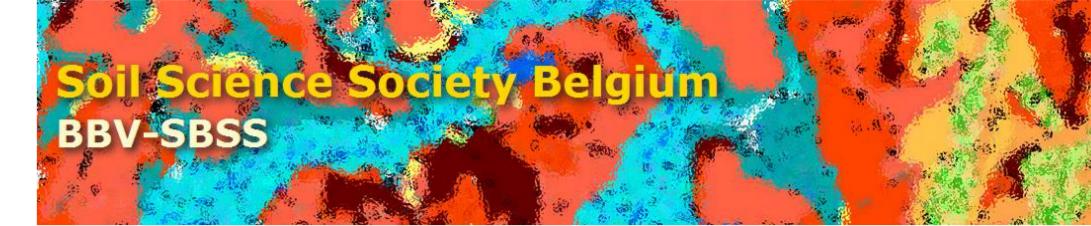


Effects of *Eisenia fetida* on metal uptake of heavy metals from polluted soils by *Vicia faba* and *Zea Mays*

A. Lemtiri, A. Liénard, F. Francis, T. Alabi, D. Cluzeau, B. Bodson, Y. Brostaux, E. Haubruge, & G. Colinet

University of Liege – Gembloux Agro-Bio Tech - Soil Science Unit
Passage des Déportés, 2. B-5030 Gembloux
alemtiri@doct.ulg.ac.be



Introduction

With industrialization and mining, heavy metals pollution of soil has become a serious environmental problem. Among metallic trace elements (MTE), cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are receiving extensive attention because of toxicological effects on plants and animals. *Z. mays* and *V. faba* plants are relatively sensitive to metals. Earthworms play a key role in terrestrial ecotoxicological risk assessment. The original aspect of this experiment consists in the utilization of industrial polluted soils. In this way, a cocktail of metals could be studied, to carry out complementary ecotoxicity tests. In order to gain better understanding of the metal uptake by plants in the presence of earthworms, the aims of this study are to : (1) evaluate the effects of a gradient of metal contamination on life cycle parameters of *Eisenia fetida*; (2) assess the impact of *E. fetida* on metal bioavailability and on the properties of the soil (3) assess if the addition of *E. fetida* lead to metal accumulation in *Vicia faba* and *Zea mays* plants. Then, earthworm and plant metal bioaccumulation studies will be performed to better understand the effects of earthworms on the phytoremediation by two plants.



Metalliferous grassland of Sclaingneaux calaminary site

Materials and methods

Soil characteristics

Three contaminated soils were used in this study (Table 1). They were collected from the upper layer (0 to 20 cm) of the soil horizon. Soils were also collected from uncontaminated sites. The soil was primarily air dried, homogenized by sieving (8 mm) and stored at 4° C until analysis. Soil pH was measured in distilled water and in 1N KCl. Total organic carbon (TOC) and nitrogen content were determined. For several decades, the industrial activities at the site have produced high concentrations of Zn, Cd, Pb and Cu. Pseudo-total elemental composition was determined after aqua-regia-digestion. The different element contents were measured by flame atomic absorption spectrometry.

Table 1. Physico-chemical properties of the collected experimental soils and total heavy metal concentrations (mg kg⁻¹)

	pH	Pseudo total elements (mg kg ⁻¹)					CEC (Cmol ⁺ kg ⁻¹)
		H ₂ O	KCl	Cd	Cu	Pb	
S0	8.2	7.6	0.71	15	29	102.5	15.16
S1	7.4	7.8	2.48	11.7	69	207.5	11.80
S2	7.1	6.6	4.27	21.7	129	384.1	10.67
S3	8.1	7.7	9.28	19.3	255.3	743.3	10.52

Table 2. Experimental design for ecotoxicity test.

P: plant	Treatment	Total microcosms
1	C + P0 + E0 + F1	4
2	C + P0 + E0 + F0	16
3	C + P0 + E1 + F1	16
4	C + P0 + E1 + F0	16
5	C + ZM + E0 + F0	16
6	C + ZM + E0 + F1	16
7	C + ZM + E1 + F1	16
8	C + VF + E0 + F1	16
9	C + VF + E0 + F0	16
10	C + VF + E1 + F1	16
	Total	148

Experimental design

Microcosms were prepared by weighing 2.250 kg of dry soil for each pot. Each pot was moistened to 60 % of the Water Holding Capacity (WHC). The experimental design considered three factors: metal contamination, the presence of *E. fetida*, and the presence of *Z. mays* or *V. faba*. The experiments were performed in soils containing a mixture of Cd, Cu, Zn and Pb. The experiment was carried out using processes combined with three levels of contamination mixtures (C0 : no contamination ; C1 : low contamination ; C2 : medium contamination ; C3 : high contamination) (table 2). Twenty worms were introduced into the relevant test soil. They were kept in 16h light and 8h dark at 20 ± 1 °C in a climate chamber for 42 days. The worms were fed weekly with dried mixture of horse manure (75%) and oat flakes (25%) to provide 0.5 g per worm. After 42 days of exposure, these earthworm parameters were measured: earthworm mortality, earthworm weight, cocoon production and cocoon weight

Biological material

Z. mays and *V. faba* were seeds and germinated on plastic pot (with or without earthworms and food). Plant growth was carried out under controlled conditions : 20±1 °C and 18±1 °C day and night temperatures, 60% ± 5% relative humidity. The pots were randomly distributed.

E. fetida specimens used for this study were age-synchronized from a culture kept in the Functional and Evolutionary Entomology Unit, Gembloux Agro-Bio-Tech, University of Liege, Belgium. They were acclimatized for at least 48 h prior to exposure (OECD, 2004). Earthworms were introduced 7 days after plant seeding. After 42 days exposure, and following depuration during 48h, earthworms and plants were placed into an oven at 60 °C over night; the dry earthworms and plants were then weighed. Metal concentrations in earthworm bodies and in plants were determined after acidic digestion and metal concentrations were measured by AAS.

Results and discussion

