Effect of lead and chloride ions on methane production in arable soils

Ewa Wnuk , *Anna Walkiewicz** *and Andrzej Bieganowski*

Institute of Agrophysics, Polish Academy of Sciences, Department of Natural Environment Biogeochemistry
Doświadczalna 4, 20-290 Lublin, Poland

Abstract. Cultivated soils in high water conditions can be a source of methane (CH_4). Despite the significant introduction of lead (Pb) into soils with fertilizers or sewage sludge, there are few reports concerning its impact on methane production in arable soils. The main premise of the study was to characterize the response of methanogenesis after soil contamination with Pb. For this reason, the effect of Pb on CH_4 production in three different mineral arable soils was investigated. Lead, in the chloride form, was added at two concentrations, which were established based on the Sewage Sludge Directive (300 and 1 500 mg kg^{-1}). Additionally, two types of controls were used – water and $CaCl_2$ with chloride ions. It was observed that the process could be slowed down at the lower dose and that methane production was totally inhibited at the higher dose. Additionally, the inhibitory effect of the chloride ions on the process was also observed in the control samples. Despite the inhibition of methanogenesis in the soil, which has a positive effect on reducing the amount of the gas released, this process cannot be analysed individually. Other reactions in the soil should also be taken into consideration, and these changes which occur under the influence of the various factors should be investigated further.

Keywords: methane, methanogenesis, arable soil, lead

INTRODUCTION

The global warming effect is a very serious problem at the present time (Fronzek *et al*., 2018; VijayaVenkataRaman *et al*., 2012). The increasing temperature of our planet is causing many human tragedies and it has global consequences such as the warming of the oceans, the melting of glaciers and the extinction of numerous animal species (Kirschke *et al*., 2013). The main reason for this situation is the increasing atmospheric concentration of greenhouse gases (GHGs), including methane (CH_4). After carbon dioxide (CO_2),

methane is the second GHG responsible for global warming, despite having a much lower atmospheric content than CO_2. The CH_4 concentration has more than doubled over the last 200 years and is now at about 1.8 ppm (Brzezińska *et al*., 2012; IPCC, 2014). This increase has been caused by increased emissions of methane from anthropogenic sources, such as rice production, the use of fossil fuels and the utilization of wastes, but also by an imbalance between the methane emitted and that absorbed by sinks, including soils (Contin *et al*., 2012; Nosalewicz *et al*., 2011; Stępniewska *et al*., 2018). Measurements carried out over the years show that the total emission of methane to the atmosphere (~558 Tg year^{-1}) is greater than the total amount of methane absorption (~548 Tg year^{-1}), leading to the continuous accumulation of this gas in the atmosphere (Saunois *et al*., 2016).

Soil is a unique environment, which can act as a CH_4 sink or source, depending on the prevailing conditions. Under aerobic conditions we may observe the activity of methane-oxidizing bacteria (methanotrophs), which naturally colonize soil (Szafranek-Nakonieczna *et al*., 2018). These absorb CH_4 from the environment and then use it as a source of the carbon and energy necessary for their survival (Bodelier and Steenbergh, 2014; Einola *et al*., 2007). Under anaerobic conditions, e.g., in flooded soils, the process of methane production is observed (methanogenesis). Methanogens use carbon from, e.g., methanol or formic acid to produce methane (Malyan *et al*., 2016; Serrano-Silva *et al*., 2014; Szafranek-Nakonieczna and Stępniewska, 2015). As a consequence, soil plays a crucial role in the final atmospheric CH_4 concentration. The amount of methane emitted by soils accounts for approximately 40% of the

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*Corresponding author e-mail: a.walkiewicz@ipan.lublin.pl

total emissions of this gas (Saunois *et al.*, 2016). The processes of CH_4 production and consumption are regulated by many chemical and physical soil parameters, such as pH, moisture, texture and nitrogen content (Majdinasab and Yuan, 2017; Tate, 2015). The common factors in arable soils are heavy metals, which may come from a parent material or may be introduced into soils via fertilizers or the sewage sludge used in agriculture (Pawłowska *et al.*, 2011). One of the most common soil contaminants is lead (Pb). Generally, the Pb concentration in soil depends on the country and varies from 18 to 27 mg kg^{-1}. In addition to agricultural origins, it can also be present in the soil as a side effect of industrial activity (mining, smelting and the metal processing industry) (Kuźniar *et al.*, 2018; Sankhla *et al.*, 2016). The natural content is also determined by the parent rock (Kabata-Pendias, 2010).

The mobility of heavy metals in the soil and the ecological effects of their presence depends on various factors, such as pH, temperature, particle-size distribution, water-air conditions in the soil, pore structure, fertilization or soil organic matter (Łukowski and Wiater, 2016; Sherene, 2010), and also on physicochemical processes, e.g., sorption/desorption, solution complexation, oxidation-reduction and precipitation-dissolution reactions (Caporale and Violante, 2016). Due to the low solubility of Pb and its high affinity for absorption, Pb accumulates at the soil surface.

Unlike some heavy metals, Pb has no essential function in the life cycles of organisms. It only has a negative influence on living organisms. Depending on the concentration of the heavy metal, it can behave as an activator, a stimulator or a poisonous substance (Kugelman and Chin, 1971). It is well known that heavy metals such as Pb, in higher concentrations, may cause cell-membrane disruption, the inhibition of cell division and enzyme activity as well as protein denaturation. On the other hand, the presence of heavy metals in the environment may change the properties or activate components present in the environment, which can also have an effect, negative or positive, on the activity of microorganisms and thus on the final result of the biological process (Abdu *et al.*, 2016; Blagodatskaya *et al.*, 2006). In addition, a reduction in soil enzyme activity (dehydrogenase, urease) has been observed in the presence of high levels of Pb contamination (Furtak and Gajda, 2017; Pająk *et al.*, 2016).

Although methane may be produced in arable soil in anaerobic zones (e.g. as a result of flooding or rainy periods), the issue of the influence of Pb on methanogenesis in the temperate zone has been poorly investigated. We were able to find only one study in which the authors had determined the impact of Pb on methane production. However, this research was carried out on tropical rice soils with the addition of only one dose of lead salt (Mishra *et al.*, 1999). Therefore, the aim of this study was to investigate methanogenesis in arable soils contaminated with Pb doses at the maximum permitted level and at a dose far exceeding this limit.

MATERIALS AND METHODS

Three mineral arable soils were used: Eutric Cambisol, Haplic Podzol and Mollic Gleysol. They were collected from a depth of 0-20 cm, air-dried and sieved to <2 mm. The soils were characterized by similar air-water conditions, as a result of their similar textures (sandy loam and loamy sand). The main difference between the soils was the C_{org} content (Walkiewicz *et al.*, 2018; Wnuk *et al.*, 2017). The basic properties of the tested soils are given in Table 1.

The particle-size distribution (PSD) was measured for texture determination using the laser diffraction method and a Mastersizer 2000 (Malvern, UK) with a Hydro 2000G dispersion unit (Bieganowski *et al.*, 2018). Organic and dissolved carbon contents were determined using a TOC-VCPH analyser (Shimadzu, Japan). The soil pH was determined potentiometrically in 1 mol KCl and in H_2O (1:2.5 v/v) at room temperature, after a 24 hour period of stabilization. The measurement was made using an HQ40D portable multimeter (Hach Lange). The pH values obtained are presented in Table 2. The Pb contents in the tested soils were determined by ICP-OES (inductively coupled plasma optical emission spectrometry) using an iCAP 6500 Series apparatus from Thermo Fisher Scientific, with a charge injection device (CID) detector (Kitowski *et al.*, 2014). The phosphorus content was measured according to the PN-R-04024:1997 standard, using the colorimetric method.

Table 1. The basic properties of investigated soils

Soil	Particle-size distribution			Native Pb content (mg kg^{-1})	C_{org} (%)	DOC (mg dm^{-3})	NH_4^+	P	Initial Cl
	Sand	Clay	Silt						
	(%)						(mg kg^{-1})		
Eutric Cambisol	71.6	25.1	3.23	6.87	1.18	63.76	4.20	180	2.2
Haplic Podzol	74.6	22.3	3.04	2.32	0.43	61.05	0.49	300	6.3
Mollic Gleysol	74.8	21.7	3.93	7.49	3.93	65.7	2.84	297	8.6

Table 2. Values of pH in the tested soils (Eutric Cambisol, Haplic Podzol, Mollic Gleysol) before and after incubation for methane production

Type of soil	pH						
	Before incubation		After incubation				
	H_2O	KCl	H_2O	$CaCl_2$	$CaCl_2$x5	Pb	Pbx5
E. Cambisol	6.47	6.38	6.79	6.39	5.93	5.94	3.95
H. Podzol	6.56	6.5	6.78	6.41	6.22	6.12	4.3
M. Gleysol	6.72	7.71	7.17	6.99	6.7	6.71	5.4

The CH_4 concentration was measured chromatographically every week or every two weeks, for 160 days. For the measurements, a Shimadzu GC-2014 (Japan) gas chromatograph with an AOC-5000 auto injector was used, it was equipped with a flame ionization detector (FID) for CH_4. Helium (He) was used as a carrier gas at a rate of 33 cm^3 min^{-1}. The column temperature was 30 and 150°C.

The measurements were based on the CH_4 production (methanogenesis) in the headspace of the soil sample contaminated with Pb, during the allotted incubation time. Pb was added (in the form of $PbCl_2$) in amounts determined by the limit values established by the Sewage Sludge Directive (86/278/EEC). Lead was added in two doses – the maximum Pb concentration permitted in arable soils (denoted Pb (300 mg kg^{-1})) and a five-fold higher value (denoted Pbx5 (1.500 mg kg^{-1})). Two types of controls were used: H_2O and $CaCl_2$. $CaCl_2$ was added in an amount providing the same concentration of chloride ions as was obtained in the tested samples (Wnuk *et al*., 2017). The calcium chloride control was used to observe the influence of chloride ions on the process of methane production in the soil.

For the experiments, 10 g of air-dried soil samples were used. Samples were flooded with 5 ml of heavy-metal solution, or H_2O or $CaCl_2$ solution for the control samples. Then, all of the bottles were tightly closed with rubber stoppers and aluminium caps. In order to obtain the anaerobic conditions necessary for the CH_4 production process, the headspace of each sample was purged with pure nitrogen. The samples were incubated in 120 cm^3 glass bottles in the dark at 25°C. Three independent replicates were used for each treatment. On the last day of incubation, the bottles were opened, and the pH was measured.

Based on the results obtained, the methane production rates were calculated by subtracting the final gas concentration from the initial concentration and dividing by the number of incubation days. The results were statistically analysed using a non-parametric Kruskal-Wallis test (Statistica 10 software) to determine any significant differences.

RESULTS

The increases in methane concentration in the tested samples during the incubation time are shown in Fig. 1 and the methane production rates are shown in Table 3.

By analysing the process of methane production in the soil, three phases can be identified (Fig. 1): i) the lag phase was the same in all experiments, as there was no methane production in this time interval; ii) phase I – when a rapid increase in the concentration of the gas produced was observed, and iii) phase II – when production slowed down. The confirmation necessary to distinguish between phases I and II was the statistically significant differences in CH_4 production in all of the investigated variants: both the lead-contaminated and the control cases.

The lag phase was the same in all soils and was equal to 14 days. After that time, the production of methane began. However, the efficiency of methane production was dependent on the soil and the dose of calcium chloride. While the lag phase was generally the same for all soils, the duration of the first phase of methanogenesis in Mollic Gleysol (Fig. 1c) was shorter than that in Eutric Cambisol and Haplic Podzol (Fig. 1a, b).
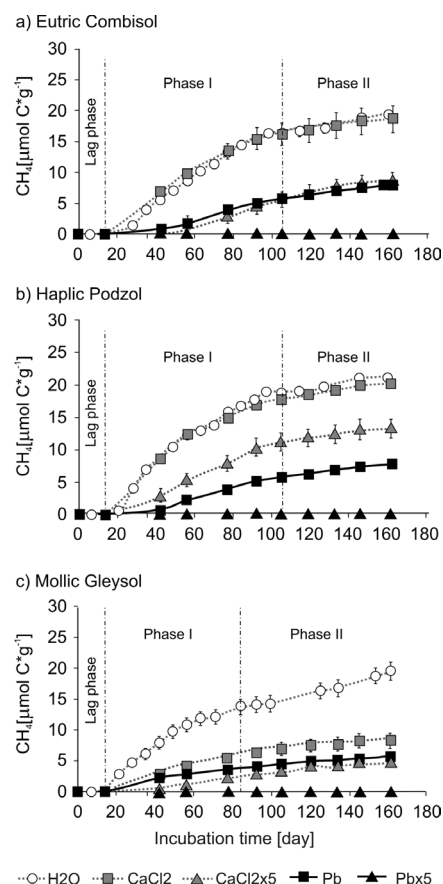
Fig. 1. Increases in the CH_4 concentration with time in the headspace of the tested soils (Eutric Cambisol, Haplic Podzol, Mollic Gleysol) in the controls (soil with water and $CaCl_2$) and in soils contaminated with Pb in two doses: the maximum Pb concentration in arable soils (Pb) and a five-fold higher value (Pbx5). As a second control, $CaCl_2$ solution was used to provide the same concentration of chloride ions corresponding to the concentration obtained in the case of lead salt in particular variants of the experiment. Points are averages of triplicate samples; bars indicate standard deviations.

The CH_4 production rates in Haplic Podzol and Eutric Cambisol were similar (Fig. 1a, b). In both cases, there were practically no differences between the controls with water and a lower dose of $CaCl_2$. Furthermore, in both cases, the emission of methane was significantly lower for a higher dose of $CaCl_2$, though for Eutric Cambisol the decrease was greater than it was for Haplic Podzol. The situation was different for Mollic Gleysol (Fig. 1c). The influence of chloride ions was much more significant in this soil, and methanogenesis was reduced even with the lower dose of $CaCl_2$. For Mollic Gleysol, the inhibitory effect of a higher dose of Cl$^-$ on CH_4 production was even greater than the effect of the lower dose of $PbCl_2$.

The influence of chloride ions on CH_4 emission is shown in Table 3. It is very clear when comparing CH_4 production rates in the first phase of the process (shown in Fig. 1). In all cases, an increase in Cl$^-$ concentration produced a decrease in methanogenesis. The rate in phase II was almost independent of the concentration of chloride

ions for Eutric Cambisol and Haplic Podzol. The situation was slightly different for Mollic Gleysol, because in this case it was difficult to identify the border between phase I and phase II. The value indicated in Fig. 1c was assumed rather arbitrarily, and this could be the reason why the value for CH_4 production in this soil in the second phase without $CaCl_2$ was higher than with the addition of $CaCl_2$ (Table 3).

Table 3. Methane production rate in the investigated soils without (controls) and with two Pb doses

Variants	Lag phase duration (days)	CH$_4$ production rate (µmol C g^{-1}d^{-1})	
		Phase I	Phase II
		Eutric Cambisol	
H$_2$O	14	0.196 ±0.011 **a**	0.063 ±0.01 **a**
CaCl$_2$	14	0.149 ±0.028 **A***	0.042 ±0.006 **A***
CaCl$_2$x5	14	0.087 ±0.019 **A***	0.044 ±0.015 **A**
Pb	14	0.078 ±0.009 **bB**	0.036 ±0.001 **bA**
Pbx5	0	0 **bB**	0 **bB**
		Haplic Podzol	
H$_2$O	14	0.196 ±0.011 **a**	0.047 ±0.004 **a**
CaCl$_2$	14	0.145 ±0.013 **A***	0.037 ±0.005 **A***
CaCl$_2$x5	14	0.135 ±0.005 **A***	0.033 ±0.002 **A***
Pb	14	0.079 ±0.004 **bB**	0.034 ±0.007 **bA**
Pbx5	0	0 **bB**	0 **bB**
		Mollic Gleysol	
H$_2$O	14	0.172 ±0.014 **a**	0.077 ±0.004 **a**
CaCl$_2$	14	0.068 ±0.013 **A***	0.029 ±0.005 **A***
CaCl$_2$x5	14	0.044 ±0.003 **A***	0.026 ±0.005 **A***
Pb	14	0.037 ±0.006 **bB**	0.022 ±0.005 **bA**
Pbx5	0	0 **bB**	0 **bB**

The same letter means no statistically significant differences among the rates; an asterisk indicates statistical differences between the control sample with H$_2$O and CaCl$_2$; small letters indicate statistical differences between the control with water and the contaminated sample; capital letters mean statistical differences between the control with CaCl$_2$ and the test sample (non-parametric Kruskal-Wallis test, p <0.05, separate for each tested soil)

The addition of chloride ions also had an influence on changes in pH in the investigated soils (Table 2). In all cases, a higher Cl$^-$ concentration caused a lower pH value. The changes varied from 0.86 units (for Eutric Cambisol) to 0.41 units (for Mollic Gleysol).

After the enrichment of the tested soils with Pb^{2+}, the lag phase preceding CH$_4$ emission lasted for the same time as in samples without these ions (Fig. 1 and Table 3).

The addition of Pb significantly changed the methanogenesis rate in all tested soils (Fig. 1). The single Pb dose caused a significant slowing of the process in all cases. The higher dose of Pb (by five times) caused a total inhibition of CH$_4$ production in all soils used in the experiments.

As in the case of the control with chloride ions, the differences in methanogenesis described above found confirmation in the analysis of the methane production rate,

as shown in Table 3. Considering the changes in rates in the first phase (shown in Fig. 1), it may be seen that for all soils, the decrease in CH$_4$ production for the control with CaCl$_2$ and for the sample with PbCl$_2$ was practically the same, at ~1.85. With a higher Pb^{2+} concentration, methane production was stopped in all soils, and therefore in these cases the methane production rate was zero.

The pH values of samples contaminated with Pb were lower in all cases than those of the corresponding controls, including the controls with CaCl$_2$ (Table 2). A greater pH decrease was observed after the application of the higher Pb dose. For instance, in Eutric Cambisol and Haplic Podzol, there was a change of almost two pH units.

DISCUSSION

The ability of soil to produce and emit methane to the atmosphere is regulated by many factors connected mainly with soil properties, such as the particle-size distribution, fertilization, the pH or the C$_{org}$ content, which simultaneously affect the behaviour of heavy metals in the soil. All of the tested soils showed the biphasic production of CH$_4$ (Fig. 1), but some differences in the process rate were detected (Table 3). Cambisol and Podzol showed a similar rate of CH$_4$ emission in both phases of the process. Mollic Gleysol showed a lower methanogenic potential. The rate in phase II was always much lower than that in phase I.

Angel *et al*. (2012) tested the methanogenic potential of soils collected from all over the world by flooding their soil samples. The results showed that in almost all samples, methane production started within seven to 14 days of incubation. What is more, the methane production rate was not connected with parameters such as organic matter content or atmospheric precipitation. A similar tendency may be observed in our results. In almost all of the tested soil samples, the time required for methane production to begin was around 14 days. Additionally, no connection between higher C$_{org}$ content and methanogenic activity was detected. The highest methane production rates were observed for Haplic Podzol where the C$_{org}$ content was 0.43% and the lowest methane production rate was found in Mollic Gleysol, which is distinguished by the highest C$_{org}$ concentration at 3.93%. A possible reason for this may be the poor quality of the available C$_{org}$ (Guérin *et al*., 2008).

The initial soil pH is another factor which may affect methanogenesis. Two out of the three soils tested had similar pH values – Eutric Cambisol at 6.38 and Haplic Podzol at 6.5. The highest pH values were detected in Mollic Gleysol, at 7.71. Despite this, the observed methane production rates were similar. One reason for this was given by Yang and Chang (1998) who tested the effects of a few factors, such as water content, organic substances, oxygen and initial pH, on methane production in paddy soil. The measurements were taken at pH values in the range of 3.2 to 9.3. The results showed no differences in the process

at pH values between 6.5 and 7.7, which is similar to the range of pH values observed in our research. Higher and lower pH values outside the given range caused a slowing or (at extreme values) a complete inhibition of the process.

It has been shown that NH_4^+ in the soil may also affect the process of methane production (Fotidis *et al*., 2013; Hao *et al*., 2017; Lv *et al*., 2014). Experimental results have shown that a lower NH_4^+ content led to higher methane production rates in the tested soils. In our measurements, differences in the methane production rate in the tested soils may be observed compared to the controls with water. The highest values were obtained with Haplic Podzol, followed by Mollic Gleysol. The lowest values were found in Eutric Cambisol. These differences may be related to the different ammonium ion content in the different soils. The highest concentration of NH_4^+ ions in our study was observed in Eutric Cambisol (4.20 mg kg^{-1}) and the lowest value was observed in Haplic Podzol (0.49 mg kg^{-1}), which is consistent with the statement above. However, it should be pointed out that the initial NH_4^+ content in the tested soil was quite low, and therefore this parameter was not treated as a key variable in our experiment.

Another possible reason for differences in the course of the methane production process might be connected with the presence of salt ions. In the research, two types of controls were used: samples mixed with water or with CaCl$_2$ solution. Calcium has a positive effect on the growth of methanogens to a limited extent (Kumar *et al*., 2016; Murray and Zinder, 1985). Chloride (Cl$^-$) is usually supposed to be unreactive in soil, but some authors have reported its participation in biogeochemical cycles (Bastviken *et al*., 2007; Gustavsson *et al*., 2012; Öberg and Sandén, 2005). Analysis of the course of methane production in all tested soils showed differences between the results of the two controls. A possible reason for this is the effect of chloride ions on methanogenesis, because the influence of calcium ions, in light of the available literature, seems to be negligible. Ahn *et al*. (2006) examined the effect of calcium ions on the anaerobic digestion of wastewater. The results showed that despite the changes in calcium concentration in the reactors, the methane yield did not change. Van Langerak *et al*. (1998) came to a similar conclusion while testing the effect of calcium on methanogenic sludge.

Although the use of chloride salts as a control is common in methane production research (Altaş, 2009; Basiliko and Yavitt, 2001; Muñoz *et al*., 1996), there are only a few reports available concerning the possible effect of these ions on the process. It has been stated that Cl$^-$ may cause a reduction in CH$_4$ production (Jagadeesh Babu *et al*., 2006; Koyama and Kimura, 1998). A possible reason for this is osmotic imbalance, which affects the cation-anion equilibrium. The disturbance of the existing balance leads to a reduction in the activity of methanogens or microorganisms which provide substrates for reactions (Mishra *et al*., 2003). In our study, the negative influence of CaCl$_2$ on CH$_4$ emission was measured in all soils enriched with a higher Cl$^-$ dose (Fig. 1, Table 3), while in Mollic Gleysol, even the lower Cl$^-$ concentration led to a reduced ability to produce CH$_4$.

As in the controls, after the addition of the lower concentration of Pb, a lag phase followed by a biphasic pattern of CH$_4$ emission was observed. The methane production curves showed the strong negative effect of Pb at a concentration of 300 mg kg^{-1}, compared to the corresponding controls. The higher heavy-metal dose (1 500 mg kg^{-1}) caused a total inhibition of the process.

The analysis of the effect of Pb on the process of methane production may be considered in several ways. However, we should take into consideration the fact that the influence of Pb on methanogenesis may be concurrent with the impact of CaCl$_2$. One important aspect to consider is the direct impact of the metal ion on soil microorganisms.

The effect of Pb contamination on soil microorganisms is regulated by the soil's physicochemical properties, since these affect the heavy metal's mobility and, as a consequence, its bioavailability. It was proven that a higher organic matter and clay content leads to lower Pb availability, and hence the reduced negative effect on the microbial community (Alvim Ferraz and Lourenco, 2000; Zeng *et al*., 2006). Mollic Gleysol, had the highest organic matter content of the samples tested, and was less sensitive to Pb contamination than the other soils investigated (Table 3). This may be the result of the creation of complexes with organic acids, thereby decreasing the solubility and mobility of heavy metals, especially Pb (Antić-Mladenović *et al*., 2017; Laing *et al*., 2009).

The impact of phosphate on the behaviour of Pb has also been noted. Phosphate in soil causes the immobilization of Pb by reducing its mobility and bioavailability. The main reason is the formation of insoluble pyromorphite complexes (Kelebemang *et al*., 2017; Sanderson *et al*., 2016). The soils used in our experiments were characterized by a very high phosphorus content (Table 1), which may also partially protect methanogens against the harmful effects of Pb at lower doses, leading to a reduction in (but not a total inhibition of) CH$_4$ production.

A study of the microbial community in the presence of Pb ions allowed the concentration limit for causing a decrease in microbial activity to be determined (above 300 mg kg^{-1}). Lower concentrations caused the stimulation of microbial activity. In our research, a concentration of 300 mg kg^{-1} caused a reduction in microbiological activity (observed as a slowdown in the production of methane). These differences probably resulted from the use of Pb in the form of lead acetate, since acetate is an organic carbon compound and hence may stimulate the activity of soil microbes (Loka Bharathi *et al*., 1990).

There are few reports on the direct effect of Pb on the process of methane production in soil. Mishra *et al*. (1999) tested the effect of a few heavy metals (including Pb) on the process of methane production in three types of Indian soils:

alluvial soil, laterite soil and acid sulphate soil. The metal was added to obtain a final concentration of 20 µg g^{-1} in the soil. The results showed that the methanogenesis process was inhibited in the soil samples after 20 days of incubation. According to our research results, CH$_4$ production did not even begin at the higher Pb concentration. At the lower concentration, CH$_4$ production was inhibited after 40 days of incubation in Haplic Podzol and Eutric Cambisol, and after 60 days of incubation in Mollic Gleysol. This showed that Mollic Gleysol was the least sensitive to Pb addition. The process of methane production can also be observed in other ecosystems where anoxic conditions occur. Muñoz *et al.* (1996) investigated methane production in sludge enriched by nickel, lead and a combination of these two heavy metals. Pb was added at three levels, one of which was close to our tested value (500 mg kg^{-1}). In contrast to our results, the authors stated that Pb did not show any long-term inhibitory effect.

When considering the influence of Pb on methanogenesis, it is necessary to pay attention to the conditions prevailing during the process. Anaerobic conditions are necessary, in order to initiate CH$_4$ production. These conditions were obtained by flooding the samples and removing atmospheric air from the headspace. In this environment, the properties of the heavy metal may change, as they are connected with a decrease in soil redox potential (Eh) (Penning and Conrad, 2007; Sun *et al.*, 2007). It has been shown that Eh has an effect on the solubility and availability of heavy metals: a low Eh value (together with an increase in pH caused by flooding) causes a decrease in Pb solubility and mobility, connected mainly with complexation with soil organic matter but also with clay minerals or Fe and Mn hydroxides (Antić-Mladenović *et al.*, 2017; Laing *et al.*, 2009; Rinklebe *et al.*, 2016; Sun *et al.*, 2007). In our research, Pb caused a slowdown in or a total inhibition of the methanogenesis process, depending on the Pb level (Fig. 1). The behaviour of Pb after flooding may depend on the duration of this condition. At very low Eh values (below –150 mV (Gambrell *et al.*, 1991; Mansfeldt, 2004) or –220 mV (Ross, 1989)) sulphide formation is also possible (Rinklebe *et al.*, 2016), and these ions may bond with Pb. The appropriate redox potential for methanogenesis is also in this range (Chowdhury and Dick, 2013; Joulian *et al.*, 1997). After a longer period (more than two months), the immobilization of trace metals by precipitation or readsorption may occur (Charlatchka and Cambier, 2000), and this may be the mechanism regulating CH$_4$ emission in phase II in our study.

As mentioned previously, pH is also an important factor affecting the process of methane production in contaminated samples. The optimal pH value for methanogenesis is in the range of 6-8, depending on the source (Yang and Chang, 1998). For each sample, the addition of Pb caused a decrease in pH, compared with the corresponding control sample. In samples with the lower Pb dose, the highest decrease was observed in Eutric Cambisol, with similar decreases being observed in Haplic Podzol and Mollic Gleysol. However, the pH values were still in the optimal range for methanogens, where it is possible for them to produce methane. In samples contaminated with Pb in the five-fold dose, the pH decrease was much higher (Table 2). In both cases, it may be stated that the changes in pH were connected with a decrease in the methane production rate. In the five-fold dose, the process was completely inhibited. The same effect has been observed by other authors. The addition of Pb^{2+} caused changes in the pH values, which in turn led to differences in methane production, mainly to inhibition (Gogo and Pearce, 2009; Keller and Wade, 2018). A possible reason for this may be the release of H$^+$ ions via cation exchange in the presence of trace metals. However, this is a subject that requires further research.

Our short-term experiment showed the importance of observing the permissible doses of heavy metals in arable soils where Pb-induced CH$_4$ imbalance may occur. On the one hand, exceeding the permitted Pb doses may reduce CH$_4$ emissions, but it also has a negative effect on its oxidation in these soils (Wnuk *et al.*, 2017). In such cases, the accumulation of heavy metals may result in CH$_4$ emissions from cultivated soils. The real environmental effect may be demonstrated by long-term field studies in this area, and precise mechanisms may be found using microbiological studies.

CONCLUSIONS

1. The lowest methane production rate was measured in Mollic Gleysol. The results obtained for Eutric Cambisol and Haplic Podzol were similar to each other.

2. The addition of Pb caused a lower production of methane (for the single dose) or the total inhibition of production (for the five-fold dose).

3. An analysis of the results for the control samples showed that the effect of changes in salt ion concentration may be observed. It is supposed that chloride ions were responsible for the slightly lower methane concentration in the sample corresponding to the control with water.

Conflict of interest: The authors declare that they have no conflict of interest.

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