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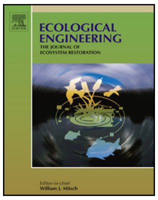

Short communication

Intra-specific variability of the guaiacol peroxidase (GPOD) activity in roots of *Phragmites australis* exposed to copper excess

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ABSTRACT

The intra-specific variability of the soluble guaiacol peroxidase (GPOD) activity in roots of 2-year-old *Phragmites australis* (Cav.) Trin. ex Steud. exposed to Cu excess (21 days) was investigated. The GPOD activity did not depend on the Cu exposure (0.08 vs. 25 μM) ($p > 0.05$) but was influenced by the sampling site ($p < 0.05$). The lowest GPOD activity (312-461 μM min^{-1}) was found in roots of *P. australis* from the Capanne site, which displayed the highest total soil Cu (375 mg kg^{-1}), while for plants from the three other sites the root GPOD activity remained steady, in the 1062-1389 μM min^{-1} range, independently of the sampling site. Copper excess did not affect the chlorophyll fluorescence for the four populations tested. Evidence of an intra-specific variability of the root GPOD activity, known for scavenging hydrogen peroxides in response to Cu excess, for *P. australis* provides new hints towards choosing relevant populations of this plant species for further use in constructed wetlands.

Capsule The response of guaiacol peroxidase activity to Cu excess in the roots of *Phragmites australis* displays an intra-specific variability.

1. Introduction

Declining wetland quality is a global issue of concern as increases in human populations, industrial and agricultural activities, and climate change can alter the hydrological cycle (UNESCO, 2015). In industrialized countries, connection to municipal wastewater treatment plants ranges from 50% to 95%, whereas in developing countries over 90% of raw sewage and 70% of untreated industrial wastes are dumped into surface waters, accumulating then in surface waters, groundwater, substrates and plants (UNESCO, 2009, 2010). Every day, 2 million tons of sewage and other effluents drain into the world's waters (UN- World Water Day, 2010). Major water pollutants include trace elements (TE, here, essential and non-essential metal(loid)s with common concentrations in plant shoots below 100 mg kg^{-1} dry weight, DW; Adriano, 2001), microbes, nutrients, organic chemicals including oils (UN-Water for Life 2005-2015).

In the 5-20 mg Cu kg^{-1} DW range suitable for cellular homeostasis in leaves, Cu acts as a cofactor in many processes in higher plants (Palmer and Guerinot, 2009). Copper excess however induces phytotoxicity symptoms (Marschner, 2011). Excessive free Cu ions can enhance the formation of Reactive Oxygen Species (ROS) (Sharma and Dietz, 2009). Main ROS-scavenging pathways in plants are based on both non-enzymatic antioxidant compounds (Sharma et al., 2012) and the enzymatic cascades including enzymes such as superoxide dismutases (SOD), ascorbate peroxidase (APX), catalases (CAT), glutathione reductase (GR) and the guaiacol (iso)peroxidases (GPOD, EC 1.11.1.7) (Cuypers et al., 2011). The GPOD belongs to the class III peroxidases, which are plant peroxidases that are glycosylated and targeted either to the apoplast, the cell wall or the vacuole (Horemans et al., 2015). The GPOD activity uses the guaiacol as a reductant and mediates the decomposition of H_2O_2 (Mika and Lüthje, 2003). Class III peroxidases are considered as biomarkers for TE stress in plants (Jouili et al., 2011) since its activity present a dose-effect relationship with increasing TE exposure (Mocquot et al., 1996; Thounaojam et al., 2014). In common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), the GPOD activity increased in both roots and shoots with increased arsenic uptake (Ghassemzadeh et al., 2008).

Common reed is considered highly invasive outside its native range (Rodriguez and Brisson, 2015) but is widely used in constructed wetlands (CWs) to remove pollutants (Anderson et al., 2015; Pedescoll et al., 2015). It was commonly admitted that selection may have shaped a constitutive TE tolerance for rooted macrophytes such as common reed (Marchand et al., 2010). However, Marchand et al. (2014) reported an intra-specific variability

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of the root biomass production depending on both the sampling location and the Cu exposure (in the 2.5-25 μM Cu range) for *P. australis*, *Juncus effusus* L., *Schoenoplectus lacustris* L., and *Phalaris arundinacea* L. Consequently the constitutive Cu tolerance may not be a species-wide trait for all rooted macrophytes and may exhibit a variability for some of them (Marchand et al., 2014). In parallel, Hego et al. (2014) reported that a Cu-tolerant population of *Agrostis capillaris* L. did not evolve a specific mechanism in roots, but its Cu resistance would result from the cooperation of various processes in roots including a higher superoxide detoxification.

This study aimed at exploring a potential intra-specific variability of the soluble GPOD activity in the roots of four *P. australis* populations exposed to two Cu concentrations (0.08 vs. 25 μM Cu) which may occur in CWs. We hypothesized that a high Cu exposure in wetlands may promote the selection of plants with either a higher root GPOD activity to cope with Cu excess or a lower one in the case of selection of tolerance mechanisms such as sub-cellular compartmentation. Additionally, potential impacts on the plant physiology were assessed by measuring the PSII photosynthesis rate (Marchand et al., 2015).

2. Material and Methods

2.1. Sampling sites

Four sampling sites were investigated (Table 1). Three are located in France (Cornubia, Jalle, and Lagnet) and one in Italy (Capanne). Total soil Cu (in mg kg^{-1} DW) varied from 27 (Lagnet) to 375 (Capanne) and soil pH ranged from 5.7 (Cornubia) to 7.5 (Jalle). High soil TE concentrations resulted from either the geochemical background or industrial and agricultural activities.

The Cornubia site (Gironde, France) is a CW collecting effluents and water runoff from a former Cu sulphate production plant, its activity dating back more than a century (BASOL, 2015). The Lagnet site is a draining ditch located in the vineyards of Saint-Emilion (Gironde, France), annually treated with the Bordeaux mixture containing Cu salts. The Jalle d'Eysines River flows into the Garonne next to Bordeaux (Gironde, France) and receives both TE-contaminated runoffs from industrial, agricultural and residential areas and effluents from two major municipal wastewater treatment plants (WTP) of the Bordeaux suburbs, serving more than 100,000 inhabitants. The polymetallic (Zn, Cu, Pb, Fe, and Ag) sulfide deposit of Fenice Capanne (Massa Marittima, Italy) was mined for 25 centuries up until 1985 and the alteration of mine waste materials has produced pollution in superficial waters and sediments (Mascaro et al., 2001). For convenience, we will name the four sites as "Cornubia", "Capanne", "Jalle" and "Lagnet" throughout the paper. Physico-chemical parameters and TE concentrations of these soils (Table 1) were previously investigated (Marchand et al., 2014). The free Cu^{2+} activity (pCu^{2+}) was calculated according to Sauvé et al. (2003) (Table 1):

$$pCu^{2+} = 3.20 + 1.47\,pH - 1.84\log 10(\text{total soil Cu})$$

[The free Cu^{2+} activity increases when pCu^{2+} decreases]

2.2. Plant sampling, vegetative reproduction, and plant exposure to Cu

Phragmites australis was mainly collected at the beginning of the growing season in 2011. Four populations corresponding to the four sampling sites were sampled with 20-30 individuals per population (below- and aboveground biomasses). Shortly after collection, rhizomes and/or stem-bearing buds were cut into small pieces (10-20 cm) in a greenhouse at the Centre INRA-Bordeaux Aquitaine, Villenave d'Ornon, France. They were then grown in separate polyethylene containers containing perlite imbibed with a quarter-strength Hoagland nutrient solution (HNS, Hoagland and Arnon, 1950, details in Marchand et al., 2014). Water was renewed and nutrients were added every month during the growing season and every two months during winter. In April 2012 (week 15), 6 standardized tillers (similar stem and root size and volume) of each population were isolated from the sprouting rhizomes and grown in pots filled with perlite imbibed with HNS for 8 weeks. In June 2012, before the test, the six individuals of each population were transferred into plastic containers (1L), filled with 1L of a quarter-strength HNS prepared with ultra-pure water (MilliQ system), during three weeks (weeks 24-26). The 24 plants (6 individuals from the 4 populations) were randomly placed on a bench in the same greenhouse [day (9-21 h) 1911 ± 1232 μM photons $m^{-2}s^{-1}$, 28 ± 5 °C, night (^{21}h–^{9}h) 19 ± 3 °C]. The quarter strength HNS was renewed every week. In week 27, the growth medium was spiked with Cu ($CuSO_4.5H_2O$) to achieve two Cu treatments: 0.08 and 25 μM Cu (**S1**). Then three individuals from each population were grown in each Cu treatment. Nutrient solutions were weekly changed during the 3-week exposure test (weeks 27-29) to maintain Cu concentrations and avoid depletion of oxygen and

Table 1
Trace element concentrations (mg kg^{-1} DW) and physico-chemical parameters in soils at sampling sites. Values in bold exceeded the background metal concentration in soils (Blum et al., 2012).

sites	geographic coordinates	C g kg^{-1}	N g kg^{-1}	Soil pH	CEC cmol kg^{-1}	pCu^{2+} ***	Cu mg kg^{-1}	Zn mg kg^{-1}	Cr mg kg^{-1}	Ni mg kg^{-1}
Cornubia*	44°54′26"N;0°32′46″W	30.9	1.5	5.7	5.4	7.3	**205**	**306**	14.0	7.04
Lagnet*	44°54′54"N;0°08′23″W	5.5	0.5	7.4	2.4	11.4	27	22.9	14.0	4.58
Jalle*	44°54′34"N;0°34′56″W	28.9	2.6	7.5	26.3	11.4	33	171	**79.6**	40.2
Capanne *	43°00′39"N;10°55′04″W	0.9	0.1	7.4	<1	9.3	**375**	**720**	35.7	29.7
Background metal concentrations in soils**							10-40	20-200	10-50	10-50

Sites	Country	Co	Pb	Cd	Mo	Mn	Fe	Ca	Al	Mg
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Cornubia*	France	3.4	**59.7**	1.10	0.4	154	8.8	6.7	27.8	1.7
Lagnet*	France	2.1	15.6	0.07	0.2	139	5.7	9.8	22.6	0.9
Jalle*	France	**19.0**	**54.9**	0.50	1.6	805	45.1	6.3	90.4	9.3
Capanne *	Italy	10.5	**66.0**	1.03	0.5	**3180**	38.9	39.3	35.6	5.9
Background metal concentrations in soils*		1-10	10-50	0.05-1	0.5-2	300-1000	10-50	*nd*	*nd*	*nd*

* From Marchand et al. (2014); **Blum et al. (2012); ***pCu^{2+} = 3.20 + 1.47pH -1.84 log10 (total soil Cu) (Sauvé, 2003); nd: not determined;