European Journal of Soil Biology 45 (2009) 363–369

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Pollution-induced tolerance of soil bacterial communities in meadow and forest ecosystems polluted with heavy metals

Anna M. Stefanowicz*, Maria Niklińska, Ryszard Laskowski

Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

ARTICLE INFO

Article history:
Received 3 October 2008
Received in revised form
2 April 2009
Accepted 4 May 2009
Available online 19 May 2009
Handling editor: Petra Marschner

Keywords:
Pollution-induced community tolerance (PICT)
Biolog
Metals
Soil
Microorganisms

ABSTRACT

Pollution-induced community tolerance (PICT) allows finding a cause–effect relationship between pollution and adverse changes in a community. In our previous study we found that functional diversity of bacterial communities decreased significantly with increasing metal concentration, in both forest humus and meadow topsoil. Thus, the aim of the present study was to test whether tolerance of soil bacterial communities had increased as an effect of long-term metal pollution. Bacterial tolerance was tested with the use of the Biolog® ECO plates in soils originating from the most polluted and the least polluted sites from three forest and five meadow transects located near smelters in Avonmouth (England), Clydach (Wales), and Głogów and Olkusz (Poland). We found that tolerance of bacterial communities was significantly increased in polluted meadow soils when compared to control meadow soils. On the contrary, no increase in tolerance was detected in polluted forest humus.

1. Introduction

Soil microbial communities play a crucial role in decomposition of dead organic matter and nutrient cycling. Any adverse alteration in soil microbial parameters, such as activity, biomass, community structure and function may potentially lead to disturbance in nutrient cycling and, thus, the functioning of the whole ecosystem. Many studies on the impact of metal pollution on soil microbial characteristics have been performed so far, yielding inconsistent results (e.g., [14,15,18,20]). However, microbial parameters may be affected not only by pollution but also by natural factors, such as nutrients content, soil acidity, temperature and others, which may obscure toxicant effects [19]. Investigations of the so-called pollution-induced community tolerance (PICT), proposed by Blanck et al. [4], allow finding a cause–effect relationship between metal pollution and adverse changes observed in communities. The concept of PICT is based on the assumptions that species differ in their sensitivity to a particular toxicant, and that due to the presence of a contaminant the survival, growth and reproduction rates of the most sensitive organisms are reduced. Thus, the community becomes dominated by less sensitive species/genotypes and, as a result, its overall tolerance increases [23]. Additionally, other mechanisms of tolerance increase are possible, that is selection for tolerance resulting from differences in competitive abilities, and acclimatisation or adaptation of organisms due to physiological and genetic changes [7,16].

Although appearance of PICT in bacterial communities due to metal pollution is well documented (e.g., [2,23]), some authors found no effects of particular metals on the level of bacterial tolerance [6,11]. Similarly, Niklińska et al. [20] found a significant ($p < 0.05$) increase in tolerance to Cu only in bacterial communities utilizing 1, while to Zn only in bacterial communities metabolizing 7 out of 31 substrates supplied in Biolog ECO plate wells.

Induction of microbial community tolerance by metals was frequently studied in soils contaminated artificially in laboratory or field experiments (e.g., [6–8,24]), but rarely in soils polluted with metals for decades due to industrial activities such as metal mining and smelting [1,20,21]. PICT studies encompassing sites contaminated by a number of different smelters in both forest and meadow ecosystems are still lacking.

In our previous study on three forests and five meadow transects near metal smelters in Poland, England and Wales [26] we found that soil bacterial functional diversity significantly decreased with increasing metal concentration in both forest humus and meadow topsoil. It is expected that the observed loss of functions in bacterial communities resulted mainly from a severe decrease in activity and/or loss of the most sensitive species from communities. Thus, the average tolerance of bacterial communities at polluted sites should have increased. The aim of the present study was, thus,

* Corresponding author. Tel.: +48 126645133; fax: +48 126646912.
E-mail address: anna.stefanowicz@uj.edu.pl (A.M. Stefanowicz).

1164-5563/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejsobi.2009.05.005

to test whether tolerance of soil bacterial communities indeed increased due to the long-term metal contamination of forest humus and meadow soils.

2. Materials and methods

2.1. Research sites and soil sampling

Three forest and five meadow transects were established in areas polluted with heavy metals as an effect of metal mining and smelting. They were located near an abandoned Pb/Zn smelter in Avonmouth (England), Ni refinery in Clydach (Wales), Zn/Pb smelter in Olkusz (Poland), and Cu smelter in Głogów (Poland) [26]. Characteristics of the research sites are given in Table 1. Mean annual temperature and precipitation in these regions are 11 °C and 600 mm (Avonmouth), 12 °C and 300 mm (Clydach), 8 °C and 550 mm (Głogów), 8 °C and 650 mm (Olkusz). Short description of vegetation at the research sites is given in Table 2. In June 2004, samples of humus (in forests) or of a 10 cm-deep topsoil (on meadows; because no distinct organic layer could be found there) were collected from 39 sites located along the transects. Five samples were randomly taken from the area of ca. 100 m^2 at each site, sieved (10 mm mesh, as commonly used for highly organic soils) and mixed. Soils on different transects were highly differentiated, from poor sandy soils and podzols (Olkusz, Głogów) to more rich, such as rendzinas (Olkusz) and alluvial gley soils (Avonmouth). Because meadows in the Olkusz region were especially differentiated, two meadow transects were traced in that area in order to cover this diversity. The two transects were roughly distinguished according to sand content in soil and soil humidity. For each distance from the smelter (that is, at presumably similar pollution level) two meadow sites were thus established, differing only in edaphic conditions. These are distinguished in further text as "dry" and "wet" meadows. The soil samples were analyzed for concentrations of the most important pollutants on the transects, that is Cd, Cu, Ni, Pb and Zn [26], and the soils originating from the most and the least polluted sites of each transect were used in the present study. All chemical and microbiological analyses were performed on mixed samples and started a week after collection of the samples (storage temperature 17 °C).

Table 2
Vegetation at the research sites.

Site		Dominant plants species/genera/communities[a]
AM	P	ChAss.: *Arrhenatheretum elatioris*
	UP	ChAss.: *Lolio-Cynosuretum*; ChAss.: *Arrhenatheretum elatioris*
CF	P	*Quercus robur*, *Rhododendron sp.*, *Vaccinium myrtillus*, *Calluna vulgaris*
	UP	*Quercus robur*, *Fraxinus excelsior*, *Betula verrucosa*, *Rhododendron sp.*
CM	P	*Vaccinium myrtillus*
	UP	*Acer campestre*, *Anthyllis vulneraria*, *Dactylis glomerata*, *Trifolium pratense*
GF	P	*Pinus sylvestris*, *Sorbus aucuparia*, *Festuca sp.*
	UP	*Pinus sylvestris*, *Vaccinium myrtillus*, *Calluna vulgaris*
GM	P	*Arrhenatherum elatius*, *Festuca rubra*, *Melilotus officinalis*, *Tanacetum vulgare*, *Thymus pulegioides*,
	UP	*Arrhenatherum elatius*, *Lathyrus pratensis*, *Tanacetum vulgare*, *Thymus serpyllum*
OF	P	ChAss. *Leucobryo-Pinetum*, ChAll *Fagion sylvaticae*, ChAll. *Alno-Ulmion*
	UP	ChAss. *Leucobryo-Pinetum*, ChAll. *Fagion sylvaticae*, ChAll. *Sambuco-Salicion*
OMX	P	ChAll.: *Koelerion glaucae*, ChCl.: *Molinio-Arrhenatheretea*, ChAss.: *Epilobio-Salicetum capreae*, ChAss.: *Leucobryo-Pinetum*, ChCl. *Nardo-Callunetea*
	UP	ChCl. *Molinio-Arrhenatheretea*, ChCl. *Koelerio-glaucae-Corynephoretea canescentis*, ChAss. *Leucobryo-Pinetum*
OMZ	P	ChCl. *Molinio-Arrhenatheretea*, ChCl. *Koelerio-glaucae-Corynephoretea canescentis*
	UP	ChAll. *Onopordion acanthii*, ChCl. *Molinio-Arrhenatheretea*, ChCl. *Stellarietea mediae*

[a] Phytosociological records were performed only for Olkusz sites; for other transects only rough botanical data were available; C – Clydach, G – Głogów, O – Olkusz, F – forest; P – polluted, UP – unpolluted.

2.2. Chemical analyses

Detailed description of chemical analyses is given in the article by Stefanowicz et al. [26]. Briefly, the dry weight of the humus and soil was measured after 12 h drying at 105 °C. Concentrations of C_{tot} and N_{tot} were measured with an essential elements analyzer (Vario EL III; Elementar Analysensysteme). The total concentrations of metals were measured after wet digestion in concentrated HNO_3 (Merck) with a gradual temperature increase from 50 to 150 °C. Water-soluble elements were extracted by shaking the samples for 1 h in deionized water (pH = 5.5) at a 1:10 ratio (w/v). Concentrations of total and water-soluble Zn, Pb, Cd, Cu, Ni were measured by flame or graphite furnace atomic absorption spectrometry (AAnalyst 800; PerkinElmer). Soil pH was measured in H_2O at a 1:10 ratio (w/v) with a digital pH-meter (CP-401, Elmetron).

2.3. Microbial analyses

Due to economic reasons, the tolerance of bacterial communities was studied in soils originating from the most and the least

Table 1
Characteristics of the research sites [26].

Site[a]		Distance (km)[b]	Soil pH(H_2O)	Soil C/N	Metals in the field	Metal tested[c]	Industrial activity
AM	P	0.8	5.4	13.3	Pb, Zn, Cu, Cd	Pb	Pb/Zn smelter since 1929, abandoned in 2003
	UP	5.3	6.4	6.91			
CF	P	0.2	4.6	17.9	Ni, Cu	Ni	Ni refinery since 1902
	UP	1.9	4.2	16.5			
CM	P	0.1	5.2	19.1			
	UP	9.5	6.0	24.9			
GF	P	8.6	4.1	20.5	Cu	Cu	Cu mines and smelters since 1969
	UP	33.3	3.9	26.3			
GM	P	2.6	6.8	2.91			
	UP	34.8	5.5	6.42			
OF	P	3.9	4.8	20.8	Zn, Pb, Cd	Pb	Pb/Zn mines and smelter since 1967
	UP	31.8	4.5	17.8			
OMX	P	3.7	6.1	6.72	Zn, Pb, Cd	Zn	
	UP	20.0	5.8	5.61			
OMZ	P	0.6	6.4	8.56			
	UP	32.6	7.5	6.25			

[a] A – Avonmouth, C – Clydach, G – Głogów, O – Olkusz; F – forest, M – meadow; X – dry, Z – wet; P – polluted, UP – unpolluted.
[b] Distance from the smelter.
[c] In PICT detection phase.