

Predicting NO, N₂O and CO₂ emission from agricultural soil through related environmental parameters

Ph.D. Thesis

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1. INTRODUCTION

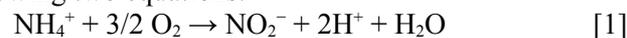
Agriculture is both a source and sink for greenhouse gases (GHGs) and intensification of land use has increased the exchange of carbon (C) and nitrogen (N) between the land and the atmosphere. Concentrations of atmospheric GHGs, such as carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), and etc. which can alter the earth's climate have risen dramatically during the past century. This has resulted in an urgent need for process-based understanding of the main factors influencing the exchange of these gases between the land and atmosphere at a range of scales, as a route to developing effective mitigation technologies. Most of the nitrogen-oxides are generated from mineral N originating from animal dung and urine, biologically fixed N₂, and mineralization of soil organic N. The exchange of N containing gases between the atmosphere and terrestrial surfaces has been an important issue in agricultural and soil research for a long time. Prominent examples are the N₂-fixation by plants and microorganisms, denitrification loss of soil N in form of N₂, N₂O, and the emission of gaseous ammonia (NH₃) from fertilized soils. Unlike these processes, the exchange of nitric oxide (NO) between biosphere and atmosphere contributes only a minor part to the N budget of most terrestrial ecosystems. However, emissions from the soil-plant system are of enormous concern for the NO concentration in the troposphere.

1.1. PROCESSES OF N TRANSFORMATIONS

The emission of NO and N₂O from forest soils is mainly the result of simultaneously occurring production and consumption processes, most of which are directly linked to the microbial N turnover processes of nitrification and denitrification (CONRAD, 1996a,b). With regard to NO also the abiotic process of chemodenitrification, during which biologically produced NO₂⁻ is chemically decomposed to NO, has been shown to be an important production process in soils at pH values lower than 4.0 (VAN CLEEMPUT & BAERT, 1984). Like most other biological processes, microbial turnover processes vary largely on spatial and temporal scales, since they are significantly influenced by a number of environmental factors such as climate and meteorological conditions, soil and vegetation properties or human management of the land surface.

1.2. NITRIFICATION

Nitrification is characterized as the process in which NH₄⁺ is converted to NO₂⁻ and then NO₃⁻. This process naturally occurs in the environment, where it is carried out by specialized bacteria. The process is described by the following two equations:



The process of creation, consumption and disposal of N₂O is described by Firestone & Davidson (1989) as the "hole-in-the-pipe" model (Figure 1.).

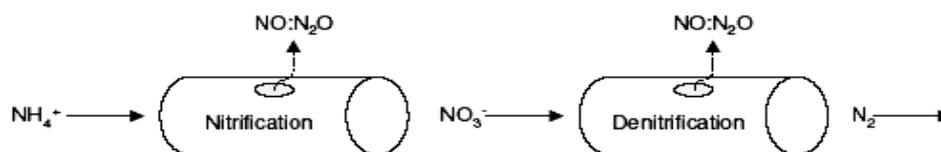


Figure 1. The "hole-in-the-pipe" model (modified from FIRESTONE & DAVIDSON, 1989).

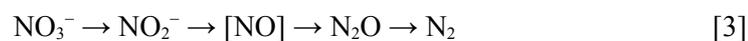
In agricultural soils, the nitrification is mainly carried out by *Nitrosomonas*, *Nitrospira* and *Nitrobacter* bacteria (ENQUÊTE-KOMMISSION SCHUTZ DER ERDATMOSPHÄRE DES DEUTSCHEN BUNDESTAGES, 1994). Soil water and O₂ content, as well as the macro pores, organic matter (OM) and pH in the soil mainly determine the development rate of N₂O. The optimum condition for the nitrification in soil is at a water content of 60%. When the water content is increased, nitrification is limited by O₂, and vice versa. Between 1% and 4% of the N input is turned into NO during the nitrification process (ENQUÊTE-KOMMISSION SCHUTZ DER ERDATMOSPHÄRE DES DEUTSCHEN BUNDESTAGES, 1994), and about 0.5% is turned into N₂O (VELDKAMP & KELLER, 1997).

Nitrification is commonly defined as the biological oxidation of NH₄⁺ to NO₃⁻ with NO₂⁻ as an intermediate (BREMNER, 1997). Although the capacity for nitrification is restricted to a few genera of strictly aerobic, mainly chemoautotrophic bacteria, this process is of major importance for the N cycling

in most cultivated and many natural soils. Overall nitrification rates, however, will increase in well-aerated soils, provided that the soil is not very acidic ($\text{pH} > 4 - 5$). If these requirements are met, the nitrification rate is predominantly controlled by the availability of NH_4^+ (Robertson, 1989).

1.3. DENITRIFICATION

Microbial denitrification requires an anaerobic environment, whereas aerobic conditions are necessary for nitrification. Since 1850 denitrification is estimated to have increased from 270 to 310 Tg N yr^{-1} . Globally, hotspots for denitrification are estimated to occur in the same regions where anthropogenic N inputs are high. By 2050 denitrification rates are estimated to increase to 370 Tg N yr^{-1} . However, the most effective solution to minimizing negative environmental effects of increased N mobilization is to decrease N use/emissions and N losses at the point of application/deposition (GALLOWAY et al., 2004). Denitrification is defined as the reduction of NO_x to molecular N_2 or NO_x with a lower oxidation state of N by bacterial activity. NO_x are used by bacteria as terminal electron acceptors in place of O_2 in anaerobic respiratory metabolism. Denitrification often occurs when the soil is wet or compacted or warm, because these are situations where O_2 is a limiting factor. The denitrification is described by the following equation:



1.4. FORMATION OF NO

Nitrous oxide is generated by nitrification and denitrification when microbes transform inorganic N, including NH_3 and NO_3^- (GRANLI & BÖCKMAN, 1994). Both processes are governed by the soil water content. Nitrification mainly occurs when 30–60% of the pore space is water-filled, and denitrification mainly occurs when 50–80% or 60–90% of the pore space is filled with water, depending on the soil properties (BOUWMAN, 1998). The faculty to reduce NO_x , when O_2 becomes limiting, enables denitrifying bacteria to grow in anaerobic environments.

Basically, the complete set of environmental factors that regulate the underlying processes of NO production and consumption in soils has the potential to affect the exchange of NO between soil and the atmosphere significantly.

Soil moisture governs whether nitrification or denitrification is the dominant process in a given soil and strongly influences the corresponding turnover as well as the ratio of NO production over NO consumption rates. Soil moisture, moreover, controls transport of microbial substrates and products of microbes. Thus a simple relationship between the flux of NO and the soil water content may not be expected (DAVIDSON, 1993).

Considering the dominance of soil microbial processes for the production of NO, one has to expect an influence of soil temperature on NO emission rates. Indeed, the bulk of existing studies (YANG & MEIXNER, 1997) has shown an increase of NO emissions with increasing soil temperatures.

1.5. PROCESSES INFLUENCING NITROUS OXIDES EMISSION

Soil microbial processes are primarily responsible for soil N_2O emissions. BEAUCHAMP (1997) reported that climate, soil characteristics, cropping practices and their interactions affect the nitrification and denitrification processes (Figure 2.) and hence the production and emission of N_2O .

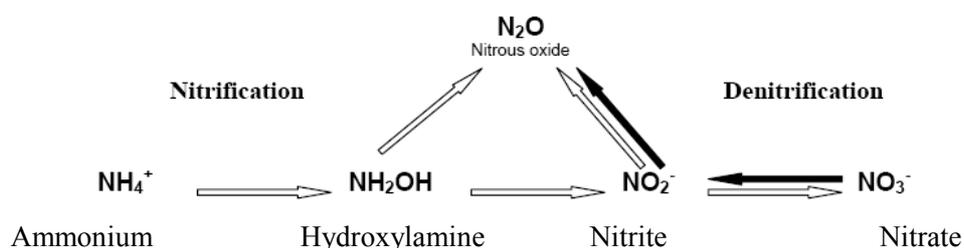


Figure 2. Conceptual model indicating the major pathways for N_2O formation regarded in this study. Nitrification is indicated with open arrows and denitrification is indicated with bold arrows. Adapted from WRAGE et al. (2001).

1.6. IMPORTANT FACTORS CONTROLLING N₂O EMISSIONS

Empirical up-scaling of the global source strength of tropical ecosystems is thus based on a very limited database that is unable to take account of the spatial and temporal variability of the soil-atmosphere exchange of trace gases (KIESE et al., 2005). The high variability in trace gas flux rates is generally caused by the underlying biogeochemical processes (e.g., mineralization, nitrification and denitrification), which are controlled by environmental factors such as soil moisture, soil temperature and nutrient availability (SMITH et al., 2003). As these environmental factors vary in time and space, site specific estimates of annual trace gas exchange are likely to contain large uncertainties. Soil temperature and soil moisture were the most important factors controlling N₂O emissions. Those parameters affect microorganisms and their metabolism and, hence, the production and consumption of N trace gases in soils (CONRAD, 1996a,b). The air-filled porosity controls the movement of the gases towards and away from the atmosphere; it also affects soil aeration, and, thus, indirectly controls the capacity of the soil for producing or consuming soil-produced trace gases (DAVIDSON et al., 2000).

1.7. EMISSION OF CO₂

JOLÁNKAI & BIRKAS (2005) mentioned that the climate change phenomena may be related to the rise in atmospheric CO₂. Long-term rise in atmospheric CO₂ highlights crop production regarding both adaptation and mitigation (JOLÁNKAI et al., 2005). The gradual increase in atmospheric CO₂ concentration and potential climatic changes are likely to affect plant, soil and ecosystem processes, including carbon flux from plants to soil and from soil to atmosphere (PAJARI, 1995). In a typical forest ecosystem, the components of soil CO₂ efflux include respiration due to litter decomposition, root respiration, rhizomicrobial respiration, and microbial respiration utilizing native SOM (CHENG, 1999). BAYOUMI HAMUDA & KECSKÉS (2003) mentioned that the biological activity in a soil is usually evaluated by measuring CO₂ evolution. In sewage sludge amended soil with high level of Pb, Cd and Zn the CO₂ evolution was increased. Soil respiration and microbial biomass can be useful indicators of soil contamination, combining the two measurements to give amounts of CO₂ evaluated per unit of biomass (µg CO₂-C/g soil). GRÖNLUND et al. (2008) reported that drainage and cultivation of peat soils stimulates SOM mineralization, which substantially increases CO₂ emissions from soils.

1.8. IMPACTS OF HEAVY METALS ON TRACE GASES EMISSIONS

Trace amount of some heavy metals are required by living organisms, however any excess amount of these metals can be detrimental to the organisms (BERTI & JACOBS, 1996). Non-essential heavy metals include arsenic, antimony, cadmium, chromium, mercury, lead, etc; are of particular concern to surface water and soil pollution (KENNISH, 1992). Heavy metals exist in colloidal, ionic, particulate and dissolved phase. These metals also have a high affinity for humic acids, organo-clays, and oxides coated with OM (ELLIOT et al., 1986). The soluble forms are generally ions or unionized organo-metallic chelates or complexes. The solubility of metals in soil and groundwater is predominantly controlled by pH (HENRY, 2000), amount of metal (GAREIA, 1984), cation exchange capacity (MARTINEZ & MOTTO, 2000), OC content (ELLIOT et al., 1986), the oxidation state of the mineral components, and the redox potential of the system (CONNELL & MILLER, 1984).

In general, soil pH seems to have the greatest effect of any single factor on the solubility or retention of metals in soils. With a greater retention and lower solubility of metal cations occurring at high soil pH (BASTA et al., 1993). Under the neutral to basic conditions typical of most soils, cationic metals are strongly adsorbed on the clay fractions and can be adsorbed by hydrous oxides of iron, aluminium, or manganese present in soil minerals. Elevated salt concentration creates increased competition between cations and metals for binding sites. Also competitive adsorption between various metals has been observed in experiments involving various solids with oxide surfaces, in several experiments, Cd adsorption was decreased by the addition of Pb or Cu (BENJAMIN & LECKIE, 1980).

2. OBJECTIVES OF THE PRESENT Ph. D STUDY

According to the above mentioned reasons, the main aim of the dissertation is to determine the effects of some environmental factors in a complex design on soil respiration, soil heterotrophic bacterial population and the emissions of trace gases such as NO, N₂O, and CO₂ which play an important role in global warming.

All activated and non-activated (control) of cultivated and uncultivated soil samples were contaminated with three concentrations of each heavy metal:

- 1.5, 3, and 6 mg Cd kg⁻¹ soil in form of CdCl₂·2.5H₂O,
- 4, 8, and 16 mg Co kg⁻¹ soil in form of CoCl₂ and
- 40, 80, and 160 mg Pb kg⁻¹ soil in form of PbCl₂.
- The control microcosms were heavy metals free.

The heavy metal amended soil samples were incubated for six weeks at 28°C. The population density of aerobic heterotrophic bacteria and the amount of CO₂-release in each soil sample were investigated after first, third and six weeks of incubation.

PART (B): Predict the ecological factors influencing the NO, N₂O and CO₂ emissions

The soil samples collected in the upper 200 – 250 mm layer after removing the first top 20 – 30 mm from a sample site. A 200 g per microcosm was homogenised (2 mm) soil samples of the Ramann-type and clay loam brown forest soil originated from Keszthely and Gödöllő respectively, were placed into the microcosm of 1200 cm³. The main physico-chemical properties of the (200 –250 mm) two soil samples were:

1. Ramann-type (Keszthely) soil samples:

pH_(KCl) 7.55, total salt content 0.054%, humus 1.48%, total organic C 1.08%, total N 0.08%, NH₄⁺-N 0.53 mg 100 g⁻¹ soil, NO₃⁻-N 0.18 mg 100 g⁻¹ soil, K₂O 136 mg 100 g⁻¹ soil, P₂O₅ 130 mg 100 g⁻¹ soil, soil density 2.45 g cm⁻³ and C:N ratio 13.5.

2. Clay loam (Gödöllő) soil samples:

pH_(KCl) 5.56, total salt content 0.037%, humus 3.51%, NH₄⁺-N 0.40 mg 100 g⁻¹ soil, NO₃⁻-N 0.28 mg 100 g⁻¹ soil, K₂O 82 mg 100 g⁻¹ soil, P₂O₅ 34 mg 100 g⁻¹ soil, and soil density 2.52 g cm⁻³.

The soil samples were contaminated with different doses of heavy metal:

- 40, 80 and 160 mg Pb kg⁻¹ soil of Pb(CH₃COO)₂·3H₂O
- 6, 12 and 24 mg Cd kg⁻¹ soil of CdCl₂·2.5H₂O.
- The control microcosms are heavy metal free.

The water filled pore space (WFPS) of soil samples was adjusted to be 30 and 60%. The gravimetric moisture content was determined from the collection sample (from the upper 200 – 250 mm soil layer, five replicates from each field, 50 g fresh weight) by drying the soil samples for 24 h at 65°C. Bulk density was determined according to BLAKE (1965). Soil moisture was adjusted to the desired content (30 and 60% WFSP) for each set of microcosms by addition of equivalent amount of distilled water.

3.1.3. Microcosm model

This version of the CO₂, N₂O, NO, nitrification and denitrification sub-model was developed to enable a finer simulation of the climate soil conditions interaction in the top few centimeters of soil. We thus reduced the thickness of the topsoil layer from 5 to 15 cm, and assigned a particular functioning to this ‘microcosm-layer’. For this version trace gases emissions are calculated in the 0–5 cm layer. The nitrification is evaluated in the 0–5 cm from soil temperature and soil moisture content for these layer thicknesses under the stress of different Cd and Pb concentrations. Static chambers (microcosms) are the most commonly used tools in the World (Tate et al., 2007). The soil sub-sample was mixed with different concentrations of Cd or Pb and was homogenized and the WFPS was adjusted to 30 or 60% in order to promote N mineralization as well as trace gases emissions originating from nitrification and denitrification processes (Merino et al. 2001). A WFPS around 60% favours nitrification because the diffusion of substrates and O₂ is not restricted (Parton et al. 1996).

3.1.4. Cultural medium

For counting the population density of the aerobic heterotrophic bacteria the Nutrient agar medium was used with the following composition (Sharma & Johri, 2003) in g l⁻¹: 5 peptone, 3 beef extract, 18 agar-agar, and pH 7.

3.2. METHODS

Part (A):

3.2.1 Determination of bioavailability of Pb, Cd, and Co and their effects on CO₂-release and density of the aerobic bacterial population density.

A. Sample preparation for determination of soluble fraction of heavy metals

MI-08-1735-1990 is the Hungarian technical directive method which was used to detect Pb, Cd and Co content in the soil samples. Five gram of air-dry and fine grounded soil sample was weighed and shaken with 25 ml of 1.5 M nitric acid at 20°C for two hours. The element analysis of the filtrate was performed by Jobin-Yvon 24 type ICP atomic emission spectrometer.

B. Element analysis by ICP-AES:

In the extracts made by the Hungarian standard procedures the following elements (Pb, Cd, Co) were determined with Jobin-Yvon JY-24 of sequential ICP-AES instrument as described in its operating manual.

3.2.2. Determination of CO₂-production

For measurement of CO₂-production, a 0.5 kg of the heavy metal treated soil was filled in about 1500 ml glass vessels and in the middle of the soil a fixed plastic tube, containing 50 ml of 10 M NaOH solution for trapping the evolution of CO₂ and vessel was closed tightly. The NaOH was titrated with HCl (1M) to calculate the volume of CO₂ released as soil respiration. Applied method of Wardle & Parkinson (1991) was used for simultaneous determination of NaOH and Na₂CO₃ content in our experimental soil samples.

3.2.3. Determination of total number of aerobic bacteria

Under sterile conditions, a 10 g of fresh soil sample was suspended with 90-cm³ water. The soil suspension was diluted gradually to 10⁻³ and 10⁻⁶ and from the diluted suspensions 1 cm³ was pipetted in (Petri dish, and mixed thoroughly with Nutrient agar). The plates were incubated on 27°C for 48 hours. After that, the developed bacterial colonies were counted.

Part (B): Predict the ecological factors influencing the NO, N₂O and CO₂ emissions

3.2.4. The Soil Incubations Experiments Performed

Separate incubations were also performed in order to determine CO₂, N₂O and NO emissions. Static system incubations were carried out in microcosm (~1200 ml) with a gas-tight septum fitting in the lid according to Bollmann et al. (1999). Heavy metal contaminated soil sub-samples with various soil moisture (30 or 60% WFPS) were incubated at different temperatures (15, 37°C) in the dark thermostatic incubator. Changes in the concentration of N₂O, NO and CO₂ in the headspace of the microcosms were determined by periodic sampling of the headspace with gas tight syringes and subsequent trace gas analysis throughout 35 incubation days. Mainly, three experiments were conducted. In addition, the NO, N₂O and CO₂ emissions were measured during time intervals of incubation under different ecological parameters.

Experiment 1: Effects of temperature

The hypothesis for this experiment was that the emissions of NO, N₂O and CO₂ will decrease when the incubation temperature was at low, and higher when the temperature was close to 30°C. We determined if there is any difference in the trace gases emissions at low and high soil temperatures. There were 3 replicate soil microcosms for each soil type per each treatment with different soil water content (30 and 60% WFPS). Gas samples (0.250 ml) were taken through the septa by injection needles.

Experiment 2: Effects of soil moisture

Here, we ran this experiment at low and high soil moisture (30% and 60% of the WFPS). Three replicate microcosms per each treatment per for two soil types (Keszthely and Gödöllő) were used. Soil

was moistened, and to omit a possible major N₂O pulse (Jørgensen & Jørgensen, 1997), the soil microcosms were stabilized for 4 h before incubation. The fluxes of the trace gases were measured at the various temperatures (15°C and 37°C). During the incubation time intervals, the development of gases concentrations in the microcosms were followed by the gas samples (0.250 ml) were taken through the septa by injection needles.

Experiment 3: Effects of heavy metals

In this experiment, the effects of different concentrations of Cd and Pb on the release of NO, N₂O and CO₂ from different soil types, with different water content and incubated at different soil incubation temperatures were studied, using three replicate microcosms per treatment. The microcosms with their two different water content (30 and 60% WFPS) were incubated at 15°C and 37°C for 35 days as incubation time intervals. The fluxes of NO, N₂O and CO₂ were measured throughout 35 days as the incubation period with time intervals. Gas samples (0.250 ml) were taken through the septa by injection needles.

3.2.5. Sampling and measurement of the trace gases

The microcosms were independently incubated in a laboratory thermostat at 15 or 37°C for 35 days under different treatments. Measurements of NO, CO₂ and N₂O were made in triplicate for each microcosm by the help of injection needle. During the run of the experiments and during incubation time intervals, NO, N₂O, and CO₂ gas samples were taken from the headspace of each microcosm and determined regularly by chemiluminescent detector (for NO) and gas chromatographic method (for N₂O and CO₂). A 250 µl gas sample in the closed atmospheric condition in each microcosm containing the specific treated soil sub-sample of the incubation headspace were used for each analysis of N₂O, NO and CO₂ was taken by gas tight Hamilton syringes and injected to the HP 5890 gas chromatography. Packed columns (Porapak Q) used to separate the different constituents of gas samples. Electron Capture Detector (ECD) and Thermal Conductivity Detector (TCD) detected N₂O and CO₂ concentrations, respectively (see Table 1. for instrumental conditions).

Table 1. The most important characteristics of gas chromatography for N₂O and CO₂ measurements

GC analysis of gas samples	HP 5980 Series II type gas chromatography	
Analysed gases	N ₂ O	CO ₂
Carrier gases (types and flow rates):	N ₂ : 23 ml/min	He: 27 ml/min
Temperature of Injector	70°C	
Columns (oven temperature is 50°C)	Porapak Q (80/100 mesh, 6 ft)	
Detectors (temperature, detection limit)	ECD (250°C, >5-10 ppm)	TCD (150°C, >100 ppm)
Retention time	1.0-1.2 min	1.1 – 1.3 min
Duration of a run	3.5 min	2.5 min
Calibration	external standard	
Calibration gas mixture contains	7.9 vpm N ₂ O	9.7 v/v% CO ₂
Evaluation of chromatograms	HP 3390 Ser. II integrator, HP CHEM	

The separated gas content was analyzed three times per day whenever measurements carried out using external standard and one point linear calibration. The NO gas emission detected by chemiluminescent detector (Model 7050 analyzer of ANTEK Instruments L.P., USA) which is specifically designed for the analysis of NO in samples.

3.3. Statistical analysis

All experimental investigations in three replicates, and the results were represented by the means of three replicates. Group differences across metric dependent variables based on set of categorical (non-metric) variables were assessed by multiple analyses of variance (MANOVA). Differences in means were evaluated by F-probe according to Sváb (1981). Excel 5.0 statistical functions were used

for calculations and graphic presentation of data. Standard deviation (SD) and Least Significant Difference at 5% level (LSD at 0.05) were calculated as well. Linear regression models and correlation (R^2) were used to assess the additive effects of ecological parameters and soil characteristics on trace gases emissions. Pearson's correlation coefficients (r) and the coefficients of variability or variation (CV%) were calculated. CV was defined as standard deviation/mean x 100. Analysis of variance was performed for emission at all parameters to examine differences in emission between and within the experimental conditions. The non-systematic error (coefficient of variability or variation) of sampling and analysis with repeated sampling performed on each gas sampling from each soil treatment and for each soil types.

4. RESULTS

The results of this study is concerned with determination the bioavailability of Pb, Cd and Co, the impacts of the different concentrations of Pb, Cd and Co on CO_2 -release as well as enumeration of aerobic heterotrophic bacterial populations in wheat cultivated and uncultivated brown forest soil. Results were collected and analyzed after 1, 3 and 6 weeks of incubation in greenhouse and under laboratory conditions at 28°C. The second investigation of the present study provides information concerning the results of the microcosm models. These models described the influences of various concentrations of Cd and Pb on the emissions of NO, N_2O and CO_2 under the impacts of soil moisture regimes and incubation temperatures of two brown forest soil samples of Ramann-type and clay loam originated from Keszthely and Gödöllő, respectively. The results showed that:

BIOAVAILABILITY OF HEAVY METAL, DETERMINATION OF CO_2 -RELEASE AND POPULATION DENSITY OF AEROBE HETEROTROPHIC BACTERIA

1. The recoveries of the amounts of Pb, Cd and Co were significantly higher at the 6th week of the incubation intervals than at 1st and 3rd incubation week. Linear regression and correlation indicated no significant differences between the metal recoveries in the uncultivated and wheat cultivated brown forest soil samples originated from Gödöllő.
2. The CO_2 -released from uncultivated soil samples was more than CO_2 -released from cultivated soils. Soil samples treated with SIR activated the biological processes in both soil samples. It was recognized that Cd was more inhibiting metal than Co and Pb in investigated soil samples. The amounts of CO_2 -released were reduced by increasing the concentrations of heavy metals.

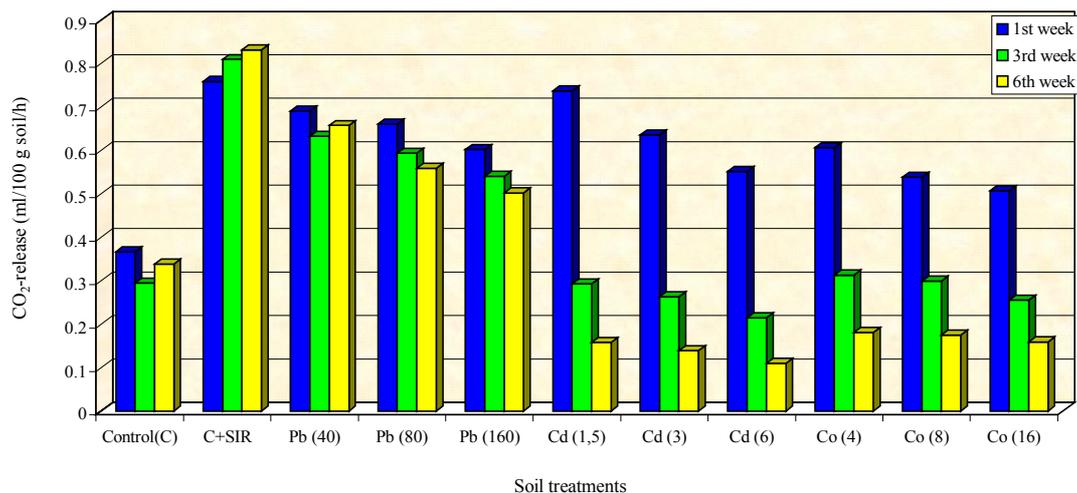


Fig. 3. CO_2 - release from cultivated brown forest soil (Gödöllő) contaminated by different concentrations (mg/kg soil) of Pb, Cd, and Co

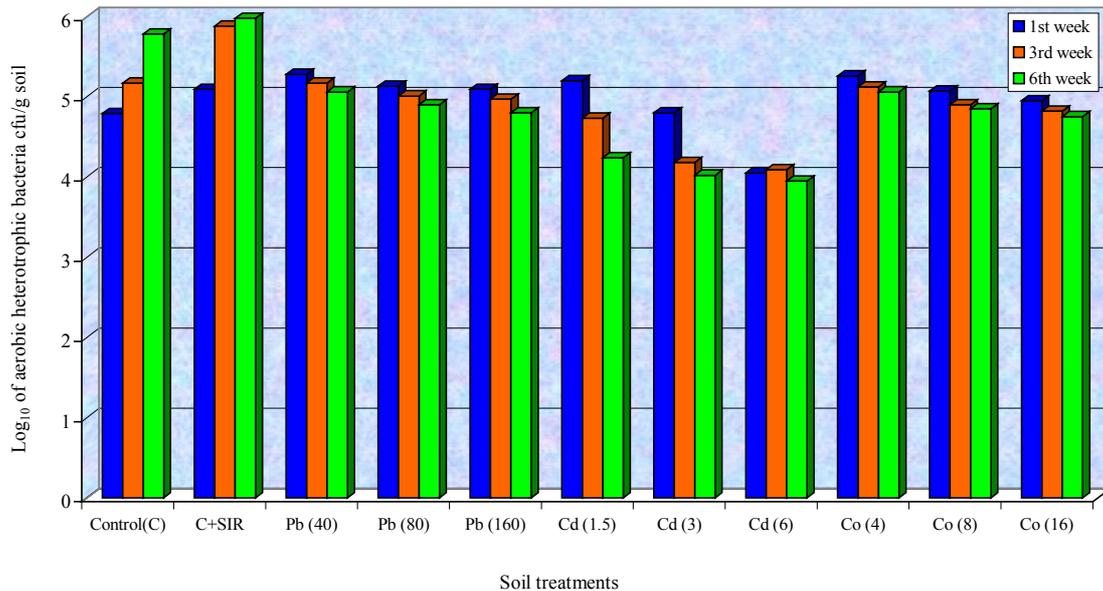


Fig.4. Log₁₀ of aerobic heterotrophic bacterial count (cfu/g soil) from cultivated brown forest soil (Gödöllő) contaminated by different concentrations (mg/kg soil) of Pb, Cd, and Co

3. Cd was more toxic metal and causes a decrease in the population density of aerobic bacterial structure in both soil samples followed by Pb and Co. The inhibition of population density of aerobic bacterial structure was increased by increasing the incubation periods. The toxicity decreasing order of the tested metals was Cd > Co > Pb.

EMISSIONS OF NO UNDER THE STRESSES OF HEAVY METALS

1. **At 30% WFPS, and when the soil microcosms incubated at 15°C:** The NO emission in Gödöllő soil samples was approximately 2 times more than the amounts emitted from Ramann-type brown forest soil samples of Keszthely when contaminated by Cd at different concentrations. NO emission rates were decreased by increasing the concentration and the time of incubation. But when Pb contaminated soil samples incubated in microcosms at 15°C, the detected NO emissions were higher in Ramann-type of Keszthely microcosms than those emitted from clay loam brown forest soil of Gödöllő soil samples. The amounts of NO were influenced by the metal contaminated doses and time of incubation. The results showed that when the soil microcosms incubated at 15°C, NO emission from Ramann-type soil microcosms was more inhibited by Cd than by Pb. But Pb more inhibited the NO emission than Cd in clay loam soil microcosms.

2. **At 60% WFPS, and when the soil microcosms incubated at 15°C:** When soil samples contaminated by Cd and incubated at 15°C, the NO emissions from Ramann-type were less than the NO emitted from clay loam soil samples. Clay loam brown forest soil samples were more sensitive to the highest concentration (24 mg Cd).

Pb concentrations were more inhibiting the NO emission in clay loam brown forest soil originated from Gödöllő than from Ramann-type brown forest soil type collected from Keszthely. Also, the NO emission rates from Keszthely and Gödöllő were more inhibited by Pb than Cd.

3. **When Ramann-type soil microcosms of 60% WFPS incubated at 37°C:** The amounts of NO detected from microcosms of soil contaminated by Pb is smaller than those detected when the soil contaminated by Cd.

4. It was found that when the soil samples had 60% WFPS and incubated at 15°C, the amounts of NO detected were the lowest when compared with the soil samples incubated at 37°C or had 30% WFPS and incubated at 15°C.

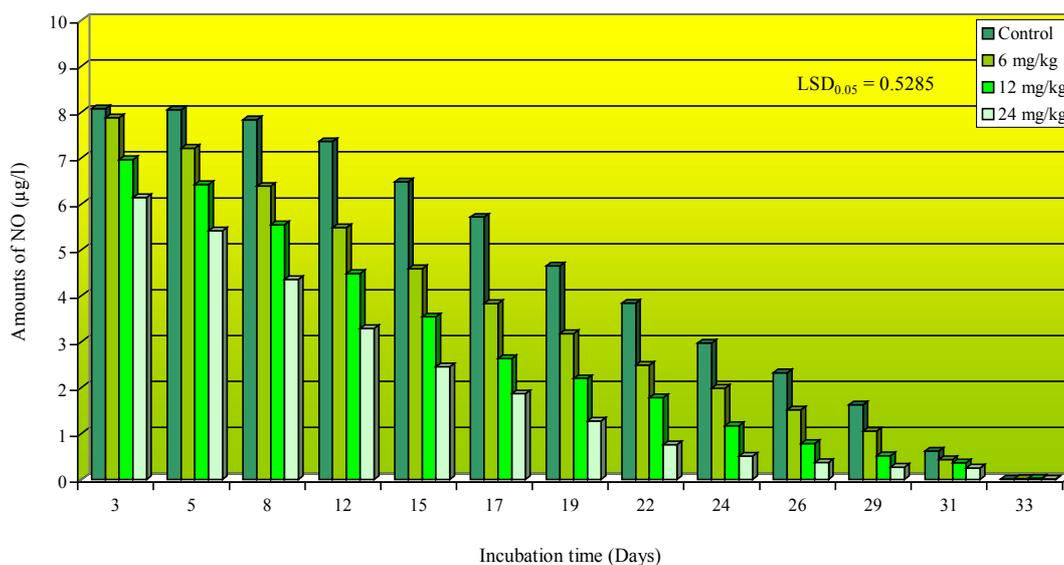


Fig. 5. Nitric oxide amounts detected in microcosm containing Ramann's brown forest soil (Keszthely) of 60% WFPS treated with different concentrations of Cd and incubated at 15°C

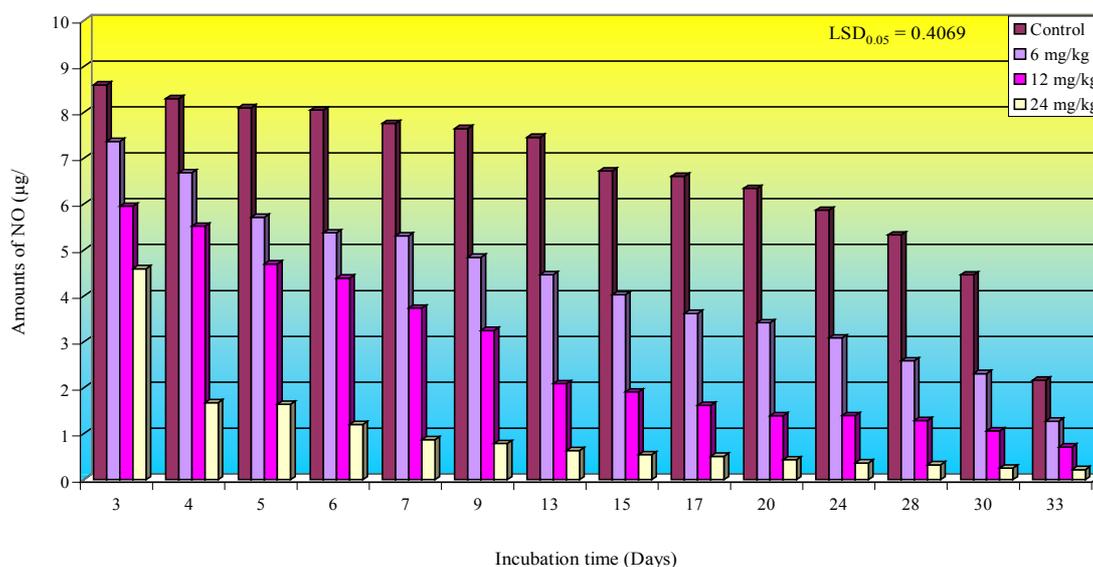


Fig. 6. Nitric oxide amounts detected in microcosm containing brown forest clay loam soil (Gödöllő) of 60% WFPS treated with different concentrations of Cd and incubated at 15°C

4.5. EMISSIONS OF N₂O UNDER THE STRESSES OF HEAVY METALS

1. At 60% WFPS, it was found that N₂O emission rates were more inhibited by highest concentrations of Cd than of Pb, when the soil samples incubated at 15°C. The amounts of N₂O emitted from Ramann-type from Keszthely soil samples were more sensitive to Cd than the amounts of N₂O emitted from clay loam soil samples from Gödöllő. The amounts of N₂O emitted from Keszthely soil samples were less than the amounts of N₂O emitted from Gödöllő when the soil samples contaminated by Pb.

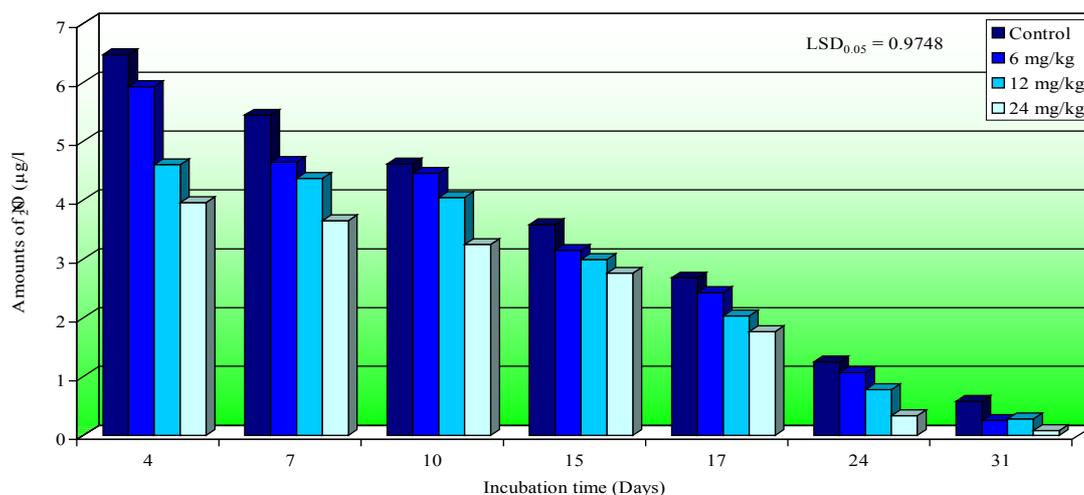


Fig. 7. Nitrous oxide amounts detected in microcosm containing Ramann's brown forest soil (Keszthely) of 60% WFPS treated with different concentrations of Cd and incubated at 37°C

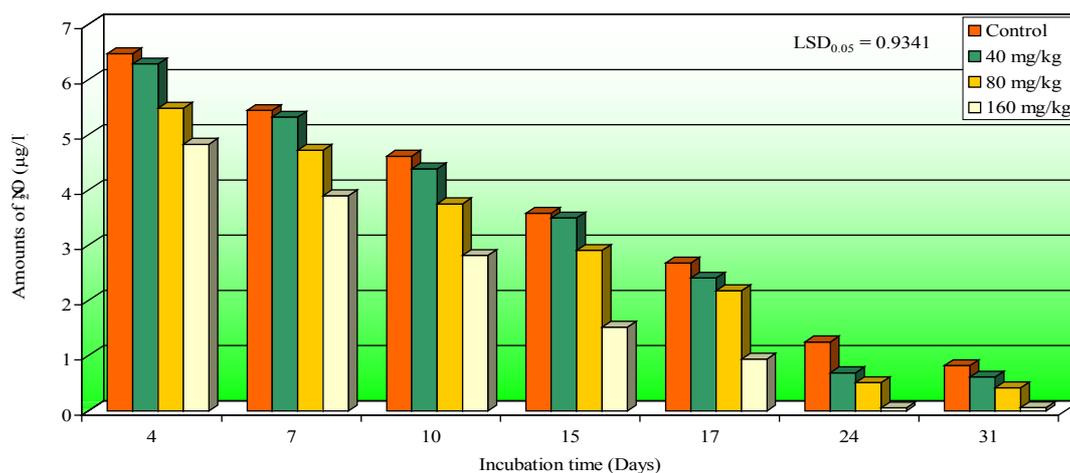


Fig. 8. Nitrous oxide amounts detected in microcosm containing Ramann's brown forest soil (Keszthely) of 60% WFPS treated with different concentrations of Pb and incubated at 37°C

2. In Ramann-type brown forest soil of Keszthely, N_2O emission rates were more inhibited by Pb than Cd when the soil microcosms contain 60% WFPS moisture regime and incubated at 37°C.

4.6. EMISSIONS OF CO_2 UNDER THE STRESSES OF HEAVY METALS

1. When the two brown forest soil samples of Ramann-type and clay loam contained 30% WFPS, contaminated by different concentrations of Cd and Pb and incubated at 15°C, the detected amounts of CO_2 were lower in Keszthely Ramann-type soil samples than those amounts of CO_2 emitted from microcosms of clay loam of Gödöllő.

2. The amount of CO_2 emitted was less in Cd contaminated soil samples than those contaminated by Pb from Ramann-type and clay loam soil originated from Keszthely and Gödöllő, respectively, at moisture regime 30% WFPS and incubation temperature 15°C.

3. At moisture regime was 30% WFPS and incubated at 15°C, the rates of CO_2 emissions were increased by Pb contamination in the soil microcosms of both soils from Keszthely and Gödöllő. But

the rates were decreased by increasing the concentrations of Cd in Gödöllő soil samples and in Keszthely soil samples; the emissions were decreased at 24 mg Cd.

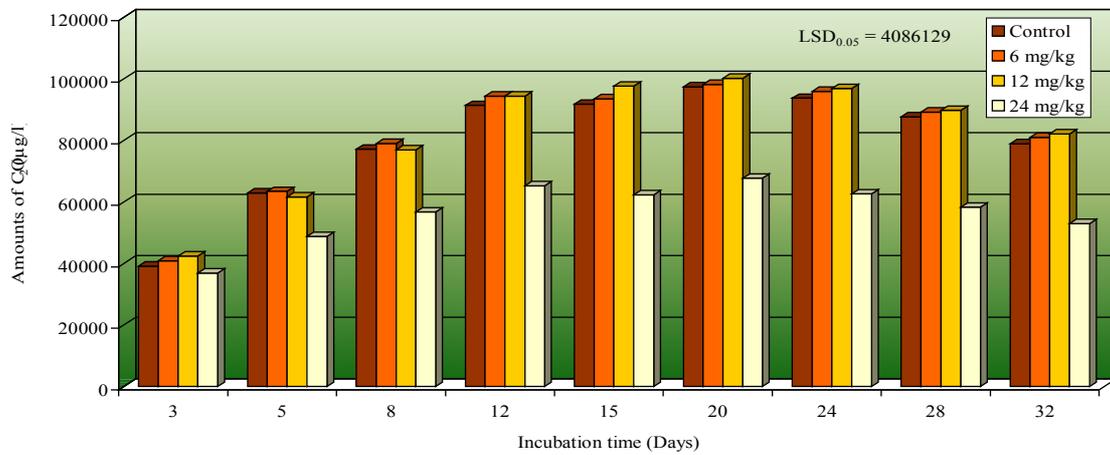


Fig. 9 Carbon dioxide amounts detected in microcosm containing Ramann's brown forest soil (Keszthely) of 30% WFPS treated with different concentrations of Cd and incubated at 15°C

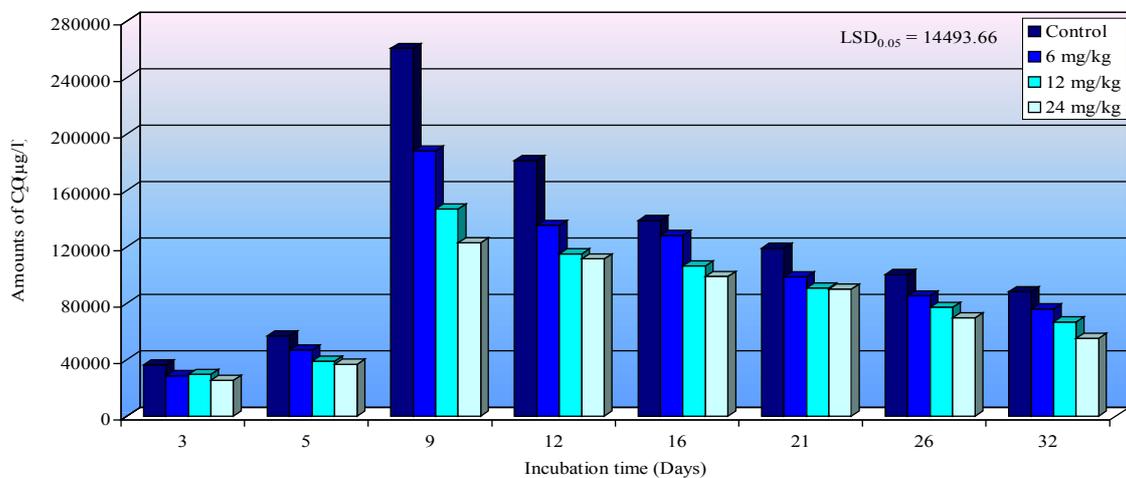


Fig. 10. Carbon dioxide amounts detected in microcosm containing brown forest clay loam soil (Gödöllő) of 30% WFPS treated with different concentrations of Cd and incubated at 15°C

4. When the soil moisture regime was at 60% WFPS and soil microcosms contaminated by Cd or Pb and incubated at 15°C, the amounts of CO₂ emitted from microcosms of clay loam soil of Gödöllő were more than those amounts emitted from Ramann-type soil of Keszthely. The rate of emission from clay loam soil microcosms was approximately the double amount emitted from Ramann-type soil. The increases in the emission may be due to the increases in concentrations.

5. Emission rates of CO₂ from Ramann-type soil with 60% WFPS contaminated by Cd and incubated at the 37°C were higher than those emitted from microcosms of Pb contaminated soil.

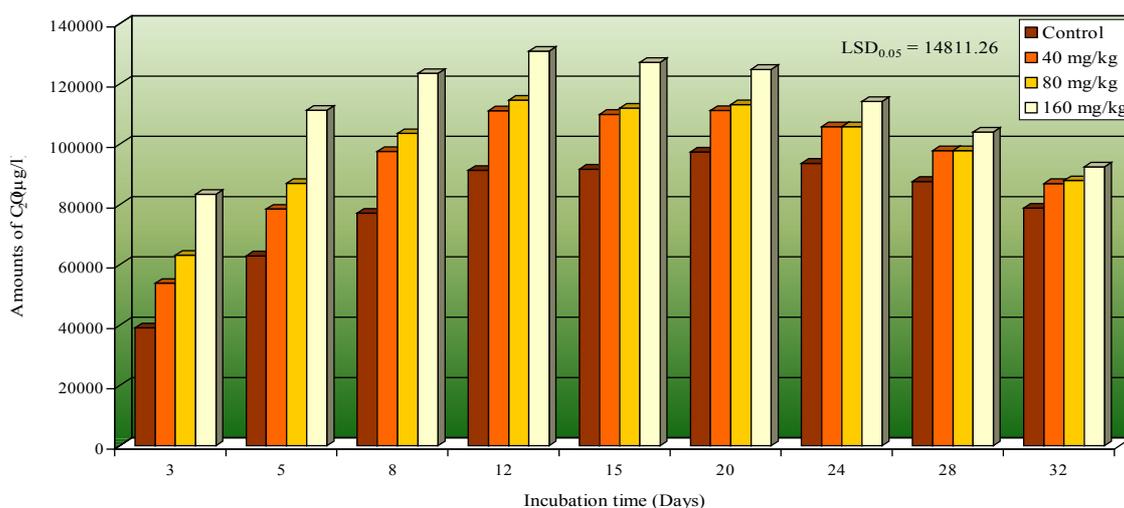


Fig. 11. Carbon dioxide amounts detected in microcosm containing Ramann's brown forest soil (Keszthely) of 30% WFPS treated with different concentrations of Pb and incubated at 15°C

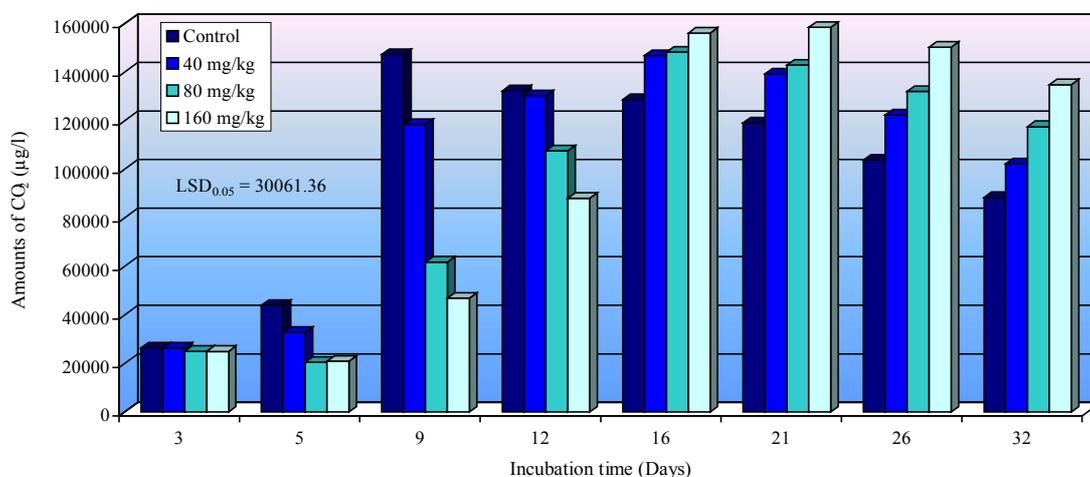


Fig. 12. Carbon dioxide amounts detected in microcosm containing brown forest clay loam soil (Gödöllő) of 30% WFPS treated with different concentrations of Pb and incubated at 15°C

5 CONCLUSION

Further research is required to investigate how the large heavy metal concentrations might affect plant characteristics. The inhibitor/suppression technique used was confirmed to be flawed as negative values for nitrifier denitrification were obtained.

Our study showed that the main factors that regulate the NO emissions in our studied soils are pH and WFPS. Our results can be used to improve current estimates of NO and N₂O emissions from the investigated ecosystems where information is limited.

Incorporation of heavy metals reduced NO, N₂O and CO₂ emissions (in some cases). This reduction was quantitatively dependent on heavy metal concentration, lower concentrations of heavy metals inducing higher emission rates than higher heavy metal concentrations.

A further conclusion is that the trace gases emissions in soils amended with heavy metal is not a constant but dependent on other environmental factors such as soil conditions, moisture, temperature, etc.

6 NEW SCIENTIFIC RESULTS

According to objectives the new scientific results are presented in two groups:

- A. Heavy metal contamination strongly influences the respiration and aerobic bacterial community in agricultural soil. The recoveries and bioavailability of contaminant amounts of Pb, Cd and Co were significantly higher at the 6th week of the incubation intervals than at 1st and 3rd incubation week. Linear regression and correlation indicated no significant differences between the metal recoveries in the uncultivated and wheat cultivated clay loam brown forest soil samples originated from Gödöllő. Also, the CO₂-released was more from uncultivated than cultivated soil samples. Cd was more toxic metal and causes a decrease in the bacterial population density in both soil samples followed by Pb and Co. The toxicity decreasing order of the tested metals was Cd > Co > Pb.
- B. The microcosm's experimental model proved to be a suitable tool for detecting the effect of factors (moisture, temperature and heavy metal) influencing the CO₂, N₂O and NO release from two agricultural soil types (1. Ramann-type brown forest soil originated from Keszthely, and 2. clay loam brown forest soil originated from Gödöllő). Based on these results, it appeared that soil characteristics are the primary factors affecting spatial emission variability in the soil sites. In my experiments in this sense the following result were achieved:
- 1 When the microcosms of Keszthely (pH = 7.55) and Gödöllő (pH = 5.56) soil samples of moisture regime 30% WFPS, contaminated with various contamination doses of Cd and Pb and incubated at 15°C, NO emission from Keszthely soil was more inhibited by Cd than by Pb. While Pb more inhibited the NO emission than Cd in Gödöllő soil microcosms. Moreover, at soil moisture 60% WFPS, and incubated at 15°C the NO emissions from Keszthely microcosms were less than the emission from Gödöllő soil. When Keszthely soil microcosms of 60% WFPS incubated at 37°C, the amounts of NO detected from microcosms of soil contaminated by Pb is smaller than those detected when the soil contaminated by Cd.
 - 2 The amounts of N₂O emitted from Keszthely soil microcosms were less than the amounts of N₂O emitted from Gödöllő microcosms when the soil samples had moisture regime of 60% WFPS, contaminated by Pb and incubated at 15°C. In Keszthely soil microcosms. N₂O emission rates were more inhibited by Pb than Cd when the microcosms incubated at 37°C.
 - 3 At moisture regime was 30% WFPS and incubated at 15°C, the rates of CO₂ emissions were increased by Pb contamination in the soil microcosms of both soils, and the emission rates were decreased by increasing the concentrations of Cd in Gödöllő and Keszthely microcosms. When the soil moisture regime was at 60% WFPS and soil microcosms contaminated by Cd or Pb and incubated at 15°C, the amounts of CO₂ emitted from microcosms of Gödöllő were more than the detected amounts emitted from the microcosms of Keszthely.
 - 4 The emission rates from clay loam soil of Gödöllő microcosms were approximately the double amount emitted from Ramann-type of Keszthely soil. The increases in the emission may be due to the increases in concentrations. In Keszthely soil of 60% WFPS contaminated by Cd and Pb and incubated at the emission rates of 37°C, the amounts of CO₂ emitted from Cd contaminated soil in the microcosms were higher than those amounts of CO₂ emitted from microcosms of Pb contaminated soil of Keszthely, too.

A *These results were published in:*

- ALGAIDI A.A. et al. (2005): Effect of heavy metals in soil microbial processes and population. *Magazine of Sebha University*. Sebha, Libya. **12**: 1-6.
- ALGAIDI A.A. et al. (2006): Impact of lead, cadmium, and cobalt on soil respiration and microbial content under in vitro conditions. Proc. VII. Intern. Ph.D. Students Conference. RNDr. M. Slábová, Ing. Z. Sýkorová (Eds.). 4th April 2006. University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic. pp.: 7-16. ISBN 80-7040-847-2.
- ALGAIDI A.A. et al. (2007): A szennyező nehézfémek hatása a talajbaktériumok mennyiségére, és a talajlégzésre *in vitro* körülmények között. *Agrokémia és Talajtan*, **56**: 353-366.

B **These results were published in:**

- ALGAIDI A.A. et al. (2005): Effect of temperature and different C/N ratios on gas emissions (N₂O, NO and CO₂) from brown forest soil. "Agriculture in Central Europe Potentials and Risks" 47th Georgikon Scientific Conference and 15th ÖGA Annual Meeting, 2005. szeptember 29–30., Keszthely. CD-R adathordozón sokszorosítva, made by ///VTCD Gn20050929 .ISBN 963 9639 03 6
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Keywords: NO, N₂O and CO₂ emission; denitrification, nitrification; heavy metals, soil type; incubation temperature, moisture regime, soil respiration, aerobic heterotrophic bacterial population, bioavailability of heavy metals.

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8. The Scientific Publications related to the Dissertation Work

1. REFREE PAPERS PUBLISHED IN JOURNAÉS:

1.1. IN ENGLISH LANGUAGE:

KAMPFL GY. - KRISTÓF K. - ALGAIDI A.A. - BAYOUMI HAMUDA H.E.A.F. - HELTAI GY. (2007): Study of NO_x and CO₂ production of cultivated soil in closed microcosm experimental system. *Microchemical Journal*, **85**: 31-38.

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ALGAIDI A.A. - BAYOUMI HAMUDA H.E.A.F. - NÓTÁS ERIKA - KRISTÓF K. - KAMPFL GY. - ISSA IBRAHIM A. - HAMID Y.S. -.8 HELTAI GY. (2005): Effect of heavy metals in soil microbial processes and population. *Magazine of Sebha University*. Sebha, Libya. **12**: 1-6

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ALGAIDI A.A. - BAYOUMI HAMUDA H.E.A.F. - NÓTÁS ERIKA - KRISTÓF K. - KAMPFL GY. - ISSA IBRAHIM A. - HAMID Y.S. -.9 HELTAI GY. (2004): Bioavailability of Zn²⁺ and Pb²⁺ on soil microbial respiration and population. *In: Az MTA Szabolcs-Szatmár-Bereg Megyei Tudományos Testülete évkönyvei Vol.: 13. Tudományos Ülés, Nyíregyháza. S. Kókai (Ed.). Kápitális Nyomdaipari és Kereskedelmi Kft, Debrecen. pp: 411-416*

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