in broiler manure. The measured pH value of sample P0 was 5.60 and the content of P (2.01%) and other macronutrients, i.e., C (41.85%), N (5.67%), K (2.36%), Ca (2.40%), Mg (0.85%) were similar to the results obtained by other authors.

It is necessary to point out that the most favorable carbon to nitrogen ratio (C/N) for organic waste is between 20:1 and 30:1, however, in the case of manure it is usually lower and most often it is 10:1 (Sweeten and Auvermann, 1988). Basing on the analysis it was shown that for the tested chicken manure sample, the C/N ratio was lower than the value considered to be the most favorable one, amounting to 7:1.

The disk diffusion method

The size of the inhibition zone diffusing from a disk with a biocide compound enabled the selection of the type of biocide and determination of its concentration which most effectively inhibits the growth of microorganisms present in chicken manure.

The biocide on the surface of a paper disk diffused radially, forming a gradient of concentrations. Its highest concentration occurred on the edge of the disk and decreased outward. The size of the observed zone of inhibition was directly proportional to the susceptibility of bacteria to a given type of biocide. As it was forecasted, the greatest biocidal effect towards microorganisms in chicken manure was observed when paper disk with CaO_2 suspension was applied. The diameter of the zone of inhibition increased together with the biocide concentration on the disk, presenting the following average values: 17.0 mm (2.5 wt.%), 20.5 mm (3.5 wt.%), 25.2 mm (7 wt.%) and 27.3 mm (10.5 wt.%). The obtained experimental results are presented in Table 4 and Figure 2. As the use of CaO_2 at the concentrations of 1.5 wt.% and 2.5 wt.% showed no results, further analyses were conducted for the CaO_2 concentration range of 3.5–10.5 wt.%. For the other tested compounds, $Ca(OH)_2$ and CaO, no significant zone of inhibition was observed, which could be associated with either lack of diffusion of the biocide around the biocide disk or their negligible biocidal effect in the experimental conditions. The biocidal effect of $Ca(OH)_2$ and CaO results from an increase in pH and the temperature when the substances react with water. Under the influence of high pH >12, changes in the structure of proteins of the microorganisms as well as loss of their enzyme activity are observed (Walawska et al. 2017).

Calcium peroxide is an inorganic compound in the form of white powder, insoluble in water. The observed zone of inhibition, for CaO_2, is associated with the diffusion of active oxygen released during hydrolysis (4), which has oxidizing and disinfecting properties.

$$2CaO_2 + 2H_2O \rightarrow 2Ca(OH)_2 + O_2 \tag{4}$$

Table 3. Macronutrient concentration and the estimated amount of microbial count in sample P0 and reference's values

Parameter	Amount		References
	P0 sample	Source value	
Dry Matter (%)	38.32	30.00–57.60	(Nicholson et al. 1999) (Myszograj and Puchalska 2012) (Wiedemann et al. 2008) (Wieremiej 2016)
pH	5.60	6.00–8.80	(Wiedemann et al. 2008)
C (% dm)	41.85	29.00	(Wiedemann et al. 2008)
N (% dm)	5.67	1.40–5.50	(Myszograj and Puchalska 2012) (Bolan et al. 2010) (Williams et al. 1999)
K (% dm)	2.36	0.60–2.10	(Myszograj and Puchalska 2012) (Bolan et al. 2010) (Williams et al. 1999)
Ca (% dm)	2.40	0.40–3.90	(Bolan et al. 2010) (Williams et al. 1999)
P (% dm)	2.01	0.70–2.50	(Myszograj and Puchalska 2012) (Bolan et al. 2010) (Williams et al. 1999)
Mg (% dm)	0.85	0.30–0.60	(Bolan et al. 2010) (Williams et al. 1999)
*EC** (cfu/g)	3.9×10^6	10^5–10^7	(Moraru et al. 2012) (Maguire 2012)

* EC– *Enterobacteriaceae* count

According to the literature data, the purity of commercial grade of CaO_2 is 60–75%, whereas the content of active oxygen is 14–17% (Ropp 2013). Following an alternative chemical reaction equation (4), CaO_2, under influence of water, breaks down and releases dihydrogen dioxide (5), which, in turn, is a source of free hydroxyl radicals •OH (6):

$$CaO_2 + 2H_2O \rightarrow Ca(OH)_2 + H_2O_2 \quad (5)$$

$$H_2O \rightarrow HO\bullet + HO\bullet \quad (6)$$

Hydroxyl radical, •OH, is one of the strongest oxidizers, capable of instant reaction with many organic and inorganic compounds and can, therefore, deactivate microorganisms and break down antibiotics in chicken manure (Turek-Szytow et al. 2020). The maximal stoichiometric amount of H_2O_2 formed through CaO_2 breakdown is 0.47 g H_2O_2 per 1 g of CaO_2 (Janda and Marcinkowski 2019).

Taguchi method

Following the planned experiments, *Enterobacteriaceae* count was determined in chicken manure samples deactivated with CaO_2 at different configurations of all applied parameters: concentrations of the applied biocide, pH ranges, temperatures, and times of the experiment. The final results were calculated with equation (2) and presented in Table 2. The best result was obtained applying a combination of input parameters: CaO_2=10.5 wt.%, pH=9.5, T=20°C and t=180 hrs. The number of *Enterobacteriaceae* colonies, for experiments nos. 8, 17 and 26 was respectively 920, 980 and 940 cfu/g and was lower than 1000 cfu/g, i.e., the maximum permissible limits of *Enterobacteriaceae* in organic fertilizers, specified both by Polish legal regulations (Ministry of Agriculture and Rural Development 2008) and the current European Union regulation (European Commission 2002).

Additionally, it was observed that at constant CaO_2 concentration, *Enterobacteriaceae* count decreased as the value of pH and the time of the experiment increased. The obtained results confirm the initial experiments presented in Section 3.2, according to which the best biocidal effect in sample P0 was observed for CaO_2 concentration of 10.5 wt.%.

To analyze the influence of the independent variables (CaO_2, pH, temperature, time) on the dependent variable of *Enterobacteriaceae* count, an analysis of variance (ANOVA) was performed. The analysis confirmed that the values of *Enterobacteriaceae* count differed in consecutive repetitions, i.e., the parameter of "repetition" was statistically significant ($p<0.05$). The results of the ANOVA test are presented in Table 5.

The next stage served to analyze the number of defects, following the equation (2) as well as the smaller-the-better criterion for which the calculated value of S/N was 58.9189. The analysis presented in Table 6 shows that the main parameter is CaO_2, followed by the time of the experiment,

Table 4. Measured zone of inhibition in chicken manure with biocidal compounds

Biocide, %		Diameter of zone* (mm) according to Fig. 1									
		A			B			C			Total
CaO_2	1.5	17	16	17	16	17	17	17	17	16	16.5±0.5
CaO_2	2.5	18	17	17	17	18	16	17	17	16	17.0±1.0
CaO_2	3.5	20	19	21	22	20	21	19	21	22	20.5±1.5
CaO_2	7.0	24	26	25	25	24	25	27	25	26	25.5±1.5
CaO_2	10.5	26	27	28	27	27	29	27	27	28	27.5±1.5
$Ca(OH)_2$	3.5	16	16	16	16	16	16	16	16	16	16.0±0.0
$Ca(OH)_2$	7.0	16	17	16	16	17	16	16	16	16	16.5±0.5
$Ca(OH)_2$	10.5	17	16	17	16	16	17	17	16	17	16.5±0.5
CaO	3.5	16	16	16	16	16	16	16	16	16	16.0±0.0
CaO	7.0	16	16	16	16	16	16	16	16	16	16.0±0.0
CaO	10.5	16	16	17	16	16	16	17	16	16	16.5±0.5

* diameter of the disk paper – 16 mm

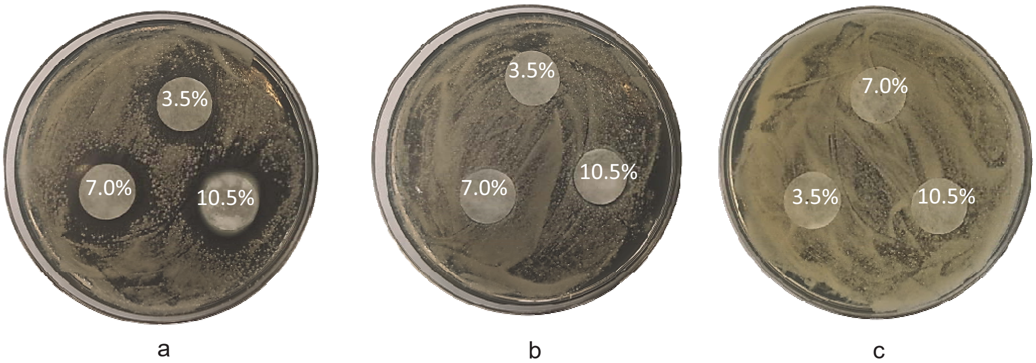

Fig. 2. Results of inhibition zone of total bacteria in chicken manure by using disks with CaO_2 (a), $Ca(OH)_2$ (b) and CaO (c)

while the temperature and pH play a less significant role in the analyzed process. The optimal conditions for the process leading to the lowest *Enterobacteriaceae* count, were created with the configuration of parameters of CaO_2=10.5wt.%, pH=9.5, T=40°C and t=180 hrs.

During the last stage of the TM analysis, the results were verified with three repetitions for such a combination of the input parameters in which the function of S/N criterion assumed the maximal values, i.e., CaO_2=10.5 wt.%, pH=9.5, T=40°C, t=180 hrs. enabling the reduction of *Enterobacteriaceae* count to 890 cfu/g. The dependency of the value of the S/N criterion function on the analyzed parameters is presented in Figure 3. The repetition parameter does not materially affect the criterion function.

Table 5. Analysis of the experiment (ANOVA) with Statistica 13

Effect/Factor	Analysis of variance, Mean=-81.510, Sigma=17.0022				
	SS	**df**	**MS**	**F**	**p**
Repetition	0.148	2	0.074	4.8	< 0.05
CaO_2 (wt.%)	7 454.149	2	3 727.075	242 626.8	< 0.05
pH	6.920	2	3.460	225.2	< 0.05
Temperature (°C)	10.969	2	5.485	357.0	< 0.05
Time (hrs)	43.519	2	21.759	1 416.5	< 0.05
Error	0.246	16	0.015	–	–

Mean – mean of S/N-value, Sigma – population standard deviation, SS – predicted residual error sum of squares, MS – mean square error, F – statistics, df – number of degrees of freedom, p – statistically significant if p<0.05

Table 6. S/N ratio in optimal conditions

Effect/Factor	Predicted S/N ratio in optimal conditions, Mean=-81.510, Sigma=17.0022		
	Parameter value	**Significance of effect**	**Standard error**
Repetition	1	0.0983	0.041314
CaO_2	3 (10.5 wt.%)	19.6683	0.041314
pH	2 (pH=9.5)	0.5088	0.041314
Temperature	3 (T=40°C)	0.6785	0.041314
Time	3 (t=180hrs)	1.6374	0.041314
Expected S/N ratio	–	-58.9189	–

Mean – mean of S/N-value, Sigma – population standard deviation

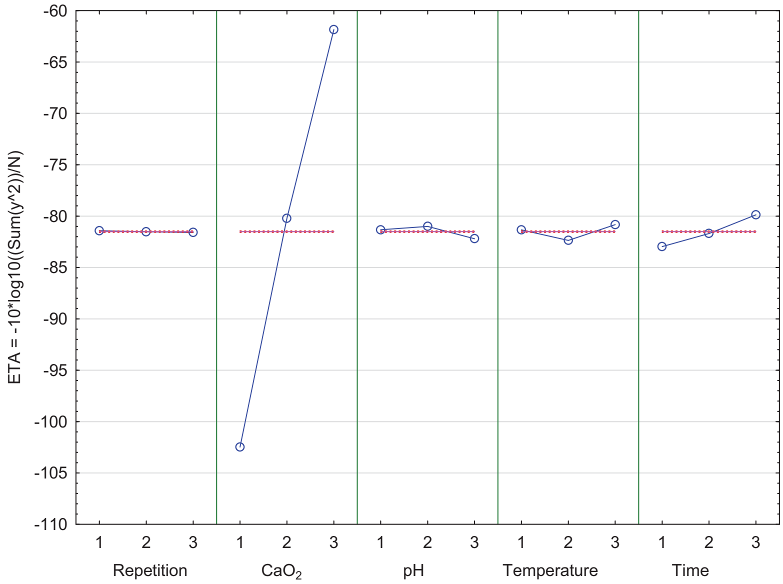

Fig. 3. The values of the S/N function versus input parameters values plot for *Enterobacteriaceae* for CaO_2=3.5 wt.% (1), 7.0 wt.% (2), 10.5 wt.% (3), pH=7.5 (1), 9.5 (2), 10.5 (3); Temperature=20°C (1), 30°C (2), 40°C (3); time=60 h (1), 120 h (2), 180 h (3). The dotted lines mean ± 2×MS error

In terms of pH and temperature values, the obtained results correspond with the data in the literature where a decrease in bacteria count by adding calcium compounds is associated with the increase of pH to 12 and of temperature to at least 50°C (Malej 2000; Walawska et al. 2007). The application of calcium peroxide causes a decrease in the values of pH and temperature, i.e. the content of nutrients (N, P) in manure after deactivation ought to be higher than in the case of CaO and $Ca(OH)_2$. The literature offers a few publications which confirm lowering bacteria count in biowaste after the addition of calcium compounds. The total count of microorganisms in fresh sludge treated with 20% quicklime after 7 days of deactivation decreased from $8.5 \cdot 10^9$ cfu/g to $8.2 \cdot 10^7$ cfu/g, and the reduction in *E. coli* (*Enterobacteriaceae* family) count from $2.0 \cdot 10^3$ cfu/g to zero (Popova et al. 2014). Maguire et al. (2006) showed that adding calcium oxide to broiler manure and laying hen manure decreased the total microbial counts by more than 99%, the results depending on the moisture content and CaO concentration. So far there has been no research into the optimization of the parameters of decreasing *Enterobacteriaceae* count in organic fertilizers with CaO_2. Nevertheless, it has been proven that, due to its strong oxidizing properties, the compound may be applied to lower the microorganism count in manure and biowaste. The effect of the addition of CaO_2 (2 g per 1 m^2 of poultry house floor area) to poultry manure on the physicochemical and microbiological parameters was studied by Mituniewicz et al. (2016). The results showed that the addition of calcium peroxide to poultry manure has no negative impact on the examined parameters and also stabilizes the manure microflora.

According to Qu et al. (2018), the addition of CaO_2 combined with CaO to cattle manure caused rapid biological drying of waste, shortened the composting time and allowed the water content decrease to 23.5%. The antimicrobial activity of calcium peroxide was studied also by Sladdin and Lynch (1983). Carbon peroxide inhibited the growth of fungi and bacteria in liquid shaken cultures. Similar amounts of $Ca(OH)_2$ alone were not effective against spore germination.

Influence of biocide addition on the content of phosphorus

The results of pH value measurement for sample P0 and for the samples P1-P9 (Table 2) with the addition of biological deactivated agents, i.e., CaO_2 (P1-P3) $Ca(OH)_2$ (P4-P6) i CaO (P7-P9) are shown in Figure 4.

For the samples P1, P4 and P7 with 3.5 wt.% of biocide agent, the pH value was 7 and comparable with pH of sample P0 (pH=6). When the content of CaO_2, $Ca(OH)_2$ or CaO in chicken manure increased, pH amounted to: pH=9.0 (P2); pH=10.5 (P5), pH=9.5 (P8) for 7.0 wt.% biocide and to pH=10.5 (P3); pH=12.0 (P6), pH=11.0 (P9) for 10.5 wt.% biocide.

Near-neutral pH value enables a decrease in the dynamics of the CaO_2 breakdown compared with CaO and $Ca(OH)_2$, and thus prolongs the oxidation process. The research conducted by Northup and Cassidy (2008) confirms that the rate of CaO_2 dissolving in reaction with water and the amount of produced H_2O_2 increase along with the decrease of pH value: 82% (pH=6), 47% (pH=9) and 0% (pH=12–13). Complete dissolution of CaO_2 at pH of 12–13, requires 62 hours; and at pH=6 only 4 hours. The increase in pH in sample P3 caused an increase in the concentration of $Ca(OH)_2$, an alkali product of the reaction (5) (Walawska et al. 2007).

The results of total phosphorus (mg/l) in the samples P1-P9 which have been leached in water and calcium lactate solution are presented in Figure 5. The results related to weight of 1 g of raw sample.

The lowest concentrations of phosphorus in water extracts of chicken manure were obtained for the samples P1, P2 and P3 (i.e. 5.91 mg/l, 4.32 mg/l and 1.56 mg/l). For the other biological compounds, $Ca(OH)_2$ and CaO, the concentration of phosphorus in water solutions amounted to 11.25 mg/l (P4) and 11.38 mg/l (P7) for 3.5 wt.% of biocide; 7.78 mg/l (P5) and 9.34 mg/l (P8) for 7.0 wt.% of biocide; 4,49 mg/l (P6) and 6.43 mg/l (P9) for 10.5 wt.% of biocide respectively. The concentration of phosphorus in water extract for the raw sample P0 was 51.15 mg/l.

Basing on the conducted tests, it may be definitely concluded that the content of phosphorus in water solutions

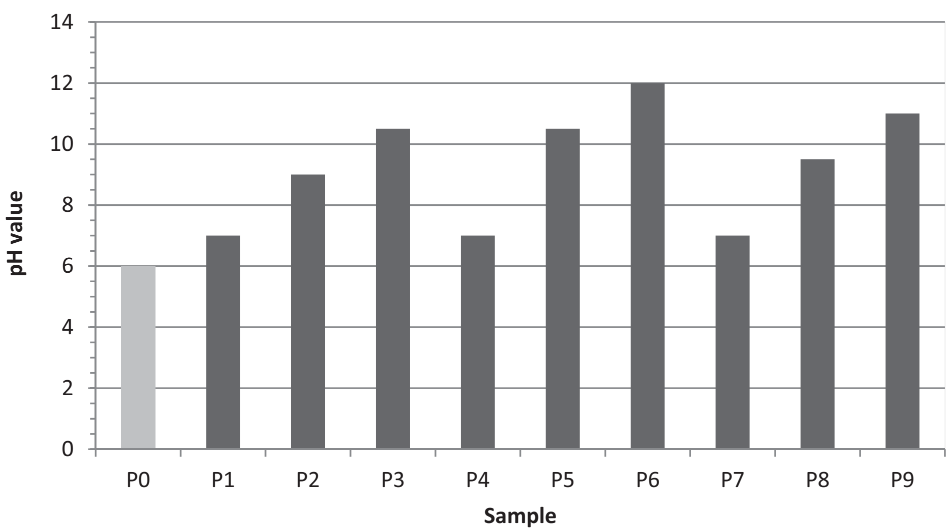

Fig. 4. Determination of the pH value for samples with the addition of biocides: CaO_2 (P1-P3), $Ca(OH)_2$ (P4-P6) and CaO (P7-P9)

decreases with the amount of applied biocide. The higher concentration of calcium compounds, the higher pH of the solution and the lower concentration of soluble phosphorus. It was observed that the amount of phosphorus in the aqueous extract for the samples with CaO_2 (P1-P3 in water extracts) was lower than in other samples. It may be associated with the formation of an insoluble crystalline compound of hydroxylapatite ($Ca_5(PO_4)_3OH$) following the reaction (7). When calcium peroxide dissolves in water, it can remove inorganic phosphate ions by forming an insoluble precipitate as follows (Cho and Lee 2002):

$$20\ CaO_2 + 12\ PO_4^{3-} + 2\ H_2O \rightarrow \rightarrow 4\ Ca_5(PO_4)_3(OH)\downarrow + 19\ O_2 \quad (7)$$

The bioavailable phosphorus content in chicken manure was measured in calcium lactate extracts using the Egner--Riehm method, applied in Poland to assess the content of available phosphorus in soil (Tyszkiewicz et al. 2019). The analysis proved that the content of bioavailable phosphorus in the samples with CaO_2 added was higher than in those with $Ca(OH)_2$ and amounted to 24.60 mg/l (P1) and 14.86 mg/l (P4) for 3.5 wt.% of biocide; 13.27 mg/l (P2) and 7.50 mg/l (P5) for 7.0 wt.% of biocide; 3.25 mg/l (P3) and 1.45 mg/l (P6) for 10.5 wt.% of biocide respectively. The concentration of bioavailable phosphorus in the extract of sample P0 was 78.25 mg/l. In the case of CaO application, the content of phosphorus in the samples was higher than in the remaining ones and amounted to 18.18 mg/l (P7), 13.20 mg/l (P8) and 9.70 mg/l (P9) respectively.

Nevertheless, the preliminary tests of the growth of the inhibition zone of the general number of microorganisms (Table 4 and Figure 1) proved CaO to have the weakest biocidal effect.

The collected literature data on the influence of calcium compounds on the solubility of phosphorus in biowaste concern CaO and $Ca(OH)_2$. So far research confirms that adding calcium compounds to animal manure results in the lowering the phosphorus solubility. According to Faridullah et al. (2009) incubating chicken biowaste with 5% CaO after eight weeks resulted in reducing phosphorus solubility by 26% for broiler and 23% for laying hen manure. According to Maguire et al. (2006) the concentration of bioavailable phosphorus in poultry manure decreased as the doses of calcium compounds, CaO and $Ca(OH)_2$, increased. It was observed that the most effective was the addition of CaO at 10 wt.% concentration. The authors proved that, when calcium is added to the manure, the proportions of the crystalline phase change into poorly soluble hydroxyapatite forms contributing to a decrease in water solubility of phosphorus in the limed samples.

It is also necessary to mention that an addition of CaO_2 favorably affects the process of soil bioremediation (López and Mueller 2009; Małachowska-Jutsz et al. 2014). Hydrogen peroxide from CaO_2 breaks down and becomes a source of active oxygen which is partly consumed by microorganisms and partly used for oxidizing organic waste in soil, i.e. aromatic hydrocarbons.

Conclusions

The studies show the possibility of applying CaO_2 for hygienization process of chicken manure as an alternative to commonly used calcium compounds. The biocidal effect of CaO_2 is associated with releasing active oxygen which makes it the ecologically friendly compound. The significant zone diameter for the inhibition of microorganisms was observed for 3.5 wt.% CaO_2 concentration. The results were verified with the Taguchi method to optimize the hygienization process within four parameters, i.e., CaO_2 amount, pH, temperature and the time of biological deactivation. The application of CaO_2 for hygienizing chicken manure enables effective reduction of *Enterobacteriaceae* count to an acceptable level, i.e. below 1000 cfu/g. The most favorable set of parameters was obtained for CaO_2 = 10.5 wt.%, pH = 9.5, temperature = 30°C, and time =180 hours.

The assessment of the content of bioavailable phosphorus, using Egner-Rhiem method, showed that phosphorus amount, in obtained extracts, was decreasing along with the higher concentration of added biocides. The tested extracts of chicken manure with CaO_2, at the concentration of 3.5–10.5 wt.%, had a higher content of phosphorus (24.60–3.25 mg/l) than the

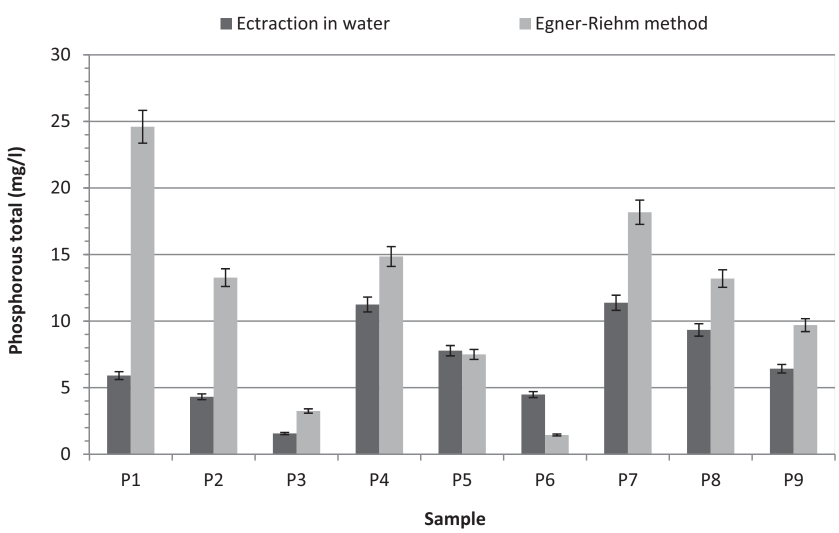

Fig. 5. Determination of the phosphorus total (mg/l) in water extracts of samples with the addition of biocides: CaO_2 (P1-P3), $Ca(OH)_2$ (P4-P6) and CaO (P7-P9). Average values (SD) ±5.0%

samples deactivated with $Ca(OH)_2$ 14.86–1.45 mg/l. The results of the study allow for the development of a hygienization technology for chicken manure for a safe use in agriculture. The economic aspect must also be considered; based on the current market prices, the costs of 1 ton of CaO_2 and $Ca(OH)_2$ are estimated at $700 and $200 respectively. Despite its high price, the latter is commonly applied in the soil and water bioremediation processes, hence it may be assumed that its application in hygienization of organic fertilizers may generate additional benefits.

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Wykorzystanie nadtlenku wapnia jako przyjaznego dla środowiska utleniacza do redukcji patogenów w nawozach organicznych i jego wpływ na biodostępność fosforu

Streszczenie: W artykule przedstawiono badania procesu higienizacji pomiotu kurzego przy użyciu nadtlenku wapnia (CaO_2) jako przyjaznego dla środowiska biologicznego środka dezaktywującego. Przeanalizowano również wpływ dodatku CaO_2 do obornika kurzego na biodostępność fosforu. Proces biologicznej dezaktywacji z użyciem CaO_2, CaO and $Ca(OH)_2$ zbadano metodą dyfuzyjno-krążkową. Dla optymalizacji wpływu parametrów procesu higienizacji (stężenie CaO_2, pH, temperatura i czas) na zmniejszenie liczby bakterii Enterobacteriaceae zastosowano metodę Taguchi. Zawartość fosforu biodostępnego zmierzono metodą Egnera-Riehma i oznaczono spektrofotometrycznie. Obniżenie liczby bakterii następowało ze wzrostem stężenia CaO_2 w próbce. Najbardziej optymalne parametry procesu higienizacji, tj.: CaO_2=10.5 wt.%, pH=9.5, T=40°C, t=180 h pozwoliły na zmniejszenie liczby bakterii Enterobacteriaceae z 10^7 cfu/g do 10^2 cfu/g. Analiza próbek metodą Egnera-Riehma wykazała, że zawartość fosforu biodostępnego w ekstraktach obornika kurzego (mg/l) malała wraz ze wzrostem ilości dodanego CaO_2, tj.: od 26,6 mg/l przy 3,5% wag. do 3,5 mg/l przy 10 %wag. Wartości te były nieznacznie wyższe w porównaniu próbkami dezaktywowanymi $Ca(OH)_2$, tj: od 11,25 mg/l przy 3,5 %wag; do 4,49 mg/l przy 10 %wag. Zastosowanie CaO_2 do celów higienizacji pomiotu kurzego pozwoliło na efektywną redukcję bakterii *Enterobacteriaceae* do poziomu dopuszczalnego (poniżej 1000 jtk/g). W porównaniu do tradycyjnie stosowanych technik higienizacji wapnem, użycie CaO_2 wpływa korzystnie na odzysk fosforu biodostępnego.