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## Depollution potential of three macrophytes: Exudated, wall-bound and intracellular peroxidase activities plus intracellular phenol concentrations

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### ABSTRACT

The aim of this study was to investigate the potential role of three macrophyte species (*Iris pseudacorus*, *Typha latifolia* and *Phragmites australis*) for detoxication of xenobiotics, and to study their variations with seasons or concentrations of sewage sludge from the food industry. For this purpose, some aspects of the green liver concept were explored through peroxidase measurements in three compartments in roots: intracellular, cell wall and extracellular. In addition, phenol concentrations were also measured in order to assess heavy metal detoxication potential. Enzyme activities and phenol concentrations were overall lower in winter according to the phenological stages and some sludge effects occurred. Results show that *P. australis* roots exuded and contained more peroxidase in all seasons: 17 U/g (1373 U/g protein), 0.8 U/g (613 U/g protein) and 4.8 U/g (1329 U/g protein) in intracellular compartments, cell wall and exudates, respectively. In contrast, the highest phenol concentration was found in *I. pseudacorus* roots: 143.3 mg eq. gallic acid/g. Hence, in constructed wetlands, *P. australis* is suitable for organic waste water treatment, while *I. pseudacorus* should be used in the case of waters highly charged with heavy metals.

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### 1. Introduction

Pollution control is the great challenge of this century, due to the dramatic increase of pollutants resulting from human activities released into the environment. Among these, organic chemicals, such as phenolic compounds, are recognized as one of the major chemical families of waste waters generated by various industrial activities (Ikehata et al., 2005; Wentz, 1989). The use of biological material through phytoremediation or bioremediation offers promising techniques for sustainable waste management (Gerhardt et al., 2009; Pilon-Smits, 2005). Constructed wetlands planted with macrophytes are potential candidates for removal of chemical pollutants, and some emergent macrophytes are efficiently used to treat waste water, such as the common reed (*Phragmites australis*), the broadleaf cattail (*Typha latifolia*) and the yellow flag (*Iris pseudacorus*) (Amaya-Chavez et al., 2006; Bragato et al., 2006; Calheiros et al., 2007; Calheiros et al., 2008). Macrophytes are beneficial for wastewater treatment mainly because of their high productivity, nutrient assimilation and favourable conditions for proliferation of soil microorganisms. While the above-ground parts of plants has been fully described (Brix, 1993), the role of

the below-ground part of these plants has remained poorly elucidated. There are two main reasons to explain this: first, studies focused on the wide diversity of microorganisms, as they have a major role in degradation, and secondly the relationship between the plant root system and microorganisms is complex.

However, plant root systems play direct and indirect roles in depollution processes. Macrophyte roots interact with the microorganism populations of their surrounding environment and stimulate their activity (Schinner and Sonnleitner, 1996; Wang et al., 2008). In addition, plants have evolved complex defense mechanisms in response to xenobiotics, which has led to the proposal of the concept of the “green liver” by analogy with the detoxication role of the liver in animal organisms. Therefore, the metabolism of xenobiotics in the rhizosphere is not solely the result of activities of root-associated microflora and fauna. Briefly, the metabolism of xenobiotics in plants is characterized by three essential phases (Sandermann, 1992). The first step (phase I) involves the transformation of chemicals by enzymes such as peroxidases and cytochrome P450 monooxygenases. This phase I occurs in the extracellular compartment, in the rhizosphere, and involves a network of oxidoreductases and hydrolases which transforms xenobiotics to facilitate their uptake into cells. Phase II consists in detoxication through conjugation reactions and phase III is the result of cellular compartmentation and storage of sequestered chemicals.

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Furthermore, plants present other detoxication mechanisms, such as exudation of phenols which act as chelate (Wang et al., 2009) towards heavy metals (Jung et al., 2003; Walker et al., 2003; Wu et al., 2001) or plant intracellular mechanisms which can involve specific scavenging of Reactive Oxygen Species (ROS) often generated in presence of xenobiotics (Marquez-Garcia et al., 2009). ROS transformation into innocuous end products depends on the occurrence of both scavenging enzymes such as peroxidases, catalases and super oxide dismutase and a wide array of metabolites as phenolic components (Mittler et al., 2004). The occurrence of these components in roots therefore indicates the potential of a species to metabolise xenobiotics and can predict its role in depollution. A particular attention should be attached to peroxidase activities that reflect a role in xenobiotic oxidation and to phenol concentrations that give indication of heavy metals and organic pollutant sequestration by plant roots. Peroxidases are ubiquitous oxidases that are expressed as extracellular, soluble and wall-bound enzymes. Their involvement in the oxidation of organic pollutants has been proven by many authors (Gonzalez et al., 2006; Ikehata et al., 2005; Singh et al., 2008). Because guaiacol peroxidase (GPX: EC 1.11.1.7) has a wide reactivity with different substrates and conserves its activity over a broad range of pH and temperature, this enzyme is considered as a potential biological catalyst in wastewater treatment applications (Karam and Nicell, 1997).

A greenhouse experiment was set up to investigate peroxydase activities and phenols concentrations in roots of three macrophyte species, *P. australis*, *T. latifolia*, and *I. pseudacorus*, under highly organic conditions (irrigations with sewage sludge from a food industry) over three seasons. The aim of this work was to assess (1) the functionalisation of xenobiotics during Phase I of the green liver concept, through measurements of exudated and wall-bound peroxidase activity; and (2) the potential for intracellular detoxication through measurements of intracellular peroxidase activity and phenol concentrations. We studied differences between the three plant species, the effects of the season (winter, spring, summer), and the effluent (water, diluted sewage sludge, and sewage sludge).

## 2. Methods

### 2.1. Experimental setup

A greenhouse experiment was carried out in the vicinity of Marseille (southern France). Young plants (one year old) of common reed (*P. australis* Cav.), broadleaf cattail (*T. latifolia* L.) and yellow flag (*I. pseudacorus* L.) were planted as mono-species (one plant per pot), as well as a mixture of *P. australis* and *T. latifolia* (one plant of each per pot), in June 2007 in artificial soil (20% peat, 40% pine bark and 40% vermiculite) in 20 L pots containing basket pots. All were irrigated with same amount of water until September 2007, and then three treatments were applied: water, diluted sludge (50% sludge + 50% water), and sludge. The sludge was a waste activated sludge from the wastewater treatment plant of a local food processing industry producing soft drinks from fruit concentrates. It contains more than 98% water and high concentrations of organic matter, which was mainly in particulate form (more than 95%). The main characteristics of this sludge are 6900 mg/L of Total Suspended Solids, a Chemical Oxygen Demand of 6060 mg/L and a Biological Oxygen Demand in 5 days of 3280 mg/L. It contains 2150 mg/L of Total Organic Carbon, 250 mg/L of Total Kjeldahl Nitrogen and 74 g/L of Total Phosphorus.

The sewage sludge contained neither heavy metals nor persistent organic pollutants, or only at ultra-trace levels. But even at trace level some xenobiotics may be toxic. The color of the waste

also suggests the presence of artificial dye, therefore, the sludge is referred as containing several types of xenobiotics for this study. The sludge showed variations over time in relation to the production of the factory. A total of 180 pots were installed with five replicates for each combination (treatment × species or unplanted) plus three sampling campaigns (February, April and June for winter, spring and summer, respectively). The pots were placed in a stratified random design.

### 2.2. Above and below-ground biomass

For each sampling campaign, plant above and below-ground materials were harvested from 60 pots, separated into above-ground parts, roots, and rhizomes, dried at 40 °C until constant weight, and then weighed for biomass measurements. Dry root materials were crushed to powder for further analyses.

### 2.3. Peroxidase (POD) analysis

#### 2.3.1. Roots and exudates collection

Root exudates were collected in February (winter), April (spring) and June (summer) 2008 for the three species in monospecific pots, and in April and June also in double-specific pots for both *P. australis* and *T. latifolia*. Basket pots were removed from the pots then placed above-ground on 2 rails. Some of the roots emerging from the basket pot grids were selected, washed, surface-sterilized in sodium hypochlorite solution (5%) for 10 min, then thoroughly rinsed for 10 min in deionised water. These roots were pooled and placed for 48 h in test tubes (20 mL) filled with deionised water and entirely covered with aluminium sheets. As controls, three tubes filled only with deionized water were prepared for each campaign. Roots in the tubes were collected, and freeze-dried to express results on a dry root basis. The collected exudates and the controls were passed through 0.2 µm membrane filters, immediately frozen then freeze-dried (Lyophilizator Lyovac GT 2-E) and stored at –20 °C until analyses.

Fresh roots were collected, then frozen until analysis of intracellular and wall-bound proteins.

#### 2.3.2. Extraction of intracellular, wall-bound and exudated proteins of roots

Fresh roots underwent a series of extractions to collect intracellular and parietal protein fractions. Fresh root sample (1 g) was ground in liquid nitrogen, and 3 mL of phosphate buffer (100 mM, pH 7) was added to extract soluble proteins. The homogenate was filtered on a double layer of gauze then centrifuged (centrifuge Sigma 3 K 15) for 15 min at 4 °C at 12 000g. The resulting pellet was resuspended in 1 mL buffer then centrifuged under the same conditions. Both supernatants obtained were pooled and represent the intracellular protein fraction. The pellet was washed by centrifugation then resuspended for 10 min in phosphate buffer at high ionic strength by the addition of KCl (1 M) to extract the proteins ionically wall-associated. NaCl and KCl (1 M) are often used to extract wall-bound peroxidases from plant materials (Csiszar et al., 2004; Zanardo et al., 2009). The suspension was centrifuged as above then the supernatant was dialysed (membrane exclusion limit: 10 kDa) overnight at 4 °C against the phosphate buffer (100 mM, pH 7). The dialysate obtained was considered as the parietal protein fraction.

Regarding the exudates, once lyophilised, they were resuspended in 2 mL of phosphate buffer (100 mM, pH 7).

Therefore, three different fractions were collected: intracellular, parietal and exudate protein fractions on which total proteins and peroxidase activity were assessed.

### 2.3.3. Peroxidase activity assays

Measurements of peroxidase activity from the three protein fractions were carried out as described by Bergmeyer (1974) but with minor modifications. Briefly, activity was determined with a spectrophotometer (Biomat 3) at 30 °C from kinetics of tetraguaiacol formation in a reaction mixture containing 100 mM phosphate buffer (pH 7), guaiacol (18 mM), H<sub>2</sub>O<sub>2</sub> (8 mM) and appropriate enzyme extract volumes. One unit of peroxidase activity (U) is defined as the amount of enzyme that catalysed the oxidation of 1 μmol of guaiacol per min at 30 °C. Peroxidase activity was expressed on the basis of dry weight (units per g) and protein (specific activity: units per mg protein). Protein concentrations were determined according to the method of Bradford (1976) using Bovine Serum Albumin as standard.

### 2.4. Total phenol measurements

Dry roots were weighed before being crushed to powder for analyses of total phenols. Total phenols were extracted from 250 mg DM of roots mixed with 10 mL of deionized water. The mixture was shaken for 1 h in the dark, then filtered through 0.2 μm membrane filters, and analysed following the method of Singleton and Ross (1965) using the Folin–Ciocalteu reagent (Sigma–Aldrich). Measurements were done after 30 min at 720 nm and the results were expressed in mg of gallic acid equivalent.

### 2.5. Statistical analyses

In order to compare the results between treatments and between species, Kruskal–Wallis tests (StatView 5.0) were performed; and when significant differences were found, Student–Newman–Keuls tests were performed. In all cases, significance was defined by  $p < 0.05$ .

## 3. Results and discussion

### 3.1. Above and below-ground biomass

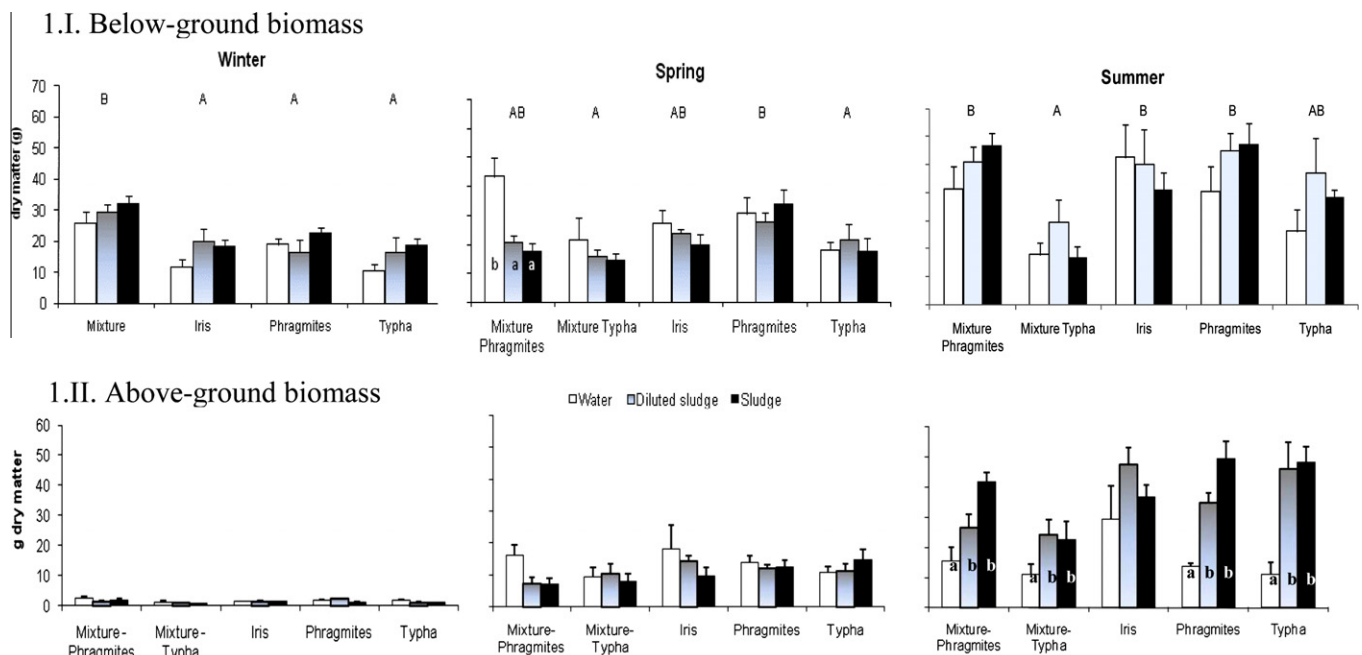
With plant development, above and below-ground biomass increased significantly over seasons (Fig. 1). The above-ground biomass was negligible in winter, developed to 11.8 g in spring to 30.5 g in summer (mean of the three species). Treatment effect on biomass was observed only in the summer for the above-ground biomass ( $p < 0.0001$ ): plants watered with sludge had a more developed shoots (37.7 g dry weight) than the others (16 g dry weight). There was no marked tendency between the three species neither for the above nor for the below-ground biomass ( $p > 0.20$ ). Nevertheless, significant differences in the proportion of rhizome and root biomass were observed. Roots represented 29%, 39% and 42% of the total below-ground biomass for *T. latifolia*, *I. pseudacorus* and *P. australis*, respectively, indicating that *P. australis* had the greatest root area. Since roots are the only effective component which release exudates to the rhizosphere, *P. australis* had a higher root metabolic activity compared to *I. pseudacorus* and *T. latifolia*.

### 3.2. Peroxidase activity

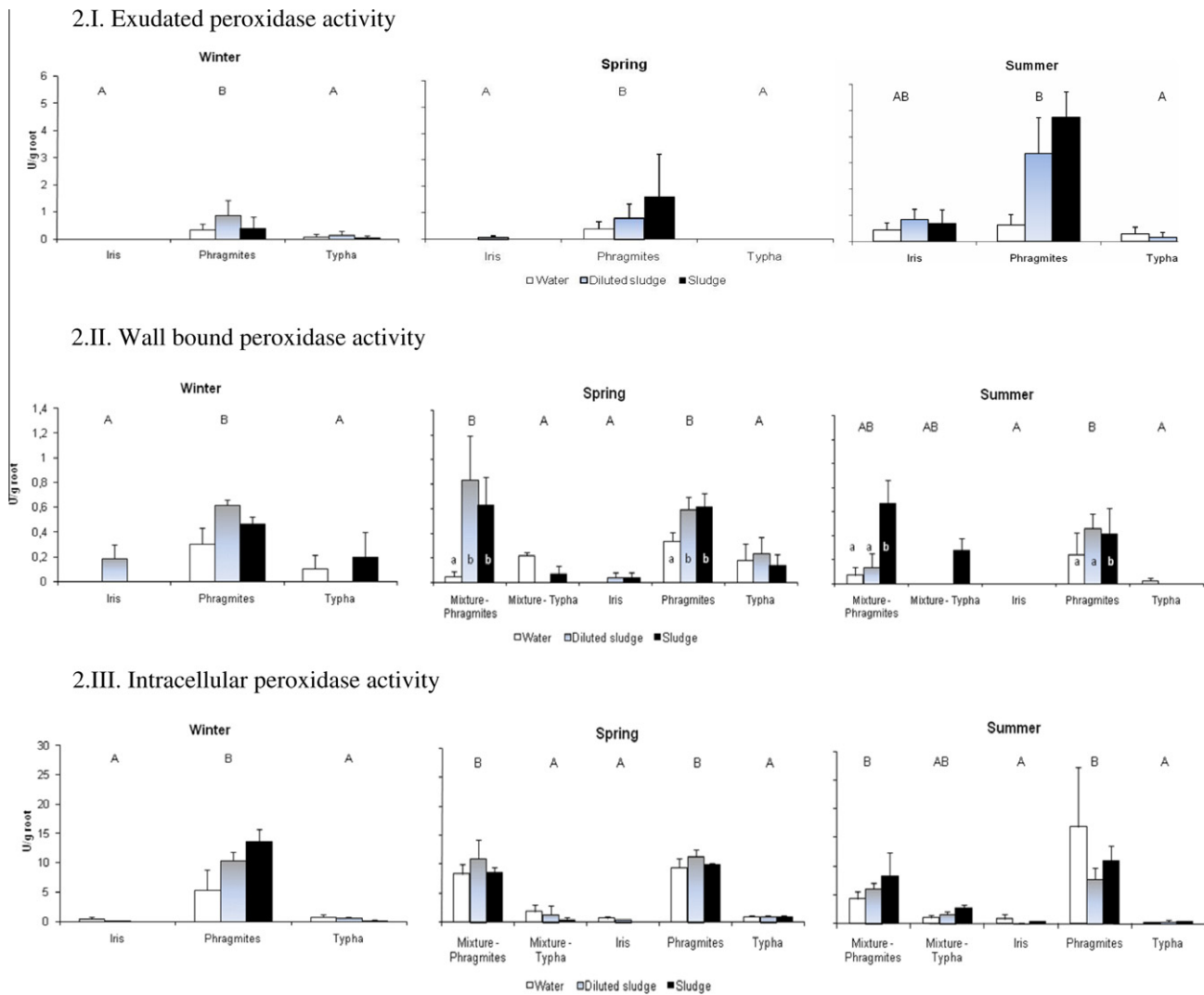
The possible effects of xenobiotic dose exposure and the season changes on exudated, wall-bound and intracellular peroxidase (POD) activities were investigated.

#### 3.2.1. Exudated and wall-bound peroxidase activity

Analysis of the collected root exudates showed that POD were released in plant rhizosphere (Fig. 2), especially from *P. australis* as its POD activity in summer reached 4 U/g root DW and 1300 U/g protein. POD secretion is recognized as an active metabolic pathway. Although the occurrence of POD from suspension cells in culture media has been widely reported (Mensen et al., 1998), the release of POD by plant roots *in vivo* were still poorly



**Fig. 1.** Above (1.I.) and below (1.II.) ground biomass (in mg of dry matter) for the three helophytes species under three different treatments (water, diluted sludge and sludge) in winter (February 2008), spring (April 2008) and summer (June 2008) (mean  $\pm$  S.D.,  $n = 5$ ). Different capital letters indicate significant differences ( $p < 0.05$ ) between species according to Kruskal–Wallis test and lowercase indicate differences between treatments within the same species.



**Fig. 2.** Exudated peroxidase (2.I.), wall-bound peroxidase (2.II.) and intracellular peroxidase (2.III.) activities (in U/g dry matter root) for the three helophytes species under three different treatments (water, diluted sludge and sludge) in winter (February 2008), spring (April 2008) and summer (June 2008) (mean  $\pm$  S.D.,  $n = 5$ ). Different capital letters indicate significant differences ( $p < 0.05$ ) between species according to Kruskal–Wallis test and lowercase indicate differences between treatments within the same species.

investigated. Only Gramms et al. (1999) studied maize grown on unsterilized soil, and found POD activity of 0.09 U/g root DW/ 2 days. Although maize is often used in phytoremediation, its exudated POD activity is by far lower than that exhibited by *P. australis* in present study, about 44-fold lower, which suggests its high waste oxidative capability. Exudated POD are mainly identified as cationic isoforms and their release into the rhizosphere is also accompanied by the secretion of  $H_2O_2$  (Kim et al., 2008). As such, exudation of POD in the rhizosphere might involve both direct and indirect oxidation processes of organic pollutants.

Comparing the three species studied, *P. australis* exhibited the highest exudated POD activity and specific activity in winter ( $p \leq 0.03$ ), spring ( $p < 0.0001$ ) and summer ( $p = 0.002$ ) (Table 1 and Fig. 2). For *P. australis*, secretions increased from winter to summer: POD activity was 1 U/g root DW in winter and reached 4 U/g root DW in summer ( $p = 0.004$ ) while that of *T. latifolia* was always at the detection limit.

Secretions for *P. australis* were higher in presence of sludge and differences between treatments were significant in spring for POD specific activity ( $p = 0.03$ ) (Table 1). It could be thought that these differences result from differences in above-ground biomass, as

5–25% of the photosynthetically fixed carbon can be released in the rhizosphere via a wide range of organic compounds including enzymes, but similar biomass was observed between treatments at this season, in spring. Most probably, POD exudation was induced by sludge exposure, which is in accordance with others studies where plants show higher POD activity in presence of xenobiotics such as azo dye acid orange (Davies et al., 2005; Carias et al., 2008) and cadmium (Fediuc and Erdei, 2002).

Regarding the cell wall compartment, the ionically bound POD activity of the plants was lower than the exudated POD. However, they may play a role in detoxication as they have been shown to be involved in oxidation of phenolic compounds (Sukalovic et al., 2005). Similarly to exudated POD, the wall-bound POD activity of *P. australis* (alone or in mixture with *T. latifolia*) was higher than that of the other species during the three seasons (Fig. 2), especially in winter, it was 0.6 U/g root DW, while the recorded activity was about 0.2 U/g root DW from *I. pseudacorus* and *T. latifolia* ( $p = 0.0003$ ). In addition, the specific POD activity (Table 1) of *P. australis* was between 50 and 600 U/g protein while for the others it was always below 200 U/g protein or even undetectable.

**Table 1**  
Exudated, wall-bound and intracellular peroxidase specific activity (in U/g protein exudated, wall-bound and intracellular respectively, mean  $\pm$  S.D.,  $n = 5$ ) for the three helophytes species under three different treatments (water–W, diluted sludge – dS and sludge – S) in winter (February 2008), spring (April 2008), and summer (June 2008). Different capital letters indicate significant differences ( $p < 0.05$ ) between species according to Kruskal–Wallis test and lowercase indicate differences between treatments within the same species.

Species	Exudated POD						Wall-bound POD						Intra-cellular POD					
	Winter		Spring		Summer		Winter		Spring		Summer		Winter		Spring		Summer	
	W	S	W	S	W	S	W	dS	S	W	dS	S	W	dS	S	W	dS	S
Mixture/Phragmites	89 $\pm$ 80B	788 $\pm$ 511B	214 $\pm$ 214B	148 $\pm$ 95Ba	637 $\pm$ 205Bb	537 $\pm$ 205Bb	221 $\pm$ 194B	787 $\pm$ 353B	1329 $\pm$ 636B	108 $\pm$ 54Ba	403 $\pm$ 57Bb	109 $\pm$ 96Aba	212 $\pm$ 164Aba	200 $\pm$ 27B	263 $\pm$ 51B	292 $\pm$ 118B	311 $\pm$ 7B	197 $\pm$ 35B
Mixture Typha	80B	511B	214B	95Ba	205Bb	537B	194B	353B	636B	54Ba	57Bb	96Aba	164Aba	27B	51B	118B	39 $\pm$ 7AB	76 $\pm$ 28AB
Phragmites	80B	511B	214B	95Ba	205Bb	537B	194B	353B	636B	54Ba	57Bb	96Aba	164Aba	27B	51B	118B	39 $\pm$ 7AB	76 $\pm$ 28AB
Typha latifolia	5A	7A	7 $\pm$ 7A	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	10 $\pm$ 10A	6 $\pm$ 6A	0 $\pm$ 0A	27 $\pm$ 27A	42 $\pm$ 42A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	1373 $\pm$ 414 $\pm$ 77B	459 $\pm$ 72B
Iris pseudacorus	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	44 $\pm$ 44A	77 $\pm$ 77A	44 $\pm$ 44A	0 $\pm$ 0A	72 $\pm$ 72A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	0 $\pm$ 0A	81 $\pm$ 81A	2 $\pm$ 2A	9 $\pm$ 9A
	0A	0A	0A	0A	0A	0A	505AB	35AB	41AB	0A	56A	0A	54A	0A	6A	80A	2 $\pm$ 2A	0A

As shown for exudated POD, the presence of sludge has also increased plants' wall-bound POD activity ( $p = 0.06$ ). Though the wall-bound POD activity of *P. australis* did not vary according to the season, the specific activity doubled in spring compared to summer. Spring is a season of high metabolism activity for growth and since POD also plays a role in lignin polymerization (Almagro et al., 2009), its increase in spring is possibly linked to this phenomenon.

Exudated and wall-bound POD are part of Phase I of the green liver concept. The measured activity proved the functionality of this phase I only for *P. australis* which even seemed to be induced by the presence of xenobiotics as sludge in this case. Moreover, the highest potential of *P. australis* for this metabolic pathway was enhanced by the greater root biomass of this species compared to *T. latifolia* and *I. pseudacorus*. Among these enzyme activities, exudated POD were much higher than wall-bound POD, which were nonetheless not negligible in spring. Considering that exudation contributes to the extension of the extracellular compartment, *P. australis* was potentially the plant that exhibits the greatest interaction with xenobiotics.

### 3.2.2. Intracellular peroxidase activity

POD activity was also found in the intracellular compartment of the macrophytes in winter, spring and summer (Fig. 2), and there was a species effect. *P. australis* (alone or in mixture) had the highest intracellular POD activity with about 10 U/g root DW compared to the other species ( $p < 0.0001$ ), and a similar pattern was observed for specific activity (Table 1) with less than 80 U/g of intracellular protein for *T. latifolia* and *I. pseudacorus*.

A season effect was only noticed for the specific activity of *P. australis* (alone) ( $p = 0.02$ ), with the lowest value in winter (110 U/g of intracellular protein) and the highest in summer (750 U/g of intracellular protein).

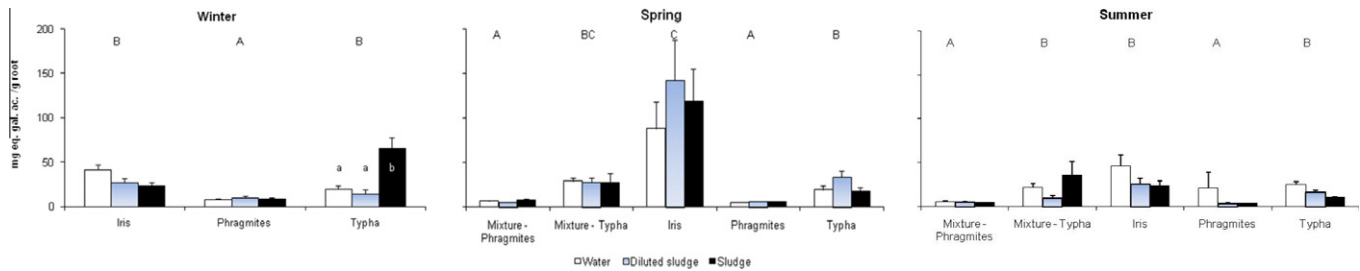
There was no effect of plant exposure to sludge on intracellular POD activity and specific activity, whatever the season ( $p = 0.7$ ). These results are in accordance with Davies et al. (2005) who showed that the POD activity of *P. australis* in a vertical flow constructed wetland was not modified with the exposure of different doses of Azo dye. However, exposure effect was observed by the authors when exogenous  $H_2O_2$  was added in the media.

### 3.3. Total phenol concentrations

Phenol concentrations were species-dependent ( $p < 0.0001$ ), but the mixture did not present differences compared to single plants (Fig. 3). On the whole, *I. pseudacorus* had the highest concentrations compared to the two other species, while *P. australis* had the lowest whatever the season. The differences were highest in spring, when phenol concentrations in *I. pseudacorus* roots reached 117 eq. gal. ac./g, compared to 23 and 6 eq. gal. ac./g for *T. latifolia* and *P. australis*, respectively. These low concentrations in the two latter species were not counterbalanced by differences in root biomass, which indicated that *I. pseudacorus* still presented the highest potential for this metabolic pathway of detoxification.

Phenol concentrations in below-ground parts were affected by the season for *P. australis* and *I. pseudacorus*, but remained stable for *T. latifolia* ( $p = 0.16$ ). *I. pseudacorus* had the highest concentrations in spring and *P. australis* had the highest in winter ( $p < 0.0001$ ). These variations were due to differences of plant physiological status (Solar et al., 2006) and of environmental factors (Ksouri et al., 2008) such as solar radiation (Toor and Savage, 2006).

Sludge did not seem to induce the production of phenols. No sludge effect was shown for *P. australis* and *I. pseudacorus* whatever the season ( $0.07 < p < 0.94$ ), and though *T. latifolia* presented different trends for the three seasons (in winter the highest concentrations were with sludge,  $p = 0.024$ , while in summer they



**Fig. 3.** Total phenol concentrations in roots (in mg equivalent gallic acid/g dry matter root, mean  $\pm$  S.D.,  $n = 5$ ) for the three helophytes species under three different treatments (water, diluted sludge and sludge) in winter (February 2008), spring (April 2008) and summer (June 2008). Different capital letters indicate significant differences ( $p < 0.05$ ) between species according to Kruskal–Wallis test and lowercase indicate differences between treatments within the same species.

were with water,  $p = 0.011$ , and no differences were found in spring). These results are in accordance with Markkola et al. (2002) who found that phenol concentrations in pine roots were similar in soils polluted with sulphur and in unpolluted soils. However, neither these soils, nor our sludge contained heavy metals or persistent organic pollutants (POP) which may be the target of phenol depollution. Therefore, it could be argued that the non-response of phenol production is due to the absence of target contaminant. Some authors have indeed found higher concentrations of soluble phenols in soil containing HAP (Cheema et al., 2009; Lee et al., 2008).

These results show the high potential of *I. pseudacorus* during the summer for depollution. Phenols can indeed act in the cell for detoxication, but can also be released into the rhizosphere (Walker et al., 2003; Wu et al., 2001) and trap pollutants such as POP (Haritash and Kaushik, 2009) and heavy metals (Jung et al., 2003). In addition, phenols can be used as a carbon source by microorganisms (Cheema et al., 2009) stimulating their activity, and therefore increasing the efficiency of the treatment through the degradation of organic matter and other compounds such as polychlorobiphenyls (PCB) (Hegde and Fletcher, 1996). On the other hand, phenols (depending on the type and concentrations) can also act as microorganism inhibitors as shown for litter degradation (Isidorov and Jdanova, 2002). Despite this negative effect of phenols for depollution processes, empirical experiments showed higher benefits in terms of pollutant removal in waste water.

#### 4. Conclusion

Our work has demonstrated the potential role of these three macrophytes for waste water treatment through constructed wetlands. *P. australis* was the most efficient plant in xenobiotics functionalisation during Phase I of the green liver concept with higher peroxidase activity in cell wall, exudates, and also in intracellular compartments. Conversely, *I. pseudacorus* exhibited the highest phenol concentrations suggesting that macrophytes operate different defense strategies in presence of xenobiotics. The presence of sludge induced changes in peroxidase activity and there was a season effect that can be correlated with plants' phenological stages.

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