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Selection of wild macrophytes for use in constructed wetlands for phytoremediation of contaminant mixtures

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A B S T R A C T

Constructed wetlands (CWs) offer an alternative to traditional industrial wastewater treatment systems that has been proved to be efficient, cost-effective and environmentally friendly. Most of the time, CWs are planted with proliferative species such as *Phragmites australis* or with plants originating from nurseries, both representing a risk for the natural biodiversity conservation of aquatic ecosystems located downstream of the CWs. For the removal of metals and organic pollutant mixtures present in industrial effluents, it is necessary to select tolerant plant species that are able to produce a high aboveground biomass and to develop a healthy belowground system. Wild plant species growing in aquatic bodies at industrial outfalls could constitute suitable tolerant species to use in CWs for industrial effluent treatment. To test this hypothesis, we assessed, under laboratory conditions (using an experimental design), the tolerance to mixtures of metals (Al, As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, Sn, Zn) or/and organic pollutants (THC, PHE, PYR, LAS) of five European sub-cosmopolitan native macrophytes (*Alisma lanceolatum*, *Carex cuprina*, *Epilobium hirsutum*, *Iris pseudacorus* and *Juncus inflexus*) that had been collected in a polluted Mediterranean wetland, after a field study (crossing ecological relevés and analyses of contaminant concentrations in water and sediments). Our results demonstrated that research on phytoremediation of industrial effluents should focus much more on the use of native macrophytes growing at short distances from industrial discharges (such as *C. cuprina* in this study), and that root/shoot ratio, aerial height and proportion of green leaves are good and cost-effective indicators of plant tolerance to metals and organic pollutant mixtures in laboratory studies.

1. Introduction

One of the burning issues in our industrial societies is the high consumption of water and the high demand for cleaning water (Schröder et al., 2007). Constructed wetlands (CWs) planted with macrophytes have proved their efficiency in treating a wide range of water pollution and are more and more widely used in European member states to treat several types of wastewater including industrial effluents (Hadad et al., 2006; Kadlec and Zmarthie, 2010; Khan et al., 2009; Vymazal, 2009). Most of the time, CWs are planted with resistant and proliferative species such as *Phragmites australis* or *Typha* spp. (Kadlec and Wallace, 2009; Vymazal, 2013). The use of such species in CWs may lead to the displacement of native vegetation in natural wetlands or disrupt the natural cycles of vegetation replacement that occur in native plant communities (Amon et al., 2007; Tulbure et al., 2007). Moreover, it has been

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recently proved that the large-scale replacement of wild plants by cultivars or hybrids of the same species represents a risk in the long-term, when implementing restoration activities, and is undesirable with regard to the conservation of native plants' biodiversity (Schröder and Prasse, 2013). It is therefore necessary to provide solutions for reconciling the objectives of pollution treatment in CWs and natural biodiversity conservation (Hsu et al., 2011).

When an area becomes moderately polluted, it is to be expected that tolerant species may replace those that have been lost (Grant, 2010). Therefore, macrophyte species that are growing in receiving bodies of water nearby effluent discharges may be tolerant to the contaminants persisting in the released effluents and are likely to constitute suitable native species to use in CWs for the treatment of these contaminants (Pilon-Smits, 2005; Ranieri and Young, 2012; Ranieri and Gikas, 2014; US EPA, 2000). Studies are needed to determine the pollution tolerance of wild macrophytes growing in contaminated water bodies and that are less competitive than the commonly used reeds and cattails.

Despite the significant progress that has been made in recent decades regarding the treatment of industrial effluents, good chemical and ecological status of water bodies located downstream of industrialized catchments are still difficult to achieve (Stalter et al., 2013). Additional retention and treatment systems such as CWs are necessary to reduce the ecotoxicity of industrial effluents and to preserve aquatic biodiversity and its ecological functions (Guittonny-Philippe et al., 2014; Schröder et al., 2007). Industrial wastewaters are characterized as complex mixtures with varying pollutants present with a wide range of concentrations (Soupilas et al., 2008). It is well known that in CWs, the growth of macrophytes and their depurative performance may be influenced by interactions among mixed pollutants (Zhang et al., 2011b). However, a limited number of data on wild macrophyte tolerance to mixtures of pollutants is available in the literature.

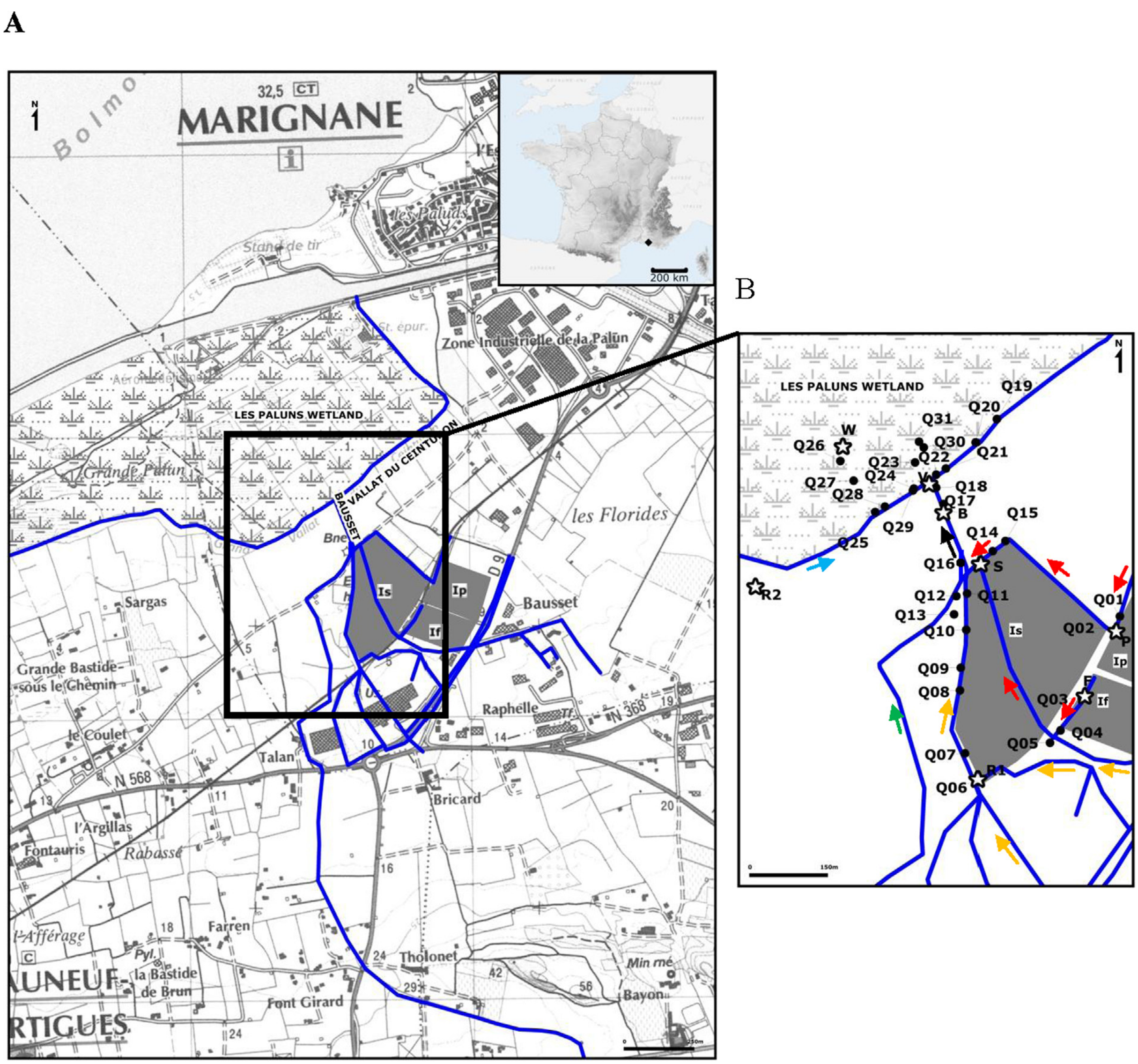


Fig. 1. (A) Localization of the study site (Marignane, South-Eastern France), interface zone between a protected wetland (Les Paluns) and an industrial zone (of which three main industrial areas, I_S, I_P and I_F, are represented in gray). Temporary streams and ditches are represented with blue lines. (B) Localization of the plots for plant ecological relevés (31 black dots: Q01 to Q31) and localization of the water and/or sediment sampling points (8 white stars: P, F, S, B, V, R1, R2, W). The direction of water flow is indicated with arrows. The color of arrows indicates the main water origin (green: catchment; orange: road-runoffs; red: industry discharges; dark: confluence; blue: wetland). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Therefore, studies on plant tolerance in the context of contaminant mixtures are of particular interest for finding new native plant species suitable for the tertiary treatment of industrial effluents.

In this perspective, the purpose of the present research was to evaluate, through field analysis and a greenhouse microcosm trial, the tolerance of five cosmopolitan wild macrophytes species (*Alisma lanceolatum*, *Carex cuprina*, *Epilobium hirsutum*, *Iris pseudacorus* and *Juncus inflexus*) to industrial contamination, with a view to selecting the most suitable of them for phytoremediation. To determine the plant species tolerance, we exposed them to three types of artificial industrial effluents composed of mixtures of organic pollutants and/or metals, during 113 days in microcosms in a full factorial design. The final aim of our study was to offer a practical and cost-effective approach for wild plant species selection for the treatment of industrial effluents in CWs.

2. Material and methods

2.1. Study site description, sampling and ecological relevés

The study site corresponds to the interface zone between a marsh wetland (Les Paluns wetland, Site of Community Importance included in the Natura 2000 habitats, south of Berre lagoon, Marignane, France) and an industrial catchment (Fig. 1A). The Les Paluns wetland is fed with brackish water by groundwater and with freshwater by the catchment of the Vallat du Ceinturon water mass. In this catchment, freshwaters are conveyed by temporary streams and most of them converge on the Bausset stream before reaching the Vallat du Ceinturon (Fig. 1). Several industrial plants have been established in the Bausset catchment and the continuous discharge of their effluents as well as road runoffs constitute a source of chronic pollution, leading to the contamination of the wetland sediments. A previous study conducted in 2008 after an accidental release of pollutants showed the presence of metallic and organic pollutants at high concentration levels in the wetland sediments (Table 1), even in zones unaffected by the accidental pollution, revealing a generalized chronic pollution input. Water samples were collected in July 2011 and in July 2012 at several points in the study site ditches and in the Vallat du Ceinturon and the Bausset streams (Fig. 1B and Table 2). A characterization of plant communities and ecological gradient was also performed. A set of 31 permanent square plots of one square meter were arranged along ditches and streams (Fig. 1B) and exhaustive censuses of vascular plant species were carried out. For each species, a cover rate was estimated using ten classes of 10%.

2.2. Planted microcosm set-up

Plantlets from five native helophytes species, i.e. *A. lanceolatum* With., *C. cuprina* (Sandor ex Heuff.) Nendtv. ex A. Kern., *E. hirsutum* L., *I. pseudacorus* L. and *J. inflexus* L., were collected in the ditches receiving industrial discharges. The plantlets were acclimated to greenhouse conditions for 4 months before experimentation. Before being replanted in microcosms, each individual was weighed and various biometric parameters were measured (Tables 6 and 7 in additional materials).

An experiment was designed for the purpose of distinguishing metals and organic pollutant effects on plants in a full factorial design as recommended by Lewis et al., 1999. Twenty-four microcosms consisting of rectangular plastic (polypropylene) tanks (413 × 345 × 294 mm, length × width × depth) were set up. The tanks were filled to 250 mm depth with 22 kg of pozzolan (7–12 mm diameter). Pozzolan was used as substrate because its mechanical characteristics are very favorable for water filtration processes (Dumont et al., 2008). In every tank, one PVC pipe (diameter 32 mm, 300 mm long) was placed upright about 150 mm from the centre of the tank for collecting water samples and immersing measuring probes. In order to avoid entanglement of the plant-root systems, the inside of the tanks was separated into six compartments of homogeneous size with intercalated flexible plastic (PVC) sheets.

Four microcosms were non-planted controls. Twenty microcosms were monospecifically planted (six plant individuals per species X condition). For each species, one microcosm was kept without contamination and served as planted control and three other microcosms were independently exposed to three different pollutant mixtures. In the same manner, among the four unplanted microcosms, an unpolluted control and three polluted microcosms were prepared (Fig. 2).

2.3. Contamination experimental design

In order to come close to realistic conditions of industrial pollution (Shen et al., 2005), we used mixtures of pollutants with relative concentrations chosen according to European regulation standards (European Union, 1976). Three types of effluents were added in the microcosms:

- A metallic pollutant mixture (MPM) containing eleven metallic salts i.e. $AlCl_3.2H_2O$; AsO_3; $CdSO_4.8H_2O$; $K_2Cr_2O_7$; $CuSO_4$; $Fe_2O_{12}S_3$; $MnSO_4.4H_2O$; $NiSO_4.7H_2O$; $Pb(NO_3)_2$; $SnCl_2$; $ZnCl_2$ that are all soluble in water in the experimental (Table 3). The stock metallic solutions were prepared individually by

Table 1
Total concentrations of contaminants in sediments (samples collected in July 2008) of the Les Paluns wetland tributaries receiving industrial discharges (P, F, S), in the Bausset (B) and Vallat du Ceinturon (V) streams, in the wetland (W) and at the reference points (R1, R2).

Chemicals	Concentrations ($mg\ kg^{-1}$) in sediments of the different stations along the pollution gradient						
	P	S	B	V	W	R1	R2
Al	5060	11 400	11 200	9540	11 300	5320	4320
Cd	6.4	<1	1.8	1.5	<1	<1	<1
Cr	64.1	19.5	25.9	20.6	20.8	13.9	9.4
Cu	794	132	79.2	69.7	22	68.2	13.5
Fe	41 100	14 400	16 000	13 400	14 300	9970	8770
Ni	54.3	15.2	18.8	18	22.1	17.4	9.7
Pb	1480	83.6	215	213	66.2	64.5	25.7
Zn	1580	244	329	316	82.8	223	37.6
THC (C10–C40)	6730	1120	2700	1560	467	638	263
PHE	0.6	0.1	0.1	<0.05	<0.05	0.1	<0.05
PYR	1	0.4	0.3	0.1	<0.05	0.2	<0.05

Table 2
Total contaminant and salt concentrations in water and dissolved oxygen, COD, suspended matters (samples from July 2011 or July 2012), electrical conductivity (measurements of January 2011) in the Les Paluns wetland tributaries receiving industrial discharges (P, F, S), in the Bausset (B) and Vallat du Ceinturon (V) streams, and at one of the reference points (R1). n.m.: not measured.

Parameters	S		P	F	B		V		R1
	2011	2012	2012	2012	2011	2012	2011	2012	2011
EC (μS cm^{-1})	1100	n.m.	n.m.	n.m.	1220	n.m.	1600	n.m.	n.m.
COD (mg L^{-1} O_2)	26.8	n.m.	n.m.	n.m.	9.7	n.m.	51.3	n.m.	12.3
Dissolved 0_2 (mg L^{-1})	4.9	n.m.	n.m.	n.m.	7.6	n.m.	9.2	n.m.	7.6
TSS (mg L^{-1})	15.8	n.m.	n.m.	n.m.	17.2	n.m.	104	n.m.	0.4
BOD_5 (mg L^{-1} O_2)	n.d.	n.m.	n.m.	n.m.	1.7	n.m.	9.2	n.m.	1.9
Fluoride (mg L^{-1})	0.5	n.m.	n.m.	n.m.	<0.1	n.m.	<0.1	n.m.	<0.1
Chloride (mg.L^{-1})	36.1	n.m.	n.m.	n.m.	77	n.m.	104.5	n.m.	84
Sulfates (mg L^{-1})	221.2	n.m.	n.m.	n.m.	265	n.m.	245.1	n.m.	266
Nitrates (mg L^{-1})	0.9	n.m.	n.m.	n.m.	5.5	n.m.	1.9	n.m.	7.8
Sodium (mg L^{-1})	43.5	n.m.	n.m.	n.m.	44.7	n.m.	57.1	n.m.	47.1
Phosphates (mg L^{-1})	<0.4	n.m.	n.m.	n.m.	<0.4	n.m.	<0.4	n.m.	<0.4
Nitrites (mg L^{-1})	<0.2	n.m.	n.m.	n.m.	<0.2	n.m.	<0.2	n.m.	<0.2
Bromides (mg L^{-1})	<0.2	n.m.	n.m.	n.m.	<0.2	n.m.	<0.2	n.m.	<0.2
Al (mg L^{-1})	<0.1	<0.1	0.41	0.41	<0.1	<0.1	0.256	<0.1	<0.1
Cd (mg L^{-1})	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cr (mg L^{-1})	<0.01	<0.01	0.02	<0.01	<0.01	0.02	0.006	<0.01	<0.01
Cu (mg L^{-1})	<0.002	0.01	0.03	0.05	<0.002	0.008	0.002	0.006	<0.002
Fe (mg L^{-1})	0.43	<0.01	0.20	0.78	0.07	<0.01	0.44	<0.01	<0.01
Mn (mg L^{-1})	0.56	0.04	0.04	0.07	0.04	0.01	0.11	0.01	0.007
Ni (mg L^{-1})	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Pb (mg L^{-1})	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
Zn (mg L^{-1})	0.05	0.02	0.12	0.24	<0.001	0.02	0.01	0.02	<0.001
THC (C_{10}–C_{40}) (mg L^{-1})	n.m.	0.60	0.87	15.8	n.m.	0.59	n.m.	0.63	n.m.
Anionic detergent LAS (mg L^{-1})	n.m.	0.18	0.18	0.29	n.m.	0.17	n.m.	0.12	n.m.
PHE (mg L^{-1})	n.m.	0.00056	0.00008	0.00195	n.m.	0.00088	n.m.	0.00093	n.m.
PYR (mg L^{-1})	n.m.	0.00001	0.00001	0.0002	n.m.	0.00002	n.m.	0.00003	n.m.

dissolving the appropriate amount of each metallic salt in deionised water.

- An organic pollutant mixture (OPM) composed of petroleum hydrocarbons (referred to as total hydrocarbons (THC)), two polyaromatic hydrocarbons i.e. phenanthrene (PHE) and pyrene (PYR) and an anionic detergent LAS (linear alkylbenzene sulfonate) named CARPHEM® (coco[polyoxyethylene(15)]methyl ammonium chloride, sodium cumene sulfonate, alkylethersulfate 2–4 EO sodium salt). The stock solutions of THC, PHE and PYR were prepared by dissolving individually an

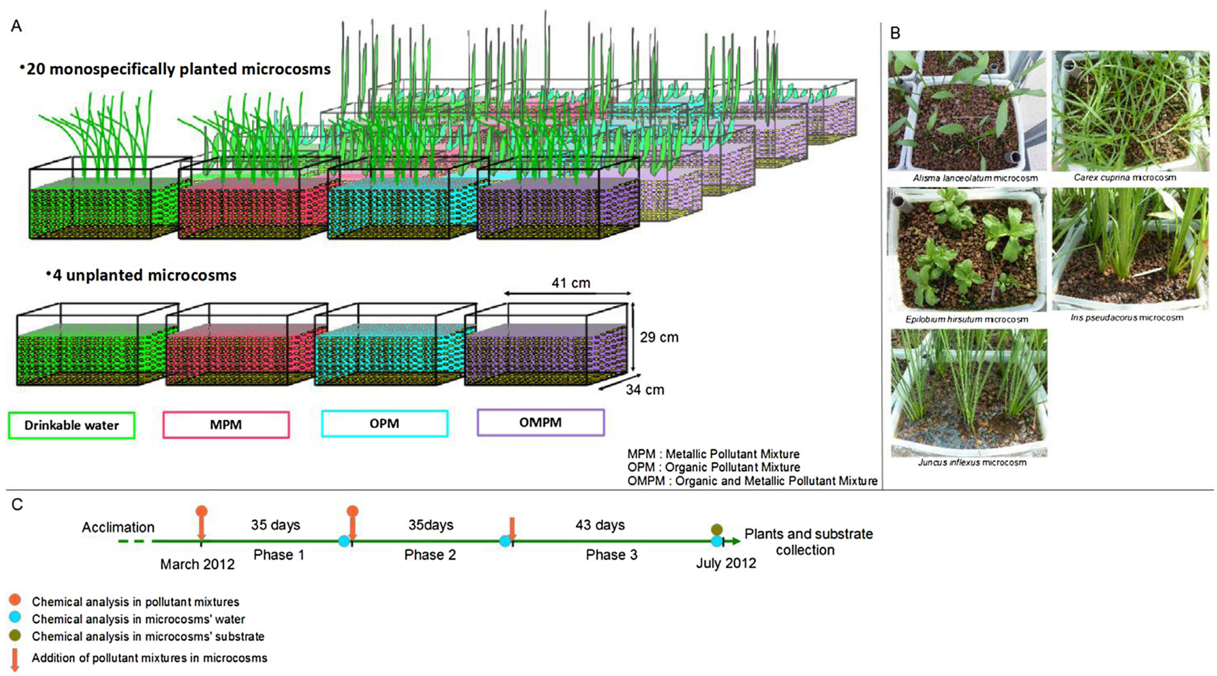


Fig. 2. (A) Experimental design of the microcosm trial; (B) photos of some of the planted microcosms; (C) Experimental schedule of the microcosm trial.

Table 3
Targeted and measured metal and organic pollutant concentrations (mg L^{-1}) in the pollutant mixtures and mean concentrations (mg L^{-1}) added in microcosms through the 3 test-phases. n.m.: not measured.

Chemicals	Chemical forms/ commercial name	European environmental quality standard	Targeted concentrations (mg L^{-1})	Measured concentrations (mg L^{-1}) ($n = 6$)	Targeted concentrations (mg L^{-1})	Measured concentrations (mg L^{-1}) ($n = 6$)	Contaminant concentrations added in microcosms in the 3 test-phases (mg L^{-1}) ($n = 6$)
			Phase 1		Phases 2 and 3		
Al	$AlCl_3.\ 2H_20$	2.5	2.5	2.02 ± 0.37	25	17 ± 2	37.5
As	AsO_3	0.05	0.05	<0.03	0.5	0.33 ± 0.06	0.7
Cd	$3CdSO_4.\ 8H_20$	0.2	0.2	0.27 ± 0.10	2	2.1 ± 0.3	4.5
Cr	$K_2Cr_20_7$	0.5	0.5	0.45 ± 0.03	5	4.5 ± 0.4	9.5
Cu	$CuSO_4$	0.5	0.5	0.47 ± 0.06	5	4.4 ± 0.2	9.3
Fe	$Fe_2O_{12}S_3$	2.5	2.5	1.90 ± 0.05	25	17 ± 1	36.0
Mn	$SO_4Mn.\ 4H_20$	1	1	1.27 ± 0.03	10	11.7 ± 0.7	24.8
Ni	$NiSO_4.\ 7H_20$	0.5	0.5	0.50 ± 0.00	5	4.9 ± 0.2	10.2
Pb	$Pb(NO_3)_2$	0.5	0.5	0.30 ± 0.04	5	3.6 ± 0.6	7.4
Sn	$SnCl_2$	2	2	n.m.	20	n.m.	n.m.
Zn	$ZnCl_2$	2	2	1.9 ± 0.4	20	16 ± 2	33.4
PHE	PHE	0.05	0.05	0.037 ± 0.005	0.5	0.32 ± 0.04	0.7
PYR	PYR	0.05	0.05	0.048 ± 0.005	0.5	0.34 ± 0.03	0.7
THC	BAL 250	10	10	7.1 ± 1.2	100	38.6 ± 3.7	84.1
Anionic detergent LAS	CARPHEM®	10	10	5.3 ± 0.6	10	4.7 ± 1.6	14.7

appropriate amount of each contaminant in dichloromethane. We used a hydrocarbon mixture of Blend Arabian Light petroleum topped at 250 °C (BAL 250) to prepare the THC stock solution, and PHE and PYR (provided by Merck (98% purity)) to prepare the PHE and PYR stock solutions. The spiking mixtures were prepared by diluting the appropriate amount of stock solutions in acetone and then in water. The anionic detergent LAS was directly added to the aqueous spiking solutions.

- The organic and metallic pollutant mixture (OMPM) containing both types of contaminants at concentration levels identical to the ones used for the MPM and the OPM.

The pollutant exposure was conducted in microcosms for 113 days in three successive pollution phases. In the first phase (days 0–34, 'chronic pollution'), pollutant concentrations corresponded to the European environmental quality standards (Table 3). In the second (days 35–69) and third (days 70–113) phases ('acute pollution'), pollutant concentrations were ten times higher than the European environmental quality standards, except for the anionic detergent LAS whose concentration was constant for the three pollution phases. In all the microcosms, 25 mL of an organo-mineral NPK 3.3.3. (NutriActiv®, NF U 42-001 produced by FLORENDI JARDIN SAS) fertilizer was added at the beginning of the first test-phase. Throughout the experiment period, the microcosms were watered with tap water at least 3 times a week, for maintaining a constant level of solution in the tanks (and a constant volume of 13 L), corresponding to the top surface of the pozzolan. At the beginning of the second and third test-phases, the liquid content of each microcosm was totally siphoned off and used to dilute the corresponding pollutant solutions of the next phase.

2.4. *Monitoring of physico-chemical and plant parameters*

Before enriching microcosms, three water aliquots were taken from the pollutant mixtures to determine the true initial pollutant concentrations added in microcosms (Sn concentration was not determined because of analytical constraints). At the end of the first and second test-phases, water samples were collected in the eighteen contaminated microcosms for determining the residual pollutant concentrations. Water samples as well as rhizospheric pozzolan samples (pozzolan in contact with plant roots) were taken at the end of the third test-phase in each of the twenty planted microcosms to determine residual metal and organic pollutant concentrations (the organic pollutant concentrations were only measured in control and contaminated microcosms planted with *C. cuprina* and *E. hirsutum* because of analytical constraints).

For each contaminated microcosm and for each contaminant, we calculated the percentage of removal rate from water at the final stage of each phase as follows:

(Tot Conc − Res Conc)/Tot Conc*100.

where "Tot Conc" is the Total Concentration added in the microcosm during the experiment (sum of concentrations per contaminant in the successive pollutant mixtures added in the microcosm) and "Res Conc" is the Residual Concentration in the microcosm water at the end of the third test phase.

During the experiment, in each microcosm, pH was monitored in the water column with a portable pH meter (Hanna Instruments®), and electrical conductivity (EC), dissolved oxygen (DO), and temperature (T°) were monitored with a WTW® device.

A wide array of growth and development parameters of the plant aerial parts was monitored during the experiment (Table 6 in additional materials). The biomonitoring data were recorded during 113 days at thirteen times of measurements. At the end of the experiment, five out of six plant individuals in each planted microcosm were harvested. Pozzolan was gently removed from the roots, and root systems were rinsed with tap water. Plants were then weighed before roots, bulb (if present), stems, leaves, and reproductive aerial parts (if present) were separated. Each plant part was also measured separately (Table 7 in additional materials) and fresh biomass was weighted (FW). Dry weights (DW) were obtained after oven drying at 80 °C for 5 days.

To enable the comparison of growth and plant development between the five plant species, results were expressed as follows:

- Total Relative Growth Rate (tot RGR) was calculated i.e. tot RGR (g/kg/day) = (ln final DW − ln initial DW)/number of days following Holopainen et al. (2010).
- Root to shoot dry weight ratio (R/S) was calculated according to Caldelas et al. (2012) from final DW data, where "shoot"

Table 4
Percentage of contaminant removed from water after the three test-phases in contaminated microcosms.

Microcosms		% Of contaminant removed from water													
		Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	HCT	PYR	PHE	Anionic detergent LAS
MOPM condition	*E. hirsutum*	99.6	100	100	100	100	99.6	99.0	98.2	99.5	99.8	98.7	100	100	98.7
	I. pseudacorus	99.8	100	100	100	100	99.4	92.6	98.5	99.5	99.8	99.0	100	100	98.9
	C. cuprina	99.6	100	100	100	100	99.4	88.9	93.9	99.5	99.0	98.7	100	100	98.0
	J. inflexus	99.6	100	100	100	100	99.8	99.7	97.7	99.5	99.5	99.4	100	100	98.7
	A. lanceolatum	98.0	100	100	100	100	99.3	95.2	98.1	94.4	99.2	99.5	100	100	98.2
	Unplanted	98.1	100	99.8	100	99.7	99.5	98.5	95.9	100	99.0	99.7	99.8	99.9	98.4
MPM or OMP condition	*E. hirsutum*	96.7	100	98.1	99.6	99.1	98.1	99.7	95.8	99.3	97.5	99.8	100	100	97.9
	I. pseudacorus	95.3	100	97.2	99.6	98.9	98.8	99.8	95.7	99.5	96.8	98.9	100	100	98.4
	C. cuprina	96.0	100	97.0	99.6	98.6	98.5	99.8	94.1	99.3	96.4	99.0	100	100	98.2
	J. inflexus	92.7	100	98.9	99.3	98.9	98.0	99.8	98.2	99.0	98.3	99.4	100	100	98.6
	A. lanceolatum	92.6	100	97.8	98.3	98.4	98.5	99.1	94.9	99.3	97.0	99.6	100	100	98.7
	Unplanted	95.2	100	98.2	94.9	98.2	99.0	99.3	96.7	99.4	98.2	99.8	99.9	99.9	98.1

designates the biomass of the emerged tissues, and "root" the biomass of the submerged organs, with rhizomes and roots summed together.

- Water content (WC) was calculated as: WC = (FW − DW)/DW (Creus et al., 1997).
- Proportion of green leaves, was calculated at each time of measurement, as follows: Number of green leaves/Total number of leaves (including senescent and dead leaves) (Holopainen et al., 2010).
- In a similar way, the proportion of leaves with chlorosis (when present), was estimated.

2.5. Chemical analyses

Analyses of *in situ* contaminant concentrations in the Les Paluns wetland sediments collected in July 2008 were conducted by an accredited laboratory (Eurofins Environment) by using inductively coupled plasma-atomic emission spectrometry (ICP-AES) following the NF EN ISO 11885 method for metals after mineralization, and by using gas chromatography-mass spectrometry (GC–MS) following a method adapted from XP X33-012 for PHE and PYR and by GC with flame ionization detector (GC-FID) according to the NF EN 14039 method for THC (sum of the total hydrocarbon between C_{10} and C_{40}).

Water samples collected in the field in July 2011, were analyzed for Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), fluoride, chloride, bromide, sulphates, phosphates, nitrites, nitrates, sodium and Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn contents. Water samples collected in the field in July 2012 were analyzed for Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn content, total hydrocarbons (THC, C_{10}–C_{40}), PHE and PYR, and anionic detergent LAS.

Details of analytical methods are given in supplementary data.

Table 5
Contaminant concentrations in substrate of planted control and contaminated microcosms at the end of the third test phase (mean between microcosms ± SD, $n = 5$ for metals and $n = 2$ for organic pollutants). For a considered contaminant, means followed by different letters significantly differ at $p \leq 0.05$ (Kruskal–Wallis test). n.m. = not measured. u.d. = unusable data.

Contaminant	Concentrations in substrate (mg kg^{-1}, dry weight) of planted microcosms			
	Control microcosms	MPM microcosms	OPM microcosms	OMPM microcosms
Al	22 839 ± 954	22 734 ± 639	n.m.	21 994 ± 588
As	11 ± 1 u.d.	11 ± 1 u.d.	n.m.	11 ± 1 u.d.
Cd	6 ± 1	7 ± 2	n.m.	6 ± 1
Cr	308 ± 42	275 ± 22	n.m.	276 ± 27
Cu	24 ± 1	25 ± 2	n.m.	23 ± 1
Fe	33 462 ± 547	33 833 ± 1802	n.m.	33 284 ± 888
Mn	529 ± 16	541 ± 39	n.m.	533 ± 30
Ni	249 ± 24	232 ± 19	n.m.	234 ± 19
Pb	5 ± 0.4	6 ± 1	n.m.	5 ± 0.4
Sn	n.m.	n.m.	n.m.	n.m.
Zn	43 ± 1a	50 ± 2b	n.m.	57 ± 14b
PHE	$0.024 \cdot 10^{-3} \pm 0.001 \cdot 10^{-3}$	n.m.	$0.027 \cdot 10^{-3} \pm 0.003 \cdot 10^{-3}$	$0.023 \cdot 10^{-3} \pm 0.0002 \cdot 10^{-3}$
PYR	$0.030 \cdot 10^{-3} \pm 0.002 \cdot 10^{-3}$	n.m.	$0.030 \cdot 10^{-3} \pm 0.001 \cdot 10^{-3}$	$0.030 \cdot 10^{-3} \pm 0.0002.10^{-3}$
THC	49 ± 5	n.m.	58 ± 16	48 ± 5

2.6. Statistical analyses

2.6.1. Ecological relevés

In order to highlight vegetation pattern over ecological gradient in the study site we performed a correspondence analysis (CA). This statistical method is adapted to matrices of contingency data (Benzécri, 1973). In our case, it allows to ordinate "n" rows (relevés) and "p" columns (plant species) by their resemblances. Hence, each relevé was characterized by its plant species composition, taking into account specific covering. The analysis releases ($n - 1$) axes that explain the total inertia (variance upon axis) of the data. Each axis corresponds to an ecological gradient. However, only the two main axes were retained here. Furthermore, two axes were retained to perform an indirect gradient analysis in relation to species ecology.

Data were analyzed statistically using R statistical software.

2.6.2. Microcosms study

For physico-chemical data (pH, T°, DO, EC), mean values were calculated for the planted microcosms with the same condition of contamination ($n = 5$) at each time of physico-chemical measurement and compared using Kruskal–Wallis analysis with post-hoc NSK test ($p \leq 0.05$).

For each contaminant, mean concentrations values of rhizospheric substrate were calculated for the planted microcosms with the same condition of contamination ($n = 5$ for metals and $n = 2$ for organic pollutants) at the end of the third test-phase and compared using Kruskal–Wallis analysis with post-hoc NSK test ($p \leq 0.05$).

We used PERMANOVA groups (Anderson, 2001; Godoy et al., 2011) to establish whether, within species, plant individuals exposed to the different pollutant mixtures could be differentiated

Table 6
Parameters monitored during the experiment on the five helophyte species.

Parameters	*A. lanceolatum*	*C. cuprina*	*E. hirsutum*	*I. pseudacorus*	*J. inflexus*
Number of green leaves	x		x	x	x
Number of young leaves	x				x
Number of leaves in senescence	x		x	x	x
Number of dead leaves	x		x	x	x
Number of leaves with chlorosis			x		
Number of young leafy stems		x			
Number of leafy stems		x			
Height of the longest leave	x			x	x
Height of the longest leafy stem		x			
Height of the longest shoot			x		
Length of the longest limb	x		x		
Number of inflorescences	x				
Number of leaves <10 cm					x
Number of leaves >30 cm					x
Number of leaves <30 cm and >10 cm					x

by a combination of the traits measured. For each species, the multivariate data were subsequently visualized using canonical analysis in principal coordinates (CAP). Constrained multivariate methods such as CAP use an a priori hypothesis to produce an ordination plot, so they enable detection of patterns that could be masked by overall dispersion with unconstrained methods such as multidimensional scaling. We generated CAP ordination plots on the basis of a matrix of pollution factor with four levels, i.e. Control, MPM, OPM, OMPM, fitted to a matrix of Euclidian dissimilarities of normalized trait values. The significance of fits was assessed with 999 permutations. PERMANOVA and CAP ordination plots were performed using Primer software v6 (Primer-E Ltd, UK).

For the plant individuals, after having detected most distinctive traits by multivariate analyses, univariate analysis of variance (ANOVA) was carried out in order to test the hypothesis that the pollutant mixtures have significant and differential effects on each of the selected traits. Prior to analyses, normality was evaluated with the Kolmogorov–Smirnov (K–S) normality test. For data which did not assume normality, a Kruskal–Wallis analysis and post-hoc NSK test were used to test the trait value differences between the conditions. For aerial height and proportion of green leaves, a repeated-measures two-way analysis of variance (RM-ANOVA) was carried out using the conditions (four types: Control, MPM, OPM, OMPM) and the time (10 times of plant biometric parameters measurements) as fixed factors. When significant differences were obtained, the Tukey's post-hoc comparison test ($p \leq 0.05$) was carried out to identify which of the pollutant mixtures induced relevant effects at the different plant measurement times. For R/S data, after having checked the normality of data by the K–S test, ANOVA analysis was performed.

Data were analyzed statistically using GraphPad Prism version 6.00 for Windows, GraphPad Software, or Primer.

3. Results and discussion

3.1. Contamination and plant species distribution in the field

In the field, sediments contained high concentrations of some metals i.e. Al, Cu, Fe, Pb, Zn, and hydrocarbons i.e. THC, PHE and PYR (Table 1), and significant concentrations of Ni, Cr and Cd locally in the industrial zone. For comparison, in sediments of the Berre Lagoon (highly industrialized site, situated near the study site), PHE and PYR were present at levels ranging from 0.05 to 0.2 mg kg^{-1}, and THC were present at around 1400–2000 mg kg^{-1} (Di Giorgio et al., 2001; Kanzari et al., 2012). Such levels are very close to those found in sediments from the study site and exceed the Canadian Interim Freshwater Sediment Quality Guidelines and also the Effect Range Low (Burton, 2002). Moreover, concentrations of PHE and PYR encountered in the wetland tributaries receiving industrial release (P) were as high as 0.6 and 1 mg kg^{-1}, largely exceeding the Threshold Effect Level (Burton, 2002). Sediments of ditches in the industrial zone (especially those sampled at the P industrial discharge level, Fig. 1) were more polluted than the wetland sediments (W, Fig. 1) and the sediments in the reference ditches (R1, R2, Fig. 1) not exposed to industrial effluents. This suggests that most of the sediment contamination originated from the chronic discharge of industrial effluents into water bodies. Nevertheless, contaminant levels in sediments of R1, R2 and W suggest that there is also background pollution probably due to the highly industrialized environment of the site (petrochemical industries, international airport, traffic, etc.).

In the Vallat du Ceinturon water, Cu and Zn concentrations exceeded the Environmental Quality Standard defined in the Water Framework Directive (0.0014 mg.L^{-1} for Cu and 0.0078 mg L^{-1} for Zn). Moreover, Cu, Mn and Zn water

Table 7
Measurements on the harvested parts for the five helophytes at the end of the experiment.

Parameters	*A. lanceolatum*	*C. cuprina*	*E. hirsutum*	*I. pseudacorus*	*J. inflexus*
Length of the longest root	x	x		x	x
Width of the bulb	x			x	
Thickness of the bulb	x			x	
Number of new bulbs	x				
Total fresh weight	x	x	x	x	x
Number of rhizomes			x		
Fresh and dry weights of roots	x	x	x	x	x
Fresh and dry weights of aerial parts	x	x	x	x	x
Fresh and dry weights of inflorescences	x				
Fresh and dry weights of bulb	x			x	

contamination levels encountered in the Vallat du Ceinturon water mass were higher than those of the southern France lagoons (Munaron et al., 2013). For these metals as well as for THC and the anionic detergent LAS, there was a south-north pollution gradient from the industrial outfalls to the Vallat du Ceinturon (P and F > S > B > V). We observed the inverse gradient for EC, TSS, DO, BOD_5, sodium and chlorides (V > B > S). PHE and PYR aqueous concentrations in the industrial ditches were below the regulatory thresholds (Table 3). However, such concentration levels of hydrophobic pollutants in the dissolved fraction are far from being negligible and are consistent with the high levels found in sediments.

Correspondence Analysis on vegetation showed two strong ecological gradients. The first gradient (explaining 12% of total inertia) corresponds to soil moisture that opposes dry conditions (negative side of first axis) – where *Dactylis glomerata* or *Sanguisorba minor* are clustered – and wet conditions with for instance *Iris pseudacorus* and *Cyperus fuscus* (positive side of first axis). This gradient showed clear spatial patterns from the industrialized zone in the southern area to the wetland in the northern area of the study site (Fig. 3A). This first gradient is understood as a disturbed *vs*. non-disturbed wetland habitat given that ruderal vegetation is observed in the ditch habitats in the south and is absent from the wetland habitats in the north. Indeed, in the wetland and in the Vallat du Ceinturon (plots Q21 to Q31; Fig. 4A), plant species are characteristic of brackish Mediterranean wetlands while in the Bausset and in industrial ditches (plots Q01 to Q20) there is a majority of ruderal plant species, characteristic of disturbed environments. Nevertheless there are also a few macrophyte species persisting in this disturbed zone, from which we selected five native wetland plant species for our microcosm trial. These five species are differently distributed along the perturbation gradient. *C. cuprina* is the most abundant of the five native species in the plots from ditches that receive industrial discharges (Fig. 4C) and is scarce in the Vallat du Ceinturon plots (only one individual in Q21). *A. lanceolatum*, *I. pseudacorus* and *J. inflexus* are mainly encountered in the Vallat du Ceinturon plots and individuals of each species are also found in one ditch plot (respectively Q14, Q16 and Q13; Fig. 4B, 4E and 4F). *E. hirsutum* was not depicted in any plot selected for the ecological relevés (Fig. 4D), however wide populations were found along the banks of the Bausset. It is worth noting that *P. australis* is abundant in the wetland and streams, as well as in the ditches exposed to industrial discharges which confirms its tolerance and its proliferative trends (Fig. 4G). *Typha latifolia* is far less abundant than *P. australis* at the study site, and is localized in the industrial ditches, specifically in the ditch receiving the Ip discharge (Fig. 4H).

The second ecological gradient (explaining 10% of total inertia) corresponds to a soil salinity gradient that does not show clear spatial patterns. Freshwater habitat (positive side of first axis) holds salt-intolerant species such as *Salix alba* or *Lemna minor*. These conditions occur almost entirely in the north, located at the confluence of Vallat du Ceinturon and Bausset (Fig. 3B). In contrast, halophytes such as *Ranunculus peltatus* or *Cyperus fuscus* are found in salt-affected soils that occur sporadically in the studied zone including in ditches receiving industrial discharges. This second gradient illustrates the diversity of the Les Paluns wetland habitats, linked to its hydrological functioning under the influence of industrial releases. Therefore, the plant species distribution at the study site is influenced by several ecological gradients interacting with industrial activity. Given the abundance of *C. cuprina* in industrial ditches where the water and sediment contaminations are the highest, we hypothesized that it is the most tolerant to industrial contamination among the five native species.

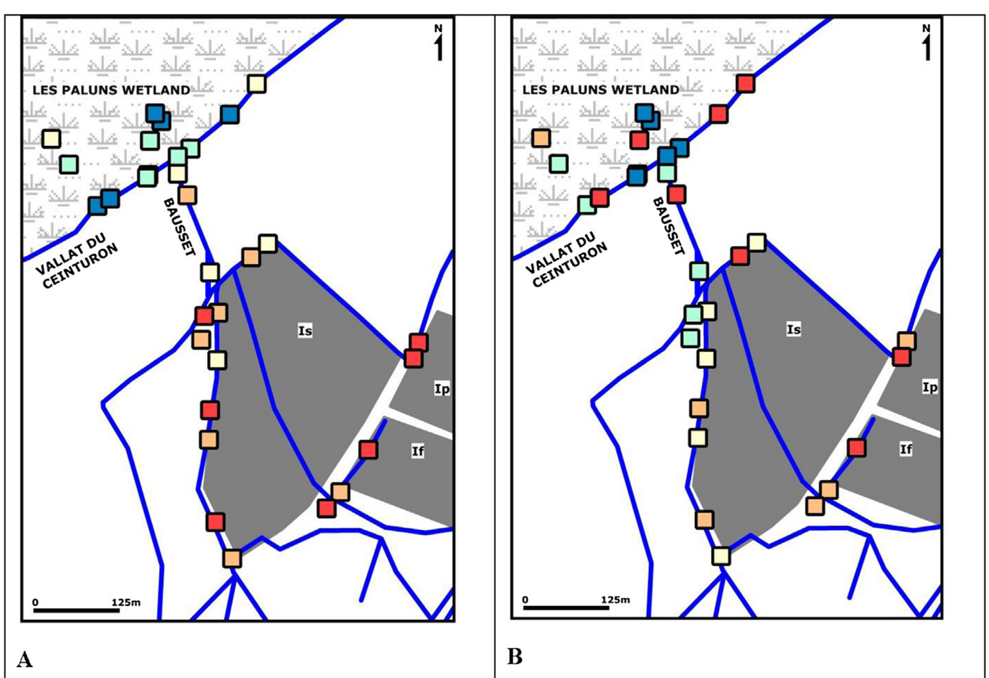


Fig. 3. Spatial projection of the coordinates of plots (squares) on (A) the first axis (soil moisture/perturbation gradient) and (B) the second axis (soil salinity gradient) of Correspondence Analysis. Extreme negative values on the axis are represented in squares with red color and extreme positive values on the axis are represented in squares with blue color. A color gradation enables representing intermediate values between the extremes (the orange squares and turquoise squares corresponding values are more similar to red and blue corresponding values, respectively). For the map legend, refer to Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

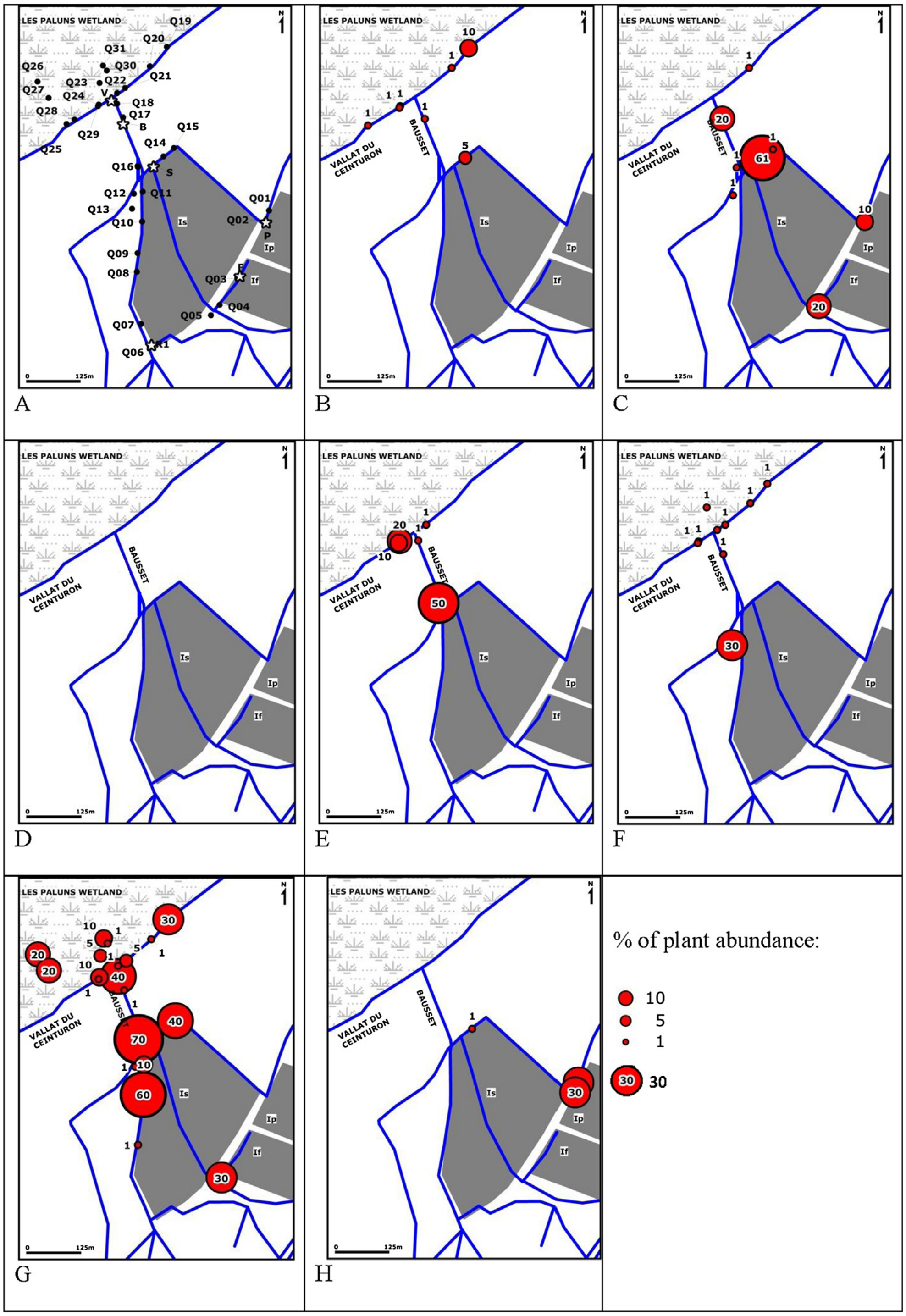


Fig. 4. (A) Localization of the 30 plots for ecological relevés. Percentages of plant abundance in each plot for (B) *A. lanceolatum*, (C) *C. cuprina*, (D) *E. hirsutum*, (E) *I. pseudacorus*, (F) *J. inflexus*, (G) *P. australis*, (H) *T. latifolia*. When there is a number inside the circle, it corresponds to the % of plant abundance.

3.2. Native plant species' tolerance to mixtures of organic pollutants and/or metals

3.2.1. Physico-chemical monitoring and depurative performance in microcosms

No significant variations of pH or T° values were detected in contaminated planted microcosms compared with control planted microcosms for each test-phase (Fig. 5C and D). Mean pH values ranged from 7.0 ± 0.2 (in OMPM microcosms at the end of the second test-phase) to 8.1 ± 0.1 (in OMPM microcosms before the first test-phase). Mean T° values ranged from 16.4 ± 0.2 °C (in MPM microcosms at the end of the second test-phase) to 23.5 ± 0.3 °C (in OMPM microcosms at the end of the third test-phase).

Before the first test-phase, mean EC values were homogeneous between control and contaminated planted microcosms, ranging from 590 ± 16 $\mu S\ cm^{-1}$ (in OMPM microcosms) to 601 ± 19 $\mu S\ cm^{-1}$ (in OPM microcosms). EC values rose at the beginning of the first test-phase in all planted microcosms, which is consistent with the fertilizer supply. In control and OPM microcosms, EC values were quite constant from the middle of the first test-phase to the end of the experiment, while EC values continued to increase in MPM and OMPM planted microcosms, to reach mean values of 1308 ± 113 μS μS cm^{-1} and 1255 ± 149 μS μS cm^{-1} respectively in MPM and OMPM planted microcosms at the end of the third test phase, linked to the high content of dissolved metallic salts in MPM and OMPM pollutant mixtures (Fig. 5B).

DO levels decreased in the beginning of the first test phase in all planted microcosms, which is consistent with the fertilizer supply, causing oxygen consumption by microorganisms for mineralisation. Mean DO values stayed low in OPM and OMPM planted microcosms until the middle of the third test phase while they began to rise in control and MPM planted-microcosms at the beginning of the second test phase. In particular, in the middle of the second test phase, mean DO values were 7.3 ± 6 and $6.4 \pm 3\%$ $O_2.L^{-1}$ in OPM and OMPM planted microcosms, respectively, while they were 73 ± 17 and $65 \pm 6\%$ $O_2.L^{-1}$, in control and MPM planted microcosms, respectively. These differences are probably due to microbial processes induced by the presence of biodegradable organic pollutants.

At the end of the third test phase, metals and organic contaminants had been efficiently removed from water in all of our experimental microcosms. The removal rates of contaminants ranged between 89% and 100% with a high proportion of them higher than 95%, even in unplanted microcosms (Table 4), which suggests that contaminants have been effectively retained in pozzolan. Pozzolan sorption of contaminants in microcosms is supported by the high availability of fixation sites for hydrophobic compounds and metals and by the total duration time of our experiment, which was probably too short to induce the saturation of adsorption sites (Dordio and Carvalho, 2013). Previous studies clearly demonstrated the efficiency of porous pozzolan in capturing and absorbing organic contaminants, such as aromatic compounds and hydrocarbons (Wang and Wu, 2006). Pozzolan can immobilize organic contaminants by a complex process consisting of both surface adsorption and porous diffusion (Wang and Wu, 2006). Moreover, pozzolan can in addition promote biodegradation of organic contaminants by acting as microorganism's substrate enabling microbial colonization of the material itself (Semple et al., 2001; Gaudin et al., 2008). It has also been shown that metals (e.g. Cd^{2+}, Cu^{2+} and Pb^{2+}) can be effectively retained by pozzolanic ash, at pH ranging from 4 to 9.

Nevertheless, no change in organic contaminant or metal concentrations linked to the artificial contamination was detected in the rhizospheric pozzolan at the end of the experiment, except for Zn (Table 5). Concerning Al, Cr, Fe, Mn and Ni, we hypothesize that the proportion of metals brought by the contaminant mixtures stayed negligible in comparison with the natural content of pozzolan (Table 5). Concerning Cd, Cu and Pb, we hypothesize that these metals were retained by another compartment of the microcosms, such as plant biomass~~,~~ or non-rhizospheric pozzolan. Concerning organic contaminants, the levels of concentrations found in the rhizospheric pozzolan correspond to trace levels of PHE and PYR and to background noise of the analytical method for THC, given that the quantification is made by integrating the chromatographic shape under the unresolved complex mixture. Absorption of organic contaminants in plant biomass is unlikely to have occurred given their physico-chemical characteristics (log Kow > 3.5 for THC, PHE and PYR and log Kow < 0.5 for the anionic

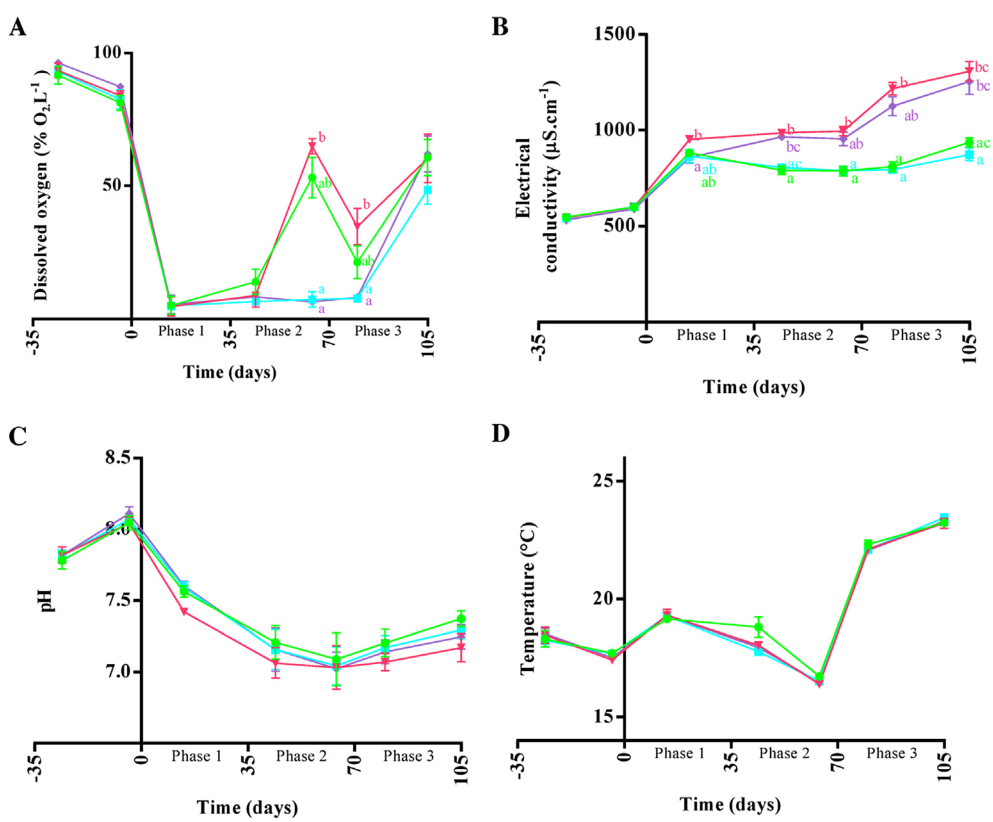


Fig. 5. Evolution of (A) dissolved oxygen (% $O_2.L^{-1}$), (B) electrical conductivity ($\mu S.cm^{-1}$), (C) pH and (D) Temperature (°C) in planted microcosms (mean between microcosms ± SEM, $n = 5$). At a given time, means followed by a same letter are not significantly different at $p \leq 0.05$ (*U*-test). Green circles = Control; Red triangles = MPM; Blue squares = OPM; Purple diamonds = OMPM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

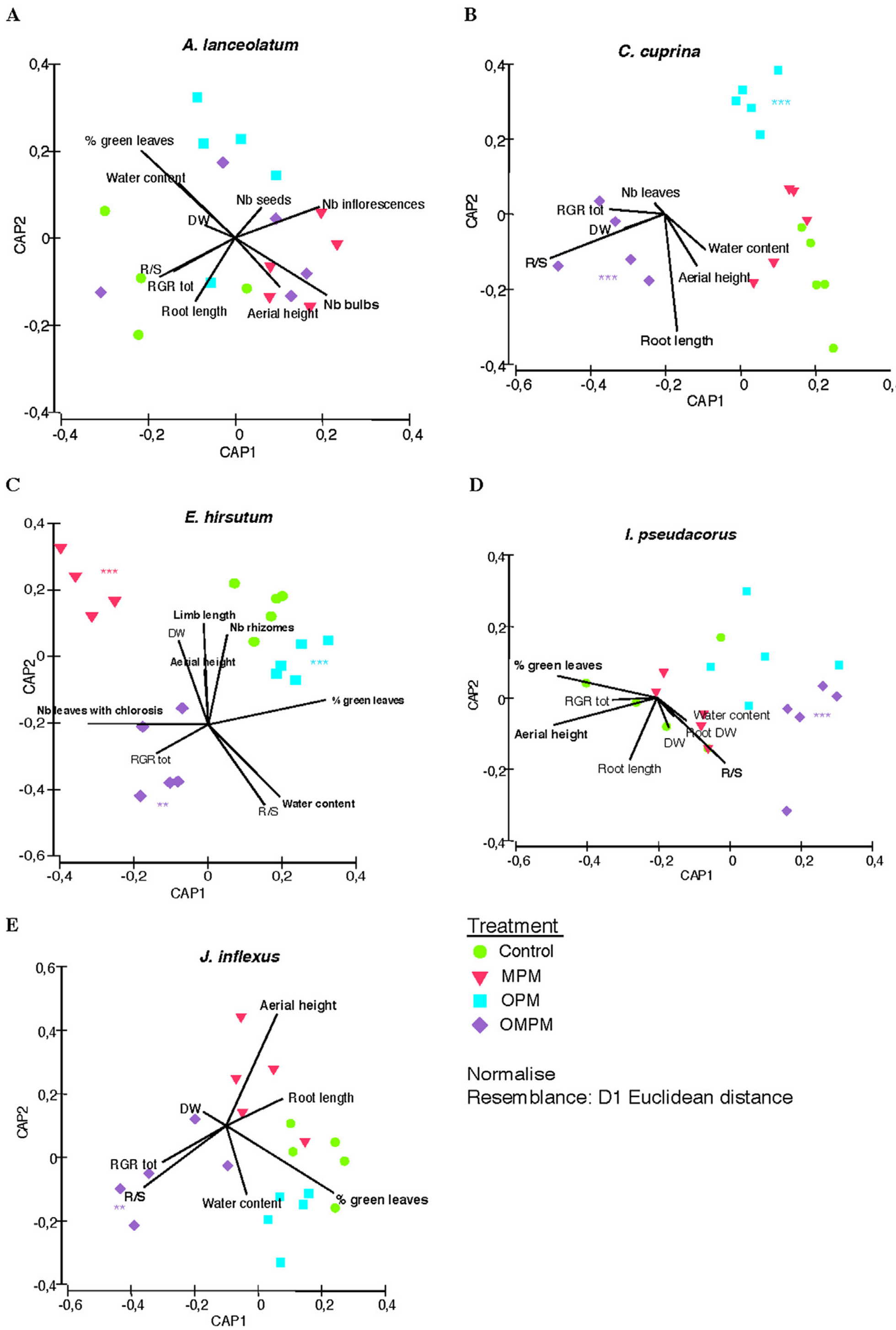
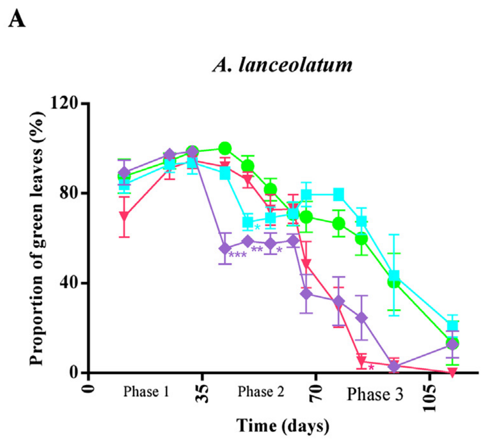


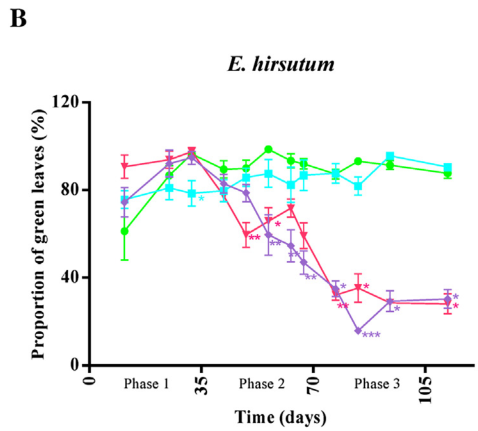
Fig. 6. Global effects of the pollutant mixtures on the growth parameters of the plant species after the 3 test-phases. DW: Dry Weight; R/S: Root to shoot ratio; RGR tot: Total Relative Growth Rate; Nb: Number; %: Percentage. When a contaminated group is significantly different from the control (PERMANOVA analysis) it is represented by asterisks (***$p \leq 0.01$; ** $0.01 < p \leq 0.05$; * $0.05 < p \leq 0.1$). The parameters that are in bold are those that were revealed significantly different from the control by ANOVA or Kruskal–Wallis tests ($p \leq 0.05$).

detergent LAS) (Dietz and Schnoor, 2001; Pilon-Smits, 2005). Therefore, organic contaminants may have been adsorbed in plant roots (Simonich and Hites, 1995), biodegraded by plant or rhizospheric microorganisms (Gramss et al., 1999), or retained by another compartment of the microcosms (e.g. dead plant biomass, plastic tank sides or non-rhizospheric pozzolan).

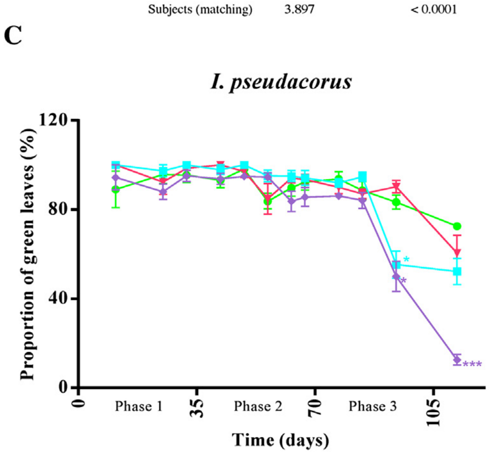
Nevertheless, even if removal rates obtained in unplanted microcosms were high in this experiment, the removal efficiency of substrate is expected to decrease with time (Imfeld et al., 2009; Semple et al., 2001), causing pollutants to become bioavailable once again. So it is important to take into account the toxicological effects of contaminants on plants for selecting the species that are able to maintain good health status and maintain their phytoremediation performance in the long term (Thullen et al., 2005). Even if it is well known that substrate acts as the primary sink for metal removal (Weis and Weis, 2004), plants may also have an



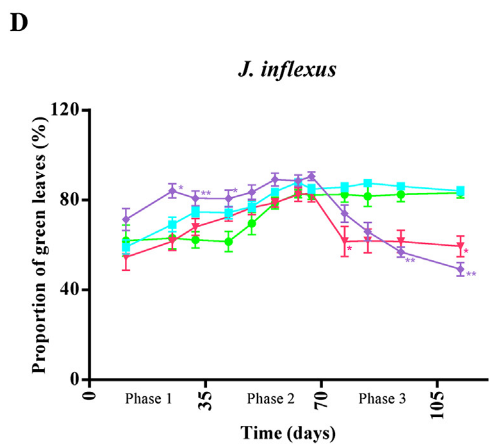
Source of Variation	% of total variation	P value
Interaction	11.46	< 0.0001
Time	61.79	< 0.0001
Pollutant mixtures	7.994	< 0.0001
Subjects (matching)	3.897	< 0.0001



Source of Variation	% of total variation	P value
Interaction	33.62	< 0.0001
Time	19.26	< 0.0001
Pollutant mixtures	28.52	< 0.0001
Subjects (matching)	2.788	0.0112



Source of Variation	% of total variation	P value
Interaction	18.42	< 0.0001
Time	55.57	< 0.0001
Pollutant mixtures	5.443	0.0001
Subjects (matching)	3.207	0.0071



Source of Variation	% of total variation	P value
Interaction	18.42	< 0.0001
Time	52.28	< 0.0001
Pollutant mixtures	9.377	0.0014
Subjects (matching)	8.265	< 0.0001

Fig. 7. Joint effects of the pollutant mixtures and time on the proportion of green leaves (mean of plant individuals ± SEM) on (A) *A. lanceolatum*, (B) *E. hirsutum* (C) *I. pseudacorus* and (D) *J. inflexus*. Asterisks associated indicate a significant difference (Kruskal–Wallis test; ***$p \leq 0.01$; ** $0.01 < p \leq 0.05$; * $0.05 < p \leq 0.1$) between the control microcosm and the corresponding contaminated microcosm at a given time. Green circles = Control; Red triangles = MPM; Blue squares = OPM; Purple diamonds = OMPM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

important role in the medium and long term. Firstly, the lifetime of the substrate can be enhanced with a frequent harvest of plants accumulating metals in their biomasses (Guittonny-Philippe et al., 2014). Secondly, given that metals can be released from the substrate of the CW to water when physical and chemical conditions change (Zhang et al., 2012), the frequent exportation of metals through plant harvesting is a necessary measure of precaution. Moreover, plant rhizospheric systems are essential for the degradation of organic contaminants retained in the CW substrate for several functions such as release of oxygen (Stottmeister et al., 2003), enzymatic and organic acid secretions or habitat furniture for microorganisms (Zhang et al., 2011a). It is therefore important to select tolerant plant species (Dordio et Carvalho, 2013) that are able to produce a high aboveground biomass and to develop a healthy belowground system, for removing metals and organic pollutants, respectively, after they have been immobilized in substrate (Kyambadde et al., 2004; Pilon-Smits, 2005). It is for this reason that, for the purpose of selecting them for further phytoremediation, we studied the tolerance of the five native plant species to mixtures of contaminants with regard to their effects on the aerial and root system development.

3.2.2. Plant responses to the contaminant mixtures' exposure in microcosms and perspectives for phytoremediation

Exposure to the MPM induced both the promotion of vegetative reproduction and an increase of leaf senescence on *A. lanceolatum*, while exposure to the OPM did not have any significant effect; and exposure to the OMPM caused leaf senescence (Figs. 6A and 7A).

Exposure to the MPM did not induce any significant effect on *C. cuprina* while exposure to the OPM inhibited its root elongation and exposure to the OMPM induced an increase in both root biomass and root to shoot partitioning (Figs. 6B and 8A).

The MPM exposure induced chlorosis and burning of leaf tips, resulting in a significant decrease of the proportion of green leaves on *E. hirsutum*. The OPM exposure caused a significant increase of water content. In addition to chlorosis and burning of leaf tips, the OMPM exposure initiated an inhibition of vegetative reproduction, a reduction of limb length and a slowdown of aerial elongation for this species (Figs. 6C, 7B and 9A).

The MPM exposure did not induce any significant effect on *I. pseudacorus* while the OPM exposure inhibited its aerial elongation and caused leaf senescence. In addition to these aerial toxicity symptoms, a root to shoot increase was induced on this species by the OMPM (Figs. 6D, 7C and 8B and 9B).

The MPM exposure caused leaf senescence on *J. inflexus*, while the OPM exposure generated a slight inhibition of aerial elongation (not significant with multiple comparisons but significant with two-tailed non-parametric comparison; Mann–Whitney *U*-test). The OMPM exposure caused leaf senescence, inhibition of aerial elongation and increase of root to shoot partitioning in this species (Fig. 6E and 7D and 8C, 9C).

Therefore, *C. cuprina* and *I. pseudacorus* were tolerant to the MPM under the conditions of our experiment while *A. lanceolatum* was tolerant to the OPM. Toxicity symptoms were detected on *J. inflexus* and *E. hirsutum* in the concomitant presence of both types of contaminants and both species were tolerant neither to the MPM nor to the OPM. Moreover *E. hirsutum* was the species that exhibited the highest number of ecotoxical symptoms in presence of the pollutant mixtures. *E. hirsutum* was thus the least tolerant of the five selected native species to industrial contamination which is consistent with its low abundance in the study site ditches. *C. cuprina* was the only species that did not show any macroscopic response of growth and development of the aerial parts. Moreover, the OMPM exposure induced an increase of root biomass and root to shoot partitioning of *C. cuprina*, which has been identified as a strategy of stress-tolerance that can enhance phytoremediation ability (Audet and Charest, 2008).

Therefore, in terms of phytoremediation potential, *C. cuprina* seems to be a suitable candidate to use in CWs for the treatment of metals and organic pollutant mixtures, as we had hypothesized on the basis of its distribution in the field (see Section 3.2.1.). *A. lanceolatum* that was tolerant to the OPM but sensitive to the MPM could be used in the downstream portion of CWs treating industrial pollutant mixtures, given that metals are mainly retained in the upstream portion (Lesage et al., 2007). *I. pseudacorus* should only be used for the treatment of industrial effluents exclusively highly polluted with metals whereas *E. hirsutum* and *J. inflexus* seem to be unsuitable species for treating highly polluted industrial effluents. Consequently, focusing on native species that are encountered at the shortest distance from industrial discharges into the aquatic ecosystem seems to be an interesting approach for the selection plants for phytoremediation.

3.2.3. Inter-specific analysis of plant responses to the contaminant mixtures' exposure and perspectives for plant selection

Our results showed that the OMPM supply altered the distribution of biomass between above- and belowground organs (in favor of belowground parts) in three out of the five species (*C. cuprina*, *J. inflexus* and *I. pseudacorus;* Fig. 8). But for these species, exposure to either the metallic or the organic pollutant mixtures separately did not induce any significant effect on the R/S, which implies that the biomass partitioning effect resulted from an interaction between the two types of pollutants.

Moreover, exposure to the OMPM had a significant inhibitive effect over time on the aerial elongation of *I. pseudacorus*, *J. inflexus* and *E. hirsutum* (Fig. 9). For the first two species, aerial elongation also decreased in plants exposed to the OPM, while such an effect was not detected in plants exposed to the MPM. The most probable hypothesis would be therefore that the negative effect on aerial elongation was mainly due to the organic pollutants, which seems consistent with previous studies concerning organic pollutants (THC, PYR, PHE or LAS) impact on plants (Chaîneau et al., 1997; Liu et al., 2004; Ma et al., 2010).

Finally, exposure to the OMPM had a significant negative effect over time on the proportion of green leaves of *A. lanceolatum*, *E. hirsutum*, *J. inflexus* and *I. pseudacorus* (Fig. 7). For the first three plant species, the proportion of green leaves decreased in plants exposed to the MPM, while such an effect was not detected in plants exposed to the OPM. Therefore, the leaf senescent effect was probably mainly caused by the metallic pollutants, which seems consistent with previous studies concerning metal impact on plants (Briat and Lebrun, 1999).

To sum up, the five species responded to the OMPM at acute concentrations, and the inter-specific analysis highlighted three patterns of response: (i) an increase of R/S caused by the interaction between organic pollutants and metals; (ii) an increase of the leaf senescent process mainly caused by metals and (iii) an inhibition of aerial elongation mainly caused by organic pollutants. These results suggest that R/S, aerial height and proportion of green leaves are good indicators of plants' tolerance, when exposed to metal and organic pollutants mixtures.

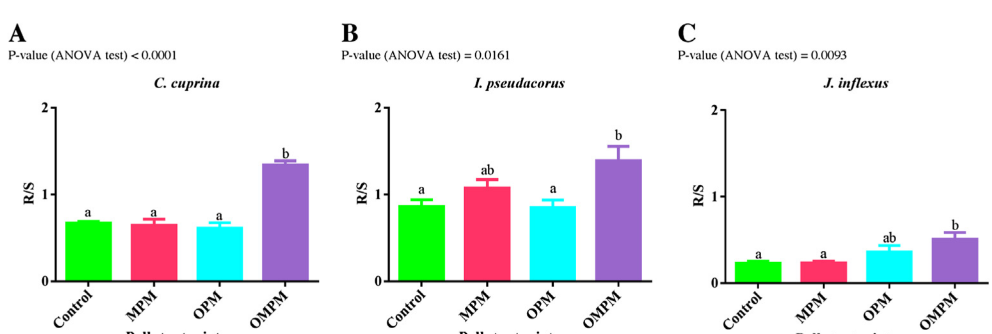
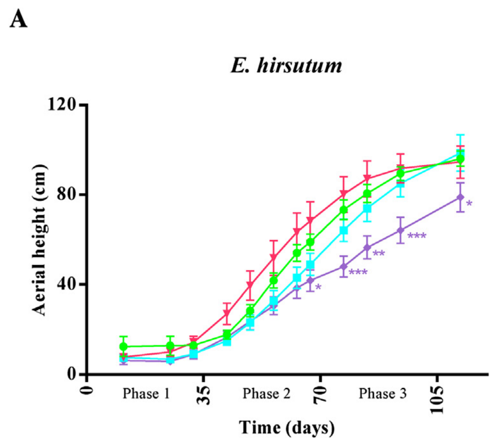
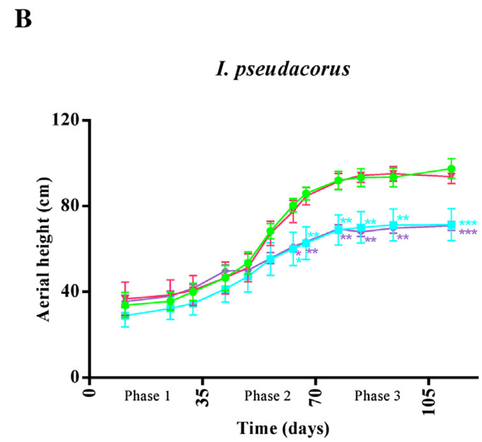


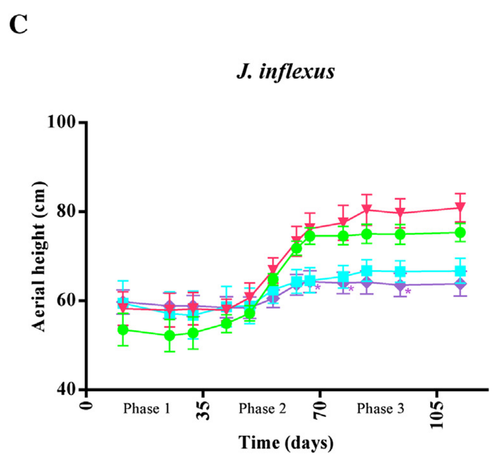
Fig. 8. Effects of the pollutant mixtures on the R/S (root to shoot ratio) (mean of plant individuals ± SEM) for (A) *C. cuprina*, (B) *I. pseudacorus* and (C) *J. inflexus* after the 3 test-phases (ANOVA analysis).



Source of Variation	% of total variation	P value
Interaction	2.166	< 0.0001
Time	81.78	< 0.0001
Pollutant mixtures	4.529	0.02
Subjects (matching)	7.339	< 0.0001



Source of Variation	% of total variation	P value
Interaction	4.812	< 0.0001
Time	63.44	< 0.0001
Pollutant mixtures	8.325	0.0509
Subjects (matching)	18.03	< 0.0001



Source of Variation	% of total variation	P value
Interaction	10.74	< 0.0001
Time	37.4	< 0.0001
Pollutant mixtures	8.003	0.1946
Subjects (matching)	30.98	< 0.0001

Fig. 9. Joint effects of the pollutant mixtures and time on the plant aerial elongation (mean of plant individuals ± SEM) of (A) *E. hirsutum*, (B) *I. pseudacorus* and (C) *J. inflexus*. Asterisks associated indicate a significant difference (***$p \leq 0.01$; ** $0.01 < p \leq 0.05$; * $0.05 < p \leq 0.1$) between the control microcosm and the corresponding contaminated microcosm at a given time (ANOVA or Kruskal–Wallis analysis). Green circles = Control; Red triangles = MPM; Blue squares = OPM; Purple diamonds = OMPM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

It has been recently emphasized that plant species selection is the best way to further maximize pollutant removal in CWs (Brisson and Chazarenc, 2009) and that native plant species of polluted sites could be good candidates. Our study confirms that research on phytoremediation should turn more towards the use of native macrophytes in CWs, and especially the species growing at short distances from industrial discharges into aquatic ecosystems, such as *C. cuprina* in our case. Therefore, we suggest the use of *C. cuprina*, *A. lanceolatum* and/or *I. pseudacorus* in CWs in Europe due to their wide distribution in European countries.

Preliminary studies in laboratories must be conducted for testing plants' tolerance before using them for phytoremediation of a specific effluent. The results of our experiment provide a basis for proposing simple and integrative morphological criteria (i.e. the aerial elongation, the leaf senescence and the R/S) which could be used in other future preliminary studies, for the selection of the best tolerant native plant species, in the case of effluents containing metals and/or organic pollutants. The validation of these morphological criteria for plant selection requires their application on other macrophytes. We recommend carrying out such studies in order to provide user-friendly tools for native plant species selection for use in phytoremediation of industrial effluents.

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Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jenvman.2014.09.009.

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