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Changes in plant communities along soil pollution gradients: Responses of leaf antioxidant enzyme activities and phytochelatin contents

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ABSTRACT

This work describes an ecological and ecotoxicological study of polluted wasteland plant communities in a former coke-factory located in Homécourt (France). Ecological analyses were performed along two transects to investigate changes in plant community structure through species richness (*S*), biological diversity (*H′*) and evenness (*J*). Five species (*Arrhenatherum elatius*, *Bromus tectorum*, *Euphorbia cyparissias*, *Hypericum perforatum* and *Tanacetum vulgare*) were then selected to assess cellular responses through antioxidant enzyme activities and phytochelatins (PCs) contents. The results showed that species richness and biological diversity correlated negatively to Cd and Hg concentrations in soil suggesting that soil concentration of non-essential heavy metals was the primary factor governing vegetation structure in the industrial wasteland. Moreover, for all studied species, abundances were partly related to metal levels in the soils, but also to plant antioxidant systems, suggesting their role in plant establishment success in polluted areas. Data for PC contents led to less conclusive results.

1. Introduction

Composition and structure of plant communities within an area depend on the geological, ecological and seasonal factors resulting from naturally occurring changes in the environment and disturbances induced by man's activity (Regvar et al., 2006). The composition of local flora, the distance from colonised areas, the germination and establishment probabilities are decisive for the formation of pioneer plant communities. The composition of vegetation on polluted sites depends on tolerance and avoidance mechanisms among which dispersal models, vitality maintaining mechanisms, phenological adaptations and mycorrhizal associations have been described (Wilcox, 1998; Prach and Pysek, 2001; Wiegleb and Felinks, 2001).

During the last century in Northern and Eastern France, coal tar processing, metallurgical industry complexes and mining activities caused extensive damages to local vegetation, including diversity loss, and important changes in soil characteristics with the highest disturbed areas found immediately in the surroundings of industrial exploitations. Such areas are generally characterised by bare and sparsely vegetated lands, dominated by pollution resistant plant species and by severely polluted soils with high levels of heavy metals and hydrocarbons (Gunn, 1995; Dudka and Adriano, 1997).

If the phytotoxicity of polycyclic aromatic hydrocarbons (PAHs) is still a matter of debate (Sverdrup et al., 2003; Smith et al., 2006; Lin et al., 2007a), heavy metal effects on plants have been largely described (Toppi and Gabbrielli, 1999). Then, the survival of a given plant species in a heavy metal contaminated environment is determined by its sensitivity to metal toxicity (Prasad, 1997) and its evolutionary and adaptive responses (Wu et al., 1975; Brej, 1998). Therefore, plants have to adapt to the prevailing conditions for their survival, resulting in the acquisition of a wide range of tolerance mechanisms and defence strategies (Hall, 2002). Among them, avoidance of metal entry into the cell via exclusion, metal binding to the cell wall and other excreted ligands like organic acids or amino acids, and sequestration through chelation with glutathione or phytochelatins (PCs) reduce metal uptake and interactions with cellular components (Krämer et al., 1996; Ma, 2000; Clemens et al., 2002; Kerkeb and Krämer, 2003). Other defence system is provided by antioxidants which can cope with reactive oxygen species (ROS) over-production caused by the interactions between heavy metals and normal metabolic functions (Foyer et al., 1994; Foyer and Noctor, 2005).

Synthesis of PCs, $(\gamma\text{-Glu-Cys})_n\text{-Gly}$, with $n = 2$–11, and their homologues, is a constitutive mechanism to cope with toxic metals in various plants (Grill et al., 1985, 1987). These peptides are synthesized by a transpeptidase named phytochelatin synthase (PCS, E.C. 2.3.2.15) using glutathione or its homologues as substrate (Grill et al., 1989; Cobbett and Goldsbrough, 2002). The enzyme

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is post-translationally activated by various heavy metals including Cd (Grill et al., 1989; Zenk, 1996). Complexes of metal with PCs are finally sequestered in the vacuole. A large number of studies had demonstrated the critical role of PCs in Cd detoxification and tolerance (Gupta and Goldsbrough, 1991; Howden et al., 1995). However, several studies with plant species that exhibit unusual hypertolerance to Cd, such as *Thlaspi caerulescens*, suggested that PCs were not responsible for the observed metal hypertolerance phenotypes (Schat and Kalff, 1992; Ebbs et al., 2002; Schat et al., 2002; Ernst et al., 2008).

Relationships between heavy metal toxicity and oxidative stress have also been studied in many systems (Prasad, 1999). The key step in oxidative stress is the production of ROS which initiates a variety of autooxidative reactions on membrane unsaturated fatty acids, producing lipid hydroperoxides and thereby cascades of reactions ultimately leading to the destruction of macromolecules and organelles (Foyer et al., 1994). Removal of ROS is regulated by antioxidant enzymes such as superoxide dismutase, catalase, or peroxidases, and a complex antioxidant system, the ascorbate-glutathione cycle and the associated glutathione metabolism enzymes (Noctor and Foyer, 1998; Cho and Seo, 2005).

High heavy metal concentrations in soils have been usually cited as one of the primary factors limiting vegetation establishment and growth in industrial wastelands (Gunn, 1995). Therefore, revegetation capacities of these areas are relatively low, with difficulties to re-establish productive ecosystems, and they are often characterised by the establishment of several exclusive and highly-plastic species. *De facto*, plant communities of post-industrial landscapes are difficult to compare to those of undisturbed areas (Prach and Pysek, 2001; Wiegleb and Felinks, 2001).

However, identification and characterization of plant species capable of growing and surviving on polluted areas could be very helpful to develop phytostabilization technologies (Kramer et al., 2000). Experimentations have already been undertaken in order to elucidate and overcome limitations to vegetation establishment, allowing large-scale revegetation schemes to be formulated (Tordoff et al., 2000). These studies were performed in a number of potentially hazardous sites including a former metallurgical landfill (Remon et al., 2005), a copper-smelter grassland (Ginocchio, 2000), uranium-mining soils (Martinez-Ruiz et al, 2001; Martinez-Ruiz and Fernandez-Santos, 2005), ancient gasworks and coke-factories (Henner et al., 1999) or open-pit coal mine (Gonzalez-Alday et al., 2008) but only took into account descriptions of plant communities and measurements of growth and heavy metal accumulation. None of these studies investigated how plants do adapt at the cellular level to their polluted environment.

Thus, it would be interesting to collect such information. Indeed, the few studies assessing plant responses to multicontaminated soils only focused on laboratory models such as *Helianthus annuus* (Singh et al., 2004), *Brassica juncea* (Singh and Sinha, 2005), *Nicotiana tabacum* and *Solanum tuberosum* (Gichner et al., 2006), or *Lolium perenne* and *Trifolium repens* (Bidar et al., 2007).

In this work, a vegetation survey was conducted on an industrial wasteland located at a former coke-factory site in Homécourt, in the North-East of France. Coke production activities from 1922 to 1980 led to the release of PAHs and heavy metals, especially Cd, Cu, Hg and Zn. The aims of the present study were: (i) to describe plant communities established on different areas of the Homécourt wasteland and try to relate ecological indices to soil parameters, and (ii) to assess the potential role of leaf cellular defence systems in the survival ability of a few species presenting varying abundances through the studied areas.

2. Materials and methods

2.1. Site description

This work was performed at a former coke-factory site in Homécourt (Lorraine, North-East of France: latitude 49°23′33″N, longitude 5°98′33″E). The climate is semi-continental with local mean annual rainfall and temperature of 720 mm and 11 °C, respectively. The natural vegetation surrounding the study area consisted in a complex matrix with grasslands (*Arrhenatherum elatius*, *Bromus mollis*, *Tanacetum vulgare*) dominated by shrubs (*Crataegus monogyna*, *Rosa canina*), and remnants of open-woodlands (*Betula pendula*, *Populus tremula*, *Robinia pseudoacacia*). Two well-established concentric horizontal vegetation gradients were identified and supposedly linked to pollutant concentrations. Two transects were drawn from central to peripheral areas for each of them and a total number of seven zones was clearly delimited by using vegetation profile.

2.2. Soil sampling and analyses

For each zone, soil physico-chemical properties and pollutant concentrations (Table 1) were analysed following the sampling and analyses methods described by Mathieu and Pieltain (1998). Soil sampling was carried out in June 2004 and four samples were randomly taken from the whole surface of each of the seven zones. Heavy metal and PAHs concentrations in soils were measured by ICP-MS and GC–MS, respectively. Quality control procedures included the participation to Wageningen Evaluating Programs for Analytical Laboratories (WEPAL) with heavy metal analyses of ISE reference materials, and the use of PAH Contaminated Soil/Sediment CRM104-100 (PAH contaminated soil/sediment from the southern Branch of the Elizabeth River, Chesapeake Bay area,

Table 1

Physico-chemical properties of soil samples taken from all transect zones. Values are presented as means (min.–max.) of four replicates.

	First transect (4 zones)				Second transect (3 zones)		
Zone *n*°	0	1	2	3	4	5	6
C/N	69.6	76.6	66.9	83.3	23.1	68.1	5.1
Clay (%)	0.9 (0.4–1.2)	0.3 (0.1–0.4)	0.1 (0.1–0.2)	0.1 (0.1–0.2)	0.3 (0.2–0.5)	0.2 (0.2–0.3)	0.6 (0.4–0.7)
Silt (%)	11.6 (8.4–13.6)	6.0 (4.4–9.7)	2.1 (1.8–4.3)	2.8 (1.9–5.6)	4.2 (2.9–7.1)	5.7 (4.8–8.4)	7.9 (5.2–9.9)
Sand (%)	87.5 (85.8–90.4)	93.7 (90.2–94.8)	97.8 (95.5–98.1)	97.1 (94.3–97.9)	95.5 (92.4–96.9)	94.1 (91.3–95.0)	91.5 (89.7–94.1)
pH H_2O	8.9 (8.8–8.9)	8.7 (8.7–8.8)	8.5 (8.5–8.6)	7.5 (7.3–7.6)	8.1 (8.0–8.1)	8.2 (8.1–8.2)	8.6 (8.6–8.7)
CEC (meq/100 g soil dw)	72.4 (59.4–86.7)	37.5 (29.8–47.9)	47.5 (41.3–68.9)	80.4 (72.3–98.7)	58.2 (53.3–76.8)	43.2 (34.2–65.1)	56.3 (52.8–67.0)
Cd[a]	4.0 (2.2–5.3)	1.1 (0.2–1.7)	1.1 (0.2–1.6)	2.0 (0.7–3.6)	28 (11.4–32.7)	9.0 (4.2–13.4)	1.0 (0.3–1.6)
Cu[a]	74 (29–114)	40 (11–57)	58 (14–96)	22 (16–34)	67 (33–78)	58 (32–89)	95 (76–137)
Hg[a]	68 (29–97)	26 (12–38)	11 (7.2–27)	4.6 (2.1–7.2)	560 (245–734)	197 (111–356)	27 (12–38)
Zn[a]	271 (115–476)	307 (143–763)	268 (123–528)	367 (237–589)	336 (218–498)	312 (210–576)	211 (131–352)
Total US-EPA PAHs[a]	4652 (3987–5432)	2584 (1978–2976)	484 (238–594)	651 (499–766)	483 (339–521)	157 (97–189)	99 (56–133)

[a] Concentrations of major pollutants are expressed in mg kg^{-1} soil dw. Other metals (Al, Cr, Ni, Pb) were also measured but their concentrations were always below 1 mg kg^{-1} soil dw.