

Impact of zinc and lead on soil respiration and microbial content under *in vitro* conditions

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ABSTRACT

The habitation and activities of soil aerobic bacteria living in the sandy clay soil were affected by the toxicity of Zinc and Lead ions at different levels. Their inhibitory effect was manifested directly in the decrease of the total aerobic bacteria counts in the soil and physiological activity of the soil aerobic bacteria. Aerobic bacteria population generally decreased similarly to the CO₂-production in response to the toxic effects of the investigated metal ions. The soil samples were metabolically activated by C,N and P in the form of sodium nitrate, potassium phosphate and glucose, respectively as substrate-induced respiration. The inhibitions of biological activities in the soil samples were appeared even after three weeks, but highly significant effects were obtained after six-week incubation period. Lead had smallest effects on decreasing CO₂-production at different incubation intervals. The strongest inhibition of the gas production was detected by the influence of Zinc ions.

Keywords: heavy metals - soil aerobic bacteria-toxicity

INTRODUCTION

The fertility of natural soil ecosystem depends significantly on the rate of turnover of soil organic matter, mediated by the soil microbial biomass. In agricultural ecosystems, soil fertility can be increased by applications of inorganic or organic fertilizer. The fertility of natural ecosystem, however, depends almost entirely on natural microbial processes, including N₂-fixation, the mineralization of organic matter of N, C, P and S and organic matter transformations, all mediated by the soil microbial biomass. Soil microbial biomass is considered to be the agent of breakdown of organic matter in the soil, although the importance of its size in relation to nutrient cycling and decomposition processes is poorly understood. It is well known that microbial biomass increases when organic materials are applied to soil. However, Brookes & McGrath (2007) showed that microbial biomass in soil

from the classical Woburn Market-garden Experiment which had been supplied with an aerobically digested lagoon-dried sewage sludge was half that in soil that had received farmyard manure. Sludge application increased soil metal concentrations up to current limits with the exception of Cd which was three to five times the maximum limit (Brookes *et al* 1999, McGrath *et al* 1988). However, in other studies with the same soils (Brookes *et al* 2000) there were no effects of metal concentration on respiration rate. Therefore, the respiration rate per unit weight of biomass was considerably greater in the metal-contaminated soil. This response may not necessarily be detrimental, but could be explained because metal-sensitive species have been replaced by other more tolerant groups that respire at a higher rate. Alternatively, the effect could be interpreted as a metal-induced stress response. Soluble metal salts have been applied to soils often as a basis for

assessing effects of heavy metals in sewage sludge on soil N transformation were summarized by Doelman (2006). It was concluded that, in general, increasing soil metal levels may reduce the composition and diversity of the total soil microbial population, causing a shift towards resistant strains that become dominant in such soils. Increasing abundance of resistant strains under elevated metal conditions enables the continuation of essential processes that maintain soil fertility (Barkay *et al* 1985).

The supply of mineralized C, N, and P from soil organic matter, the decomposition of plant and animal residues and the maintenance of soil structure are all-dependent upon the correct functioning of the soil microbial ecosystem Doelman & Haanstra (1999) carried out short-term and long-term effects of Cd, Cr, Cu, Ni, Pb and Zn on soil microbial respiration in relation to a biotic soil factor. It was found that in short-term effects of Pb in sand were distinct, while in sandy loam, the inhibitory effect was not significant, but after 43 weeks, it had increased significantly. In silty loam and clay, there were no significant inhibitory effects. In general, the heavy metals were found to be more toxic during the first eight weeks than after an extended period. It was concluded that toxicity of heavy metals in soil decreases with time, as well as the a biotic factor was found to be the dominant factor in decreasing the toxicity of Pb and to a lesser extent of Zn.

The objectives of present study were: 1) to measure soil respiration (CO₂ evolution) as bio indicator parameter of soil contamination. 2) to study the effect of heavy metal on microbial survival and activity in heavy metal amended soil under laboratory incubations. 3) to detect the bioavailability of investigated metal in assayed soil.

MATERIALS AND METHODS

Soil and treatments: The sandy clay soil samples were collected from cultivated area. The physical and chemical properties of the studied soil are: pH_(KCl) 6.97, humus content 1.21%, C:N ratio 12.4, and the following properties are in mg kg⁻¹: NH₄⁺-N (3.2), NO₃⁻-N (4.5), SO₄⁻² (4.6), K₂O (123), P₂O₃ (209), Cu (1.86), Mg (206), Mn (195), Cd (0.065), Co (1.57), Pb (8.48), and Zn (7.22). The experiment was carried out by air-dried soil. The determination of bioavailability and the effect of heavy metals (Pb and Zn) on CO₂-production and total count of the aerobic bacterial population in soil samples were carried out in control and treated soil samples. Materials in the form of sodium nitrate (170 mg kg⁻¹), potassium phosphate (50 mg kg⁻¹), and glucose (3 mg kg⁻¹) were as sources of N, P, and C, respectively, and activated the soil samples. All activated, non-activated, cultivated soil samples were treated with three concentrations of Zn in form of ZnCl₂ (1.5, 3, and 6 mg kg⁻¹), and Pb in the form of PbCl₂ (40, 80 and 160 mg kg⁻¹). The heavy metal amended soil samples were incubated for six weeks. The counting the total aerobic bacterial population and the amount of CO₂-production in each soil sample were investigated after first, third and six weeks of incubation at 28 °C.

Determination of CO₂-production: For measurement of CO₂-production, a 0.5 kg of the heavy metal treated soil was filled in 1.5 l-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, which represented the 1) respiration due to litter decomposition, 2) root respiration, 3) rhizo-microbial respiration (i.e. microbial respiration utilizing C directly derived from living roots), and 4) microbial respiration

utilizing native soil organic matter. Applied method of Wardle & Parkinson (1991) was used for simultaneous determination of NaOH and Na₂CO₃ content in our experimental soil samples.

Determination of total available fraction: MI -08-1735-1990 is the Hungarian technical directive method which was used to detect Zn and Pb content in the soil samples. Five gram of air-dry and fine grounded soil sample was weighed and shaken with 25 cm³ 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.

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.

All experiments were carried out in three replicates, and the results were represented by the means of the replicates.

RESULTS AND DISCUSSION

The effect of CNP and heavy metals on CO₂-production and bacterial population of sandy clay soil was studied in laboratory conditions. This study is concerned with the effect of CNP and heavy metals Zn and Pb on CO₂-production as well as bacteria populations of sandy clay soil during one, three and six weeks incubation. After a week incubation, the recovery of Zn and Pb concentrations added to CNP treated sandy clay soils was determined in the nitric acid soluble fraction. Data presented in Fig.1. Show that the addition of inorganic forms of Zn and Pb significantly increases the mobile (HNO₃ soluble) fraction of these metals but after week incubation their concentration does not reach the 100% recovery. It can be observed that the methods used for biomass, CO₂-evolution and total bacteria number, are

both suitable to be indicators for biomass measurements through which we can have an idea about the soil fertility. It is known that microbial biomass plays an important role in mineral nutrition of soil.

The effect of heavy metal treatments on CO₂ reduction in CNP fertilized soil is showed in Fig. 1. It is clear from data presented that CNP treated sandy clay soil showed higher rate of CO₂-production, estimated by 0.57, 0.58 and 0.63 ml/100 g soil/hour, in sandy clay soil comparing with 0.31, 0.29 and 0.30 ml/100 g soil/hour in CNP sandy clay soil during all three different incubation periods (1st week, 3rd week and 6th week).

Data recorded in Fig. 2. Show that CNP treated sandy clay soil samples have higher bacterial population (which are representing in term of log of bacterial colonies) than in untreated ones during the three different periods (1st week, 3rd week and 6th week). These previous results are in accordance with the results found by De Haan *et al.* (1999) who reported that the supply of mineralized C, N and P from soil organic matter, the decomposition of plant and animal residues and the maintenance of soil structure are all dependent upon the correct functioning of the soil microbial ecosystem. The beneficial effects of sewage sludge's due to the extra organic matter N or P supplied may be short lived. These results are also in accordance with investigation of Hossain *et al.* (2005). Hossain *et al.* (2005) have discussed the effects of fertilizer. It was reported that only the lime plus P and N plus P treatments significantly affected soil microbial biomass C content. The N plus P treatment increased biomass C content. Microbial specific respiratory activity was higher in the unfertilized treatments. Nannipieri *et al.* (1990) stated that the changes in CO₂-evolution were related to glucose concentrations of mineral nutrients. Higher initial rates of CO₂-evolution were noted after the addition of P and glucose to N amended soil at C: P ratios greater than 30:1. It's also shown from such data presented in Fig. 1. Those heavy metals, Zn and Pb have significant effect on CO₂-production of tested sandy clay soil at different incubation periods (1st week, 3rd week, and 6th week).

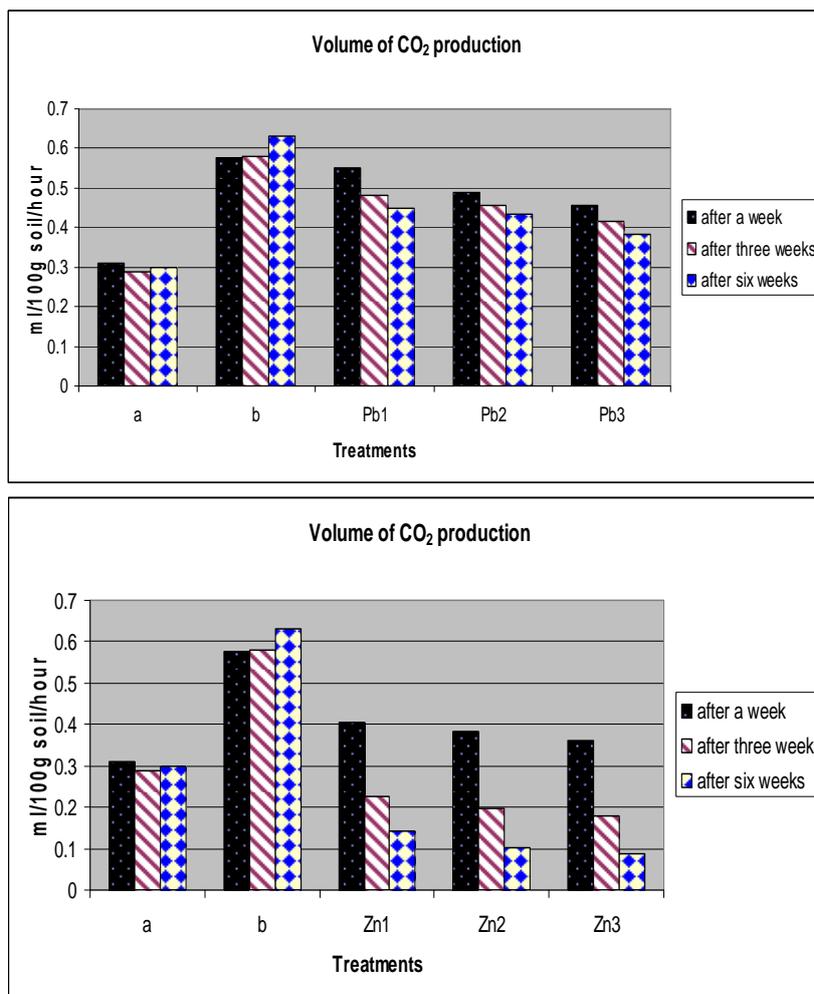


Fig. 1: Average of CO₂-production volume at 25°C, 0.1MPa (ml/100 g soil per an hour) of sandy clay soil treated by CNP and heavy metals for different three periods (a: CNP untreated soil, b: CNP treated soil).

Pb at different concentrations, 40, 80, and 160 mg/kg have the smallest effect on decreasing CO₂-production either in sandy clay soil incubated at different periods. It can be shown also from data that Zn has higher effects in decreasing the amount of CO₂-production at the different concentration used, 1.5, 3, and 6 ppm for Zn either in sandy clay soil at the three different periods. Baath (2002) studied the effect of heavy metals in soil microbial processes and populations. He established that the relative toxicity of different metals decreased in the order Cd > Cu > Zn > Pb. His results were similar to our result in which our investigation showed that the relative

toxicity of tested metals decreased in the order Pb > Zn.

A significant effect of heavy metals, lead and zinc on bacterial population has been found in CNP treated or untreated soils (Fig. 2.). It is shown that Zn has much more significant effect in decreasing bacterial population than Pb that has a little effect. It is also clear that the higher concentration of Pb and Zn cause decreases in total bacterial population. Concerning the effect of Pb and Zn on the CO₂-production and its relation to the sampling time, it is obvious that there is a clear trend. In the two heavy metal treatments, (Pb and Zn), the highest CO₂-production was measured after the 1st week incubation followed by the 3rd and 6th week

respectively. The same effect was found for the bacterial population. This result is in accordance with the result of Leita *et al.* (1995) who reported that the addition

of Pb did not have any significant inhibitory effect on the level of microbial biomass.

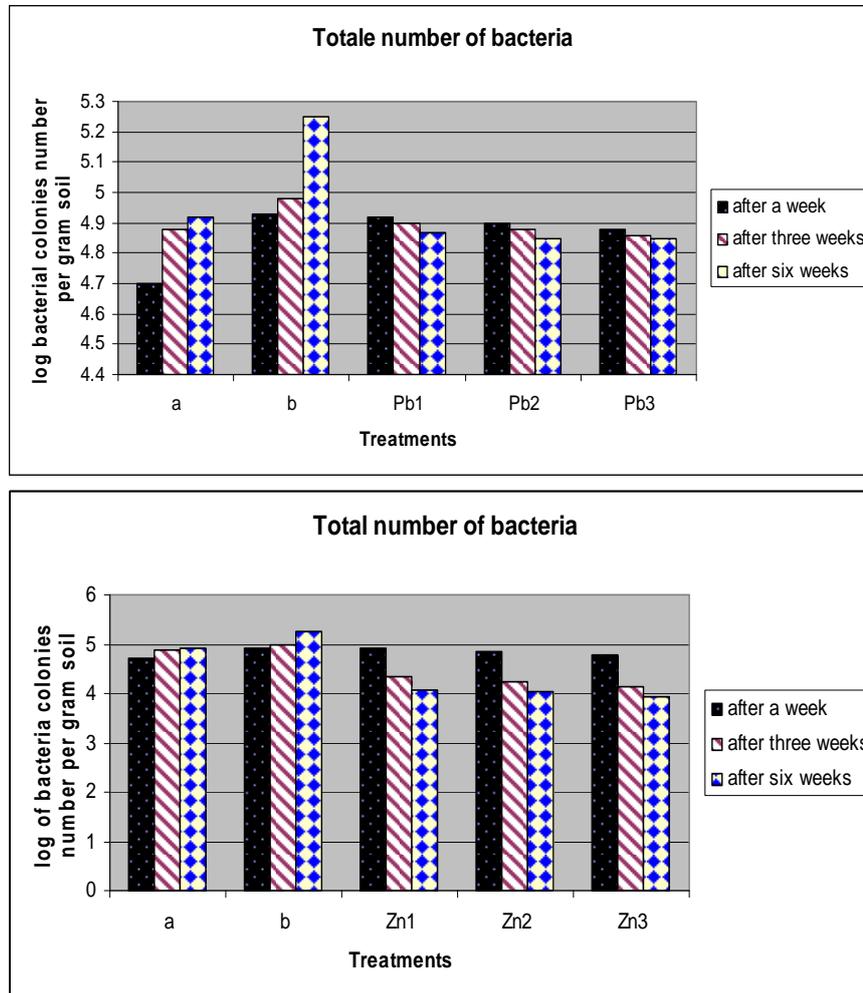


Fig. 2: Average of log of total bacterial number of sandy clay soil treated by CNP and heavy metals after different three periods (a: CNP untreated soil, b: CNP treated soil).

CONCLUSION

It can be concluded that 1. Incubation time is an important factor for metal recovery in soil, 2. Soil respiration is a detectable method for measurement the toxicity of the metal and it can be considered that this method might be an indicator of soil metal contamination, and 3. The heavy metals have the important concern in microbial populations in the investigated soil samples.

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ARABIC SUMMARY

أثر الزنك والرصاص على تنفس التربة ومحتواها الميكروبي في ظروف مختبريه

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تأثر عيش ونشاطات بكتيريا التربة الحي هوائية التي تعيش في التربة الطينية الرملية بسمية الزنك وأيونات الرصاص في مستويات مختلفة. وقد ظهر التأثير الكابح لهدين المعدين مباشرة في انخفاض بكتيريا التربة الحي هوائية التي نقص عددها عموماً بالتماثل مع إنتاج ثاني أكسيد الكربون استجابة للتأثيرات السمية للأيونات المعدنية المدروسة. وقد تم تنشيط عينات التربة أيضاً بالكربون والنيتروجين والفسفور في شكل نترات الصوديوم وفوسفات الصوديوم والجلوكوز على التوالي كتنفس مستحث بركيزة. وقد ظهرت كرابح النشاطات البيولوجية في عينات التربة حتى بعد انقضاء ثلاثة أسابيع ولكن تم الحصول على تأثيرات ملحوظة مرتفعة بعد فترة حضانة مدتها ستة أسابيع. وكان الرصاص الأقل أثراً في انخفاض إنتاج ثاني أكسيد الكربون عند فترات حضانة مختلفة. وقد تم اكتشاف أقوى كابح لإنتاج الغاز بتأثير أيونات الزنك.