10.7251/AGSY1303881DJ

EFFECT OF HEAVY METALS ON THE MICROBIAL ACTIVITY OF SOILS UNDER RED CLOVER

Dragutin ĐUKIC[1], Leka MANDIC[1*], Gordana ĐUROVIC[1],
Marijana PESAKOVIC[2], Ivana BOSKOVIC[3]

[1]University of Kragujevac, Faculty of Agronomy, Čačak, Serbia
[2]Fruit Research Institute Čačak, Serbia
[3]Faculty of Agriculture, University of East Sarajevo, Republic of Srpska, Bosnia and Herzegovina
*(Corresponding author: lekamg@kg.ac.rs)

Abstract

Heavy metals reach the environment, primarily soils, mostly through human activities. They enter certain biological links in nutrient and energy cycles, thus becoming a source of many environmental problems and health issues in both people and animals. Microorganisms as an important link in the cycling process can serve as indicators of both soil pollution and potential toxicity to other biological systems.

The objective of this study was to evaluate the effect of different concentrations of lead (60; 120; 250 mg kg^{-1} soil) and mercury (1,0; 2,0; 4,0 mg kg^{-1} soil) on total microbial count and Azotobacter in two soils, a Vertisol and an alluvium under red clover cultivation in three growing seasons. The experiment was conducted under greenhouse conditions at the Faculty of Agronomy, Čačak.

Numbers of the microorganisms tested were determined by indirect counting methods involving plating out a soil suspension onto appropriate selective culture media.

Depending on the type and concentration involved, heavy metals had a significant effect on soil microbial count in the alluvium and vertisol during the red clover growing season. Low concentrations of lead and mercury (60 mg kg^{-1} and 1 kg^{-1} soil, respectively) did not lead to significant changes in total microbial and Azotobacter counts. At 250 mg kg^{-1} soil, lead induced a decrease in total microbial and Azotobacter counts. Mercury had a markedly higher depressive effect on soil microorganisms, with concentrations of 2 and 4 mg kg^{-1} soil significantly reducing the total microbial and Azotobacter counts.

Keywords: heavy metals, microorganisms, plant, soil.

Introduction

Soil is among major natural resources invaluable to mankind as a whole, rather than to a single generation, nation, group of people or an individual. Soil formation is a slow process compared with the rapid rate of soil degradation resulting primarily from uncontrolled human-related activities. In this regard, heavy metals have received particular attention as they are very difficult to degrade, in contrast to organic pollutants (Kabata-Pendias, 2001), and show a tendency to enter the biological nutrient cycle, thus causing many environmental problems and health issues (Cornelija and Franc, 2004; Majer et al., 2002). Soil remediation must include stabilisation techniques that are typically short-term and costly (Brovn, 1997). Therefore, the central tendency of modern integrated primary production of food is to reduce and control the use of agrochemicals and other types of indirect soil pollution. A variety of methods are used to indicate the presence of heavy metals in the soil. Recent research has justified the use of microorganisms as the most active elements of the soil to this end (Rundella et al., 2005; Rajapaksha, 2004). The effects of heavy metals on soil microorganisms are dependent on their type and concentration, soil physicochemical characteristics, plant cover, cultural operations, etc. Some of them (cobalt, chromium, nickel, iron, zinc, etc.), when found at low concentrations, play an important role in plant and microbial nutrition, whereas some others, even at low concentrations, produce adverse effects on the biogeosphere (Bruins et al., 2000). High concentrations of either group are toxic to soil microorganisms (Lee et al., 2002). Toxicity is generally exhibited through their adsorption to the cell surface, resulting in changes in cytoplasmic membrane permeability, cytoplasmic protein coagulation, enzyme inactivation, DNA damage, etc. (Sobolev and Begonia, 2008). This indirectly leads to changes in the structure of microbial cenoses in the soil as well as to the emergence of heavy metal tolerant microorganisms (Spain and Alm, 2003) or toxinogenic microorganisms, which indirectly induces soil pollution.

The objective of this study was to evaluate the effect of different concentrations of lead (60; 120; 250 mg kg^{-1} soil) and mercury (1,0; 2,0; 4,0 mg kg^{-1} soil) on the total microbial and Azotobacter counts in a vertisol and an alluvial soil under red clover.

Material and methods

The experiment was established under controlled greenhouse conditions at the Faculty of Agronomy, Čačak. The test plant was red clover cv. “Kruševačka K32” sown on two soil types (factor B) viz. a degraded alluvium (pH_{KCl}- 6.4; humus-1.7%; N-0.1%; P_2O_5-0.068 mg g^{-1}; K_2O- 0.1 mg g^{-1}) and a vertisol (pH $_{KCl}$-5.01; humus- 3.59%; N-0.20%, P_2O_5-0.029 mg g^{-1}; K_2O-0.26 mg g^{-1}) in 15 dm^3 containers in five replications, according to a randomised block design. Soil samples were collected from areas confirmed to be unpolluted by laboratory analysis. Red clover was planted at a spacing of 5-7 cm and a depth of 1cm. Upon planting, the soil was subjected to experimental treatments involving the addition of an aqueous solution of heavy metals (factor A): lead [as $Pb(NO_3)_2$] at 60; 120; 250 mg kg^{-1} soil and mercury (as $HgCl_2$) at 1.0; 2.0; 4.0 mg kg^{-1} soil. Treatments without the addition of heavy metals were used as the control.

The soil was sampled for microbial analysis using a laboratory probe three times during the red clover growing season (factor C): I- at the 2-3 leaf stage; II- at the intensive plant growth stage; III- at budding. Microbial analysis involved determination of the total microbial and Azotobacter counts by indirect plating on a soil agar (Pochon and Tardieux, 1962) and Fyodorov’s medium (Anderson, 1958), respectively. Results were subjected to a three-factor analysis of variance, and the significance of differences was assessed by the LSD test (Statistica SPSS 5).

Results and discussion

The results of the present study show the dependence of the effect of heavy metals on their type and concentration, sampling date and soil type (Tabs. 1-4).

Overall, the low levels of lead (60 and 120 mg kg^{-1} soil) did not have a significant effect on the total microbial count. However, they exhibited selective effects, depending on soil type and red clover growing stage (Tab. 1). Namely, an initial depressive effect of lead at a concentration of 120 mg kg^{-1} was observed only on the alluvial soil, as opposed to the slight stimulating effect on the vertisol. During the growing season, the differences levelled off on the alluvium as well, reaching the level observed in the control treatment. The lead concentration of 250 mg kg^{-1} soil (2.5-fold higher than the permissible levels prescribed by the European Council Regulation 2092/91) led to a significant decrease in the total microbial count, particularly in the alluvium (Tab. 1). The slight decline in microbial numbers in the vertisol was likely due to its somewhat more favourable physicochemical properties (primarily the organic component or humus content). This suggestion is supported by the results of Bais et al. (2006) and Alidoust et al. (2012), who underlined the importance of organic matter and plant exudates in reducing the mobility of some heavy metals in the soil, and their availability to both plants and other biological components in the soil. The lower effect of heavy metals at final growth stages of red clover may be associated with the cometabolic effect of organic root exudates and the ability for adaptation and partial detoxication of toxinogenic components by other soil microorganisms (Khan and Scullion, 2002, Rajapaksha et al., 2004).

Tab. 1. Effect of lead (Pb_1 – 60.0; Pb_2 – 120.0; Pb_3 - 250 mg kg^{-1} soil) on total microbial count (10^6 g^{-1} absolutely dry soil) in the alluvium and vertisol during red clover growing season

A		Control		Pb_1		Pb_2		Pb_3		$\bar{X}$C
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	
C	I	88	75	90	82	71	77	70	60	76.62
	II	98	77	101	79	93	80	75	73	84.50
	III	140	96	136	101	137	102	131	103	118.75
$\bar{X}$		95.66		98.16		93.33		85.33		
$\bar{X}$		Alluvium	102.50							
		Vertisol	83.75							

Lsd	A	B	C	AB	AC	BC	ABC
0.05	4.64	2.50	3.00	6.54	8.04	5.69	11.32
0.01	6.12	3.30	3.98	8.64	10.64	7.53	14.98

The application of 60 and 120 mg Pb kg^{-1} soil did not lead to significant changes in Azotobacter count throughout the red clover growing season (Tab. 2). This may be attributed to the morphophysiological characteristics of these microorganisms (they produce a mucilaginous sheath on the exterior of the cell that ensures complexation and inactivation of different toxicants, and heavy metals as well), as evidenced by the research conducted by Govedarica et al. (1993). However, the high concentration of this metal (250 mg kg^{-1} soil) significantly reduced the count of these microorganisms, particularly at the first two experimental stages in the alluvium.

Tab. 2. Effect of lead (Pb_1 – 60.0; Pb_2 – 120.0; Pb_3 - 250 mg kg^{-1} soil) on Azotobacter count (10^2 g^{-1} absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A		Control		Pb_1		Pb_2		Pb_3		$\bar{X}$C
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	
C	I	27	14	30	16	26	13	18	10	19.25
	II	29	16	27	20	31	15	27	13	22.25
	III	41	22	44	21	39	24	33	17	30.12
X		24.83		26.33		24.66		19.66		
X		Alluvium	31.00							
		Vertisol	16.76							

Lsd	A	B	C	AB	AC	BC	ABC
0.05	2.55	1.38	1.65	3.59	4.42	3.13	6.23
0.01	3.37	1.18	2.19	4.75	5.85	4.14	8.24

Mercury was found to be extremely toxic to the soil microflora; therefore, the reduction in total microbial count was also observed in treatments with 2.0 mg Hg kg^{-1} soil. An even more depressive effect of mercury was induced by the highest application rate (4.0 mg kg^{-1} sol), particularly in the alluvial soil (Tab. 3).

Tab. 3. Effect of mercury (Hg_1 – 1.0; Hg_2 – 2.0; Hg_3 – 4.0 mg kg^{-1} soil) on the total microbial count (10^6 g^{-1} absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A		Control		Hg_1		Hg_2		Hg_3		$\bar{X}$C
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	
C	I	88	75	89	70	68	63	57	54	70.50
	II	98	77	96	81	69	70	60	64	76.88
	III	140	96	142	105	113	75	69	76	102.00
$\bar{X}$		95.66		97.16		76.33		63.33		
$\bar{X}$	Alluvium	90.75								
	Vertisol	75.50								

Lsd	A	B	C	AB	AC	BC	ABC
0.05	5.82	3.15	3.81	8.25	10.08	7.10	14.31
0.01	7.68	5.17	5.04	10.92	13.35	9.42	18.93

Azotobacter was found to be highly susceptible to mercury, with its counts decreasing by about 39 and 54% in treatments with low (2.0 mg kg^{-1} soil) and high (4.0 mg kg^{-1} soil) mercury application rates, respectively, compared to the control (Tab. 4). As in the case with the soil microflora, the depressive effect of mercury on Azotobacter counts was also particularly remarkable during the early stages of the growing season and in the alluvial soil. The markedly toxic effect of mercury on most soil microorganisms was also reported by other authors (Casucci et al., 2003; Mandic et al., 2010).

Tab. 4. Effect of mercury (Hg_1 – 1.0; Hg_2 – 2.0; Hg_3 – 4.0 mg kg^{-1} soil) on Azotobacter counts (10^2 g^{-1} absolutely dry soil) in the alluvium and vertisol during the red clover growing season

A		Control		Hg_1		Hg_2		Hg_3		$\bar{X}$C
B		Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	Alluvium	Vertisol	
C	I	27	14	23	17	13	8	8	7	14.63
	II	29	16	27	14	19	13	13	8	17.38
	III	41	22	39	25	21	17	22	11	24.75
$\bar{X}$		24.83		24.16		15.16		11.50		
$\bar{X}$	Alluvium	23.50								
	Vertisol	14.33								

Lsd	A	B	C	AB	AC	BC	ABC
0.05	2.49	1.33	1.63	3.51	4.29	3.06	6.07
0.01	3.28	1.76	2.16	4.64	5.68	4.06	8.04

Overall, the counts of the microorganisms tested were considerably higher in the alluvium than in the vertisol, and increased during the red clover growing season.

Acknowledgement

This study is part of the project “Improvement of the genetic potential of forage crops and production technologies towards the sustainable development of animal husbandry” – TR 31057 funded by the Ministry of Education and Science, Republic of Serbia.

Conclusion

Depending on the type and concentration involved, heavy metals had a significant effect on soil microbial count in the alluvium and vertisol during the red clover growing season. Low concentrations of lead and mercury (60 mg kg^{-1} and 1 kg^{-1} soil, respectively) did not lead to significant changes in total microbial and Azotobacter counts. The high application rate of lead (120 kg^{-1} soil) had a depressive effect on the microorganisms tested only at the early stages of the growing season, particularly in the alluvial soil. At 250 mg kg^{-1} soil, lead induced a decrease in total microbial and Azotobacter counts. Mercury had a markedly higher depressive effect on soil microorganisms, with concentrations of 2 and 4 mg kg^{-1} soil significantly reducing the total microbial and Azotobacter counts.

Overall, the counts of the microorganisms tested were considerably higher in the alluvium than in the vertisol, and increased during the red clover growing season.

The present findings suggest that soil microorganisms can serve as an indicator of heavy metal pollution in soils.

References

Alidoust D., Suzuki S., Matsumura S., Yoshida M. (2012). Chemical Speciation of Heavy Metals in the Fractionated Rshizosphere Soils of Sunflower Cultivated in a Humic Andosol. Communication in Soil Science and Plant Analysis, 43(17), 2314-2322.

Anderson J.P.E., Domasch K.H. (1958). A physiological method for the quantitative measurement of microbial biomas in soil. Soil Boil. Biochem., 10, 215-221.

Bais H.P., Weir T.L., Perry L.G., Gilroy S., Vivanco J.M. (2006). The Role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, 57, 233-266.

Brown K.W. (1997). Decontamination of polluted soils. In: Iskandar, I.K., Adriano, D.C. (Eds.), Remediation of Soils Contaminated with Metals. Science Reviews, Northwood, UK, pp. 47–66.

Bruins M.R, Kapil S., Oehme F.W. (2000). Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety, 45, 198-207.

Casucci C., Okeke B.C., Frankenberger W.T. (2003). Effects of mercury on microbial biomass and enzyme activities in soil. Biological Trace Element Research, 94(2), 179-191.

Cornelia L., Franz R. (2004). Evaluation of heavy metal tolerance in Calamagrostis epigejos and Elymus repensrevealed copper tolerance in a copper smelter population of C. epigejos. Environ. Exp. Bot. 51 (3),199–213.

Govedarica M., Jarak M., Milošević N. (1993). Teški metali i biogenost zemljišta. In: R. Kastori (eds), Teški metali i pesticidi u zemljištu, Institut za ratarstvo i povrtarstvo , N. Sad, 47-54.

Kabata-Pendias A., Pendias H. (2001). Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL. 413 pp.

Khan M., Scullion J. (2002). Effects of metal (Cd, Cu, Ni, Pb,Zn) enrichment of sewage-sludge on soil micro-organisms amd their activities. Appl. Soil Ecol., 20, 145-155.

Lee I.S., Kim O.K., Chang Y. Y. (2002). Heavy metals concen-trations and enzymes activities in soil from a contaminated Korean shooting range. J Biosci Bioeng, 94(5), 406-411.

Majer B.J., Tscherko D., Paschke A. (2002). Efects of heavy metals contamination on micronucleus induction inTraes-candiaand on microbial enzymes activities: a comparison investigation. Mutation Res, 515, 111–124.

Mandic L., Đukic D., Pešakovic M., Šekularac G. (2010). Microbiological indication of the presence of heavy metals in soil. Novenytermeles, Vol. 59, 81-84.

Pochon J., Tardieux P. (1962). Techniques d'analyse en microbiologie du sol (Ed. La Taurelle), 111 p. St. Mandea (Seine), Paris, France.

Rajapaksha R.M.C.P., Tobor-Kapton M.A., Bååth E. (2004). Metal Toxicity Affects Fungal and Bacterial Activities in Soil Differently. Applied and Environmental Microbiology, 70(5), 2966-2973.

Renella G., Mench M., Landi L., Nannipieri P. (2005). Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils. Soil Biol. Biochem. 37, 133–139.

Sobolev D., Begonia M.F.T. (2008). Effects of Heavy Metal Contamination upon Soil Microbes: Lead-induced Changes in General and Denitrifying Microbial Communities as Evidenced by Molecular Markers. Int. J. Environ. Res. Public Health, 5(5), 450-456.

Spain A., Alm E. (2003). Implications of Microbial Heavy Metal Tolerance in the Environment. Reviews in Undergraduate Research, 2(1-6), 3-6.

Statsoft.Inc., Statistica for Windows (computer program manual) 1995. Tulsa, OK: StatSoft.Inc.