



Experimental *in situ* exposure of the seagrass *Posidonia oceanica* (L.) Delile to 15 trace elements

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ABSTRACT

The Mediterranean seagrass *Posidonia oceanica* (L.) Delile has been used for trace element (TE) biomonitoring since decades ago. However, present informations for this bioindicator are limited mainly to plant TE levels, while virtually nothing is known about their fluxes through *P. oceanica* meadows. We therefore contaminated seagrass bed portions *in situ* at two experimental TE levels with a mix of 15 TEs (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Pb and Bi) to study their uptake and loss kinetics in *P. oceanica*. Shoots immediately accumulated pollutants from the beginning of exposures. Once contaminations ended, TE concentrations came back to their original levels within two weeks, or at least showed a clear decrease. *P. oceanica* leaves exhibited different uptake kinetics depending on elements and leaf age: the younger growing leaves forming new tissues incorporated TEs more rapidly than the older senescent leaves. Leaf epiphytes also exhibited a net uptake of most TEs, partly similar to that of *P. oceanica* shoots. The principal route of TE uptake was through the water column, as no contamination of superficial sediments was observed. However, rhizomes indirectly accumulated many TEs during the overall experiments through leaf to rhizome translocation processes. This study thus experimentally confirmed that *P. oceanica* shoots are undoubtedly an excellent short-term bioindicator and that long-term accumulations could be recorded in *P. oceanica* rhizomes.

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1. Introduction

The Mediterranean Sea is a semi-enclosed basin experiencing anthropogenic disturbances. Urban coastal development, tourism, industries, shipping, etc., threaten and pollute Mediterranean coastal environments (Laubier, 2005). The UNEP (1999, 2002) identified 101 geographic sites impacted by industrial and domestic pollutions; among them, eight hot spots mainly concentrated in the eastern Mediterranean basin are responsible for major discharges of Hg, Cd, Pb, Cr, Cu, Zn and Ni (Laubier, 2005).

Posidonia oceanica (L.) Delile, the endemic marine magnoliophyte of the Mediterranean, forms monospecific meadows along the coasts from the surface to depths of about 40 m (Boudouresque and Meinesz, 1982; Gobert et al., 2006). This seagrass presents characters of a good bioindicator: this sessile organism is abundant and easy to sample; it effectively accumulates pollutants at high levels, is resistant to pollution, persists in the vicinity of important contamination sources and reflects the status of contamination of its environment (water and sediments; Pergent-Martini and Pergent, 2000; Wright and Welbourn, 2002; Boudouresque et al., 2012).

Furthermore, *P. oceanica* is expected to play a major role in the cycling of trace elements (TEs) in Mediterranean coastal areas due to its wide abundance, high productivity and capacity to accumulate these elements (Sanz-Lázaro et al., 2012).

P. oceanica has largely been used for trace metal biomonitoring (Cr, Fe, Ni, Cu, Zn, Cd, Pb and Hg) since decades ago (reviews: Pergent-Martini and Pergent, 2000; Luy et al., 2012). Moreover, Luy et al. (2012) have also demonstrated that above-ground tissues of *P. oceanica* could effectively be used for the monitoring of a series of little studied and potentially toxic TEs (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi), and that abnormally high levels of TEs compared to baseline values could precisely be linked to specific human activities. However, ecotoxicological information for the genus *Posidonia* is limited mainly to plant TE levels, while virtually nothing is known about kinetics of TE fluxes through *P. oceanica* communities. These observations made by Warnau et al. (1996) in the mid-1990s are still topical as, to our best knowledge, no other kinetic study has been driven since then.

The aim of this study was therefore to experimentally investigate TE kinetics in *P. oceanica*. Isolated portions of a seagrass bed were contaminated *in situ* using multielement (15 TEs) exposures at two pollution scenario levels: (i) a moderate level, simulating fairly contaminated seagrass meadows, and (ii) an acute level, simulating highly contaminated seagrass meadows. Kinetics were followed in

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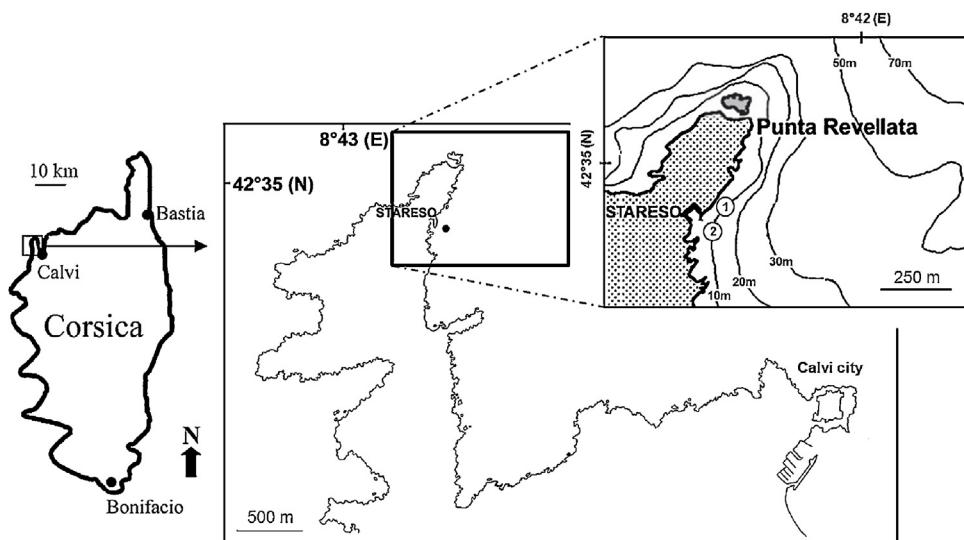


Fig. 1. Location of the *in situ* experimental contaminations in the *P. oceanica* bed in front of the oceanographic station STARESO, in the Calvi Bay (northwestern Corsica, France). Numbered circles on the 10 m isobath in the STARESO area zoom (picture right side) show the emplacement of the 2 experimental setups contaminated with a multielement solution at either moderate (1) or acute (2) levels.

entire shoots, in young and old leaves and in rhizomes. Bioavailable pollutant levels within experimental setups were measured throughout experiments. Contamination of superficial sediments and leaf epiphytes were further monitored.

2. Materials and methods

2.1. Continuous field survey

P. oceanica shoots ($n=166$) and superficial sediment samples ($n=6$) were seasonally collected between years 2008 and 2010 at 10 m depth in the Calvi Bay, northwestern Corsica (France), in front of the oceanographic station STARESO (Fig. 1), in order (i) to determine the mean natural TE levels of the studied seagrass bed and (ii) to validate (or invalidate) the selection of the Calvi Bay as a reference site for the monitoring of TE pollution in the northwestern Mediterranean (Luy et al., 2012).

2.2. Experimental design

In June 2009, isolated portions of a *P. oceanica* seagrass bed were experimentally contaminated *in situ* with a mix of 15 TEs: Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ag, Cd, As, Mo, Pb and Bi. Dissolved TEs were injected through the recirculating water pipe of experimental setups with the aid of a syringe. Experiments were driven at 10 m depth in the Calvi Bay, in front of the STARESO (Fig. 1). Two levels of contaminations were intended: (i) a moderate and (ii) an acute level. The moderate level of TEs corresponded to a final concentration in average 3.7 times higher than the world ocean seawater average concentration (and more than 20 times the Calvi Bay concentration); the acute level of TEs corresponded to a final concentration 28.8 times higher (and more than 150 times the Calvi Bay concentration). The world ocean TE average concentration (Table 1A) was calculated by compiling data from the literature (detailed in Appendix A Table A-1). Moderate levels of TEs further corresponded to what can be measured in contaminated sites of the Mediterranean such as Canari (Corsica, France; Ni contamination; Lafabrie et al., 2007), Gulf of Elefsis (Greece; Zn contamination; Scoullos, 1981) or Favigna Island (Sicily, Italy; Cd contamination; Campanella et al., 2001). Bulk multielement solutions of TEs were prepared by diluting 1000 mg L⁻¹ standard

solutions (Certipur grade, Merck). Concentrations within experimental setups after injections, according to the total amount of TEs injected, are given in Table 1B.

98 *P. oceanica* shoots were contained in the moderately contaminated cylindrical experimental setup (Appendix A Fig. A-1). It was made of a plastic jagged basis buried in sediments up to the base of its teeth. This basis was surmounted by a 120 cm high (total height = 145 cm) and 60 cm wide removable polyvinyl chloride (PVC) cylinder, closed to its top. The water volume inside the experimental setup was 410 L. A continuous circular ascending water current was created with a submerged pump recirculating all the water volume in 1 h 25. The multielement solution was injected every 12 h (at 9.00 am and 9.00 pm) for 5 days. *P. oceanica* shoots were sampled every morning before TE injection, to study their contamination kinetics, by toppling over the experimental setup, which renewed water inside. The setup was removed after 5 days; *P. oceanica* shoots were regularly sampled for 15 more days, to study their decontamination kinetics, and in November 2009 and March 2010 as post-controls.

In the second experimental setup, 91 *P. oceanica* shoots were contaminated at acute TE levels. The setup consisted of a 54 L polypropylene (PP) trapezoidal box (surface on sediment: 44 cm × 35 cm; height: 38 cm) set down with its opening side facing sediments (Appendix A Fig. A-1). The water current created with a submerged pump was horizontal through *P. oceanica* shoots enclosed in the setup. The complete water volume cycle **trough** the recirculating closed system took 11 min. The contamination period lasted 24 h. The 12 first hours, TEs were injected every 3 h (from 9.00 am to 9.00 pm). The last contamination went on all night (from 9.00 pm until 9.00 am). *P. oceanica* shoots were sampled before every TE injection, to study their contamination kinetics, by toppling over the experimental setup, which renewed the water inside. After the setup removal, *P. oceanica* shoots were regularly sampled for 15 more days, to study their decontamination kinetics, and in November 2009 and March 2010 as post-controls.

2.3. *P. oceanica* sample processing

P. oceanica shoots were treated according to the biometric method proposed by Giraud (1979). Epiphytes were scraped from leaves with the aid of a ceramic scalpel blade. Furthermore, each

Table 1 (A) Surface seawater trace element (TE) mean (\pm SD; number of references = 11 to 28) and maximal concentrations ($\mu\text{g L}^{-1}$) reported from literature (see Appendix A Table A-1 for details). (B) TE concentrations ($\mu\text{g L}^{-1}$) within experimental setups contaminated at moderate or acute levels, respectively, calculated after syringe injections of the multielement solution. (C) Average TE concentrations ($\mu\text{g L}^{-1}$) measured with diffusive gradients in thin films (DGTs) devices deployed in the *P. oceanica* control meadow and in experimental setups contaminated at moderate or acute levels, respectively; concentrations are given as means \pm SD for the *P. oceanica* control meadow (number of replicates (n) = 4) and the setup contaminated at moderate level (n = 5) and as individual measurements for the 2 DGTs deployed in the setup contaminated at acute level; italic values for V, Mo and Bi are TE concentrations measured in DGT chelex resin elutus. (D) Bioavailable fractions (%) of TE initially injected within experimental setups, calculated as the ratio between TE concentrations in experimental setups and TE concentrations in the *P. oceanica* control meadow. nd = not determined.

		As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
A.	Literature															
	Seawater conc. (mean \pm SD)	1.6 \pm 0.5	0.80 \pm 0.61	1.8 \pm 0.5	0.19 \pm 0.14	1.1 \pm 1.6	0.42 \pm 0.55	0.08 \pm 0.11	0.37 \pm 0.38	0.31 \pm 0.35	1.3 \pm 1.8	11 \pm 16	0.10 \pm 0.12	0.048 \pm 0.056	0.13 \pm 0.19	0.0001 \pm 0.0002
	Seawater maximal conc.	17.8	9.60	2.5	0.62	14.4	141.0	0.39	11.0	76.0	309.0	16	1.01	2.80	1.70	0.0906
B.	Injected TEs															
	Moderate level	9.4	8.9	22	2.3	4.4	4.7	0.89	4.4	4.5	57	88	2.4	3.9	4.6	0.16
	Acute level	94.4	90.1	222	23.0	44.2	47.2	89.9	44.8	45.5	57	891	24.3	39.0	46.3	1.63
C.	Measured TEs															
	Control (mean \pm SD)	nd	0.06 \pm 0.12	0.44 \pm 0.14	0.13 \pm 0.09	0.23 \pm 0.04	0.23 \pm 0.04	0.01 \pm 0.00	0.15 \pm 0.02	0.04 \pm 0.02	25 \pm 3.9	0.6 \pm 0.2	0.0003 \pm 0.0001	0.009 \pm 0.002	0.05 \pm 0.02	nd
	Moderate level (mean \pm SD)	nd	2.00 \pm 0.78	2.60 \pm 0.89	0.16 \pm 0.01	0.34 \pm 0.06	1.46 \pm 1.60	0.20 \pm 0.10	1.14 \pm 0.44	0.94 \pm 0.16	7.7	7.7 \pm 2.6	0.0184 \pm 0.0035	0.036 \pm 0.109	1.00 \pm 0.34	0.15 \pm 0.06
	Acute level (2 measurements)	nd	24.36; 19.10	71.54; 30.59	1.38; 0.82	2.96; 2.48	13.59; 9.18	2.02; 1.27	9.94; 6.76	9.76; 7.36	37.3; 112.5	189.0; 86.2	0.0930; 0.0270	3.082; 2.014	5.46; 5.23	0.77; 0.40
D.	Bioavailability															
	Moderate level	—	22%	—	7.1%	8%	31%	22%	26%	21%	29%	—	0.76%	7.9%	22%	—
	Acute level	—	24%	—	4.8%	6%	24%	18%	19%	19%	23%	—	0.24%	6.5%	12%	—
E.	Contamination factors															
	Moderate level	nd	35	5.9	1.3	1.5	6.2	38	7.8	24	4.7	14	55	35	21	nd
	Acute level	nd	381	115.2	8.7	12.0	48.8	319	57.3	218	37.4	242	175	289	113	nd

shoot was sorted as follows: intermediate leaves, adult leaves, rhizome 2 first cm (scales removed) and epiphytes. Sorted tissues were lyophilized (BenchTop 3L, VirTis Company Inc.), weighed and ground in an agate mortar. Contrary to rhizomes and epiphytes, *P. oceanica* leaves were cryogenically ground and then re-lyophilized to eliminate condensed ambient water vapour. Dried powders were mineralized in Teflon bombs in a closed microwave digestion lab station (Ethos D, Milestone Inc.). The digestion procedure performed was a nitric acid-hydrogen peroxide mineralization ($\text{HNO}_3/\text{H}_2\text{O}_2$; suprapure grade, Merck). Finally, digestates were diluted to an appropriate volume of 50 ml prior to being analyzed. TE levels measured in *P. oceanica* intermediate and adult leaves were weighted by their respective dry weight to calculate shoot TE levels.

2.4. Bioavailable trace element concentrations

Bioavailable TEs in experimental setups (*i.e.* the fraction of injected TEs that are effectively accessible to bioaccumulation processes) were measured throughout the used of diffusive gradients in thin films (DGTs) devices (DGT Research Ltd., UK). DGT technique is based on the diffusion of metals through a diffusive layer until a cation-exchange resin selective for trace metals (Davison and Zhang, 1994; Zhang and Davison, 1995). DGT units, fixed with a nylon fishing line to a plastic stage buried in sediments (Appendix A Fig. A-1), could float freely in the *P. oceanica* bed canopy. As trace metal diffusion coefficients through the diffusive layer depend on water temperature, average temperatures were recorded with Minilog temperature data loggers (VEMCO Division, AMIRIX Systems Inc., Canada). DGT units were replaced every day (at 9.00 am) for the moderate contamination (5 DGTs deployed), every two days in the reference *P. oceanica* bed (4 DGTs deployed), and after 12 h for the acute contamination (2 DGTs deployed). Collected DGTs were carefully rinsed with milliQ water and stored at 4 °C. Elution of metals from the Chelex 100 binding phase was carried out by immersing resins in 1.0 M HNO_3 (Suprapure grade, Merck) for a minimum of 24 h prior to being analyzed.

2.5. Sediment samples collect and processing

Superficial sediments (1st cm) were collected at the end of both contamination periods in order to quantify a possible seafloor accumulation of sorbed and precipitated TEs following injections in experimental setups. A significant accumulation of TEs in superficial sediments would represent a second route of exposure (in addition to the dissolved phase) of *P. oceanica* to TEs, *i.e.* their accumulation by the buried roots-rhizomes system. Control sediments were collected a hundred metres away in the reference seagrass bed. Frozen sediments were lyophilized (BenchTop 3L, VirTis Company Inc.) and sifted through PP sieves with nylon mesh. The different fractions of the sediments corresponding to medium to very coarse sand (0.5–2 mm), very fine to medium sand (**0.625**–0.5 mm) and mud (**<0.625** mm; Wentworth, 1922) were eluted for 4 h at room temperature with 30 ml of HCl 1N (Suprapure grade, Merck), according to Townsend et al. (2007). A 4-h extraction time in HCl 1N ensures the removal of the available precipitated TEs while not favouring the extraction of natural geogenic metals (Snape et al., 2004). Eluates were then diluted to an appropriate volume of 50 ml, centrifuged 10 min at 2000 rotations per minute and separated from their remaining culot prior to being analyzed. TE levels, measured in the 3 size fractions of dry sifted sediments, were weighted by their respective percentage to calculate total sediment (<2 mm) TE concentrations.

2.6. Trace element analysis

TE analyses were carried out by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer Inc.). Analytical accuracy was checked by analysing Certified Reference Materials (CRMs; Appendix A Table A-2); BCR 60 (*Lagarosiphon major*) and BCR 62 (*Olea europaea*) from the JCR's Institute for Reference Materials and Measurements, GBW 07603 (brush branch and leaves) from the Chinese Institute of Geophysical and Geochemical Exploration, V463 (maize) from the French National Institute of Agronomic Research and PACS-2 (harbour sediments) from the National Research Council Canada. PACS-2 TE concentrations were compared to Townsend et al. (2007) mean values obtained from as many as 25 individual HCl 1M elutions over a 5 year period. Mean TE recoveries, all CRMs together, ranged from $74 \pm 25\%$ for Al to 135% for Bi, for a global mean recovery of $101 \pm 21\%$, all elements together. For each TE, detection decision (L_C), detection limit (L_D) and quantification limit (L_Q) were calculated according to Currie (1999) or Grinzaïd et al. (1977), depending on their specific blank distribution (normal or not).

2.7. Statistical and mathematical analysis

Significant differences between initial (before contaminations) and final (at the end of contaminations) TE concentrations in epiphytes or sediments were highlighted through parametric one-way analysis of variance (one-way ANOVAs) followed by Tukey HSD pairwise comparison test of means ($p < 0.05$), after testing for normality and homogeneity of variances (Levene test) on raw or log-transformed data. Non-parametric analysis of variance (Kruskal-Wallis test) was performed when assumptions prior to one-way ANOVAs (normality and/or homoscedasticity) were not achieved, followed by Dunn pairwise comparison test of means ($p < 0.05$) (Zar, 1984).

Different models were investigated to describe the uptake and loss kinetics of the 15 studied TEs in *P. oceanica* shoots and leaves. Uptake kinetics were described using either a linear regression, an exponentially increasing function, a logarithmically increasing function or a one-phase association function. Loss kinetics were described using either a linear regression, an exponentially decreasing function or a logarithmically decreasing function. Equations of the different models are: linear regression: $y = c_1 \cdot x + c_2$ and logarithmic function: $y = c_1 \cdot \log(x) + c_2$, where c_1 = slope and $c_2 = y_0$; exponential function: $y = c_1 + c_2 \cdot \exp(c_3 \cdot x)$ and one-phase association function: $y = c_2 + (c_1 - c_2) \cdot (1 - \exp(-c_3 \cdot x))$, where c_1 = plateau, $c_2 = y_0$ and c_3 = constant rate.

To select the most adequate model that best described the uptake or loss kinetics of each TE in *P. oceanica* shoots and leaves, an Akaike information criterion (AIC) analysis was performed. In particular, the second order information criterion, often called AICc, which takes into account sample size, was used (Burnham and Anderson, 2002). In addition, F-statistics tested the overall significance of the regression model ($p < 0.05$) using the Welch approximation when heteroscedasticity was detected (Welch, 1951). Statistical analysis was performed with STATISTICA (Statsoft, Inc.; one-way ANOVAs and testing the significance of regressions) and GraphPad Prism (GraphPad Software, Inc.; regression modelling) softwares.

For the moderate contamination, shoots sampled at T0, i.e. prior contamination, were sampled a few metres beside the seagrass bed portion isolated in the experimental setup. These shoots were not incorporated in the TE uptake modelling as some TEs (Fe, V, As, Al, Mo and Mn) displayed an important patchy heterogeneity of their concentrations at the scale of a few metres. For the acute contamination, shoots were sampled at T0 within the portion of seagrass bed isolated in the experimental setup and were therefore

incorporated in the TE uptake modelling. In the particular case of rhizomes, showing little significant evolution of their TE concentrations on the whole, only the linear regression model was applied distinctly to both contamination and decontamination periods, and to entire experiments (i.e. contamination and decontamination periods together) as well.

The total amount of each TE trapped per m^2 of *P. oceanica* shoots (in $g\ m^{-2}$) at 10 m depth in the studied seagrass bed was further estimated by multiplying the 4 following parameters: (i) *P. oceanica* density, regularly monitored with a $25\ cm \times 40\ cm$ random quadrat, which is 407 ± 141 shoots m^{-2} ($n = 141$; Gobert and Lepoint, pers. comm.); (ii) the mean annual dry weight of one *P. oceanica* shoot, which is $0.877 \pm 0.494\ g$ (Richir, unpub. data); (iii) the mean annual concentration of the 15 studied TEs in *P. oceanica* shoots (present study), and (iv) the contamination factor (ratio between final and initial TE concentrations) of *P. oceanica* shoots at the end of both exposure periods (present study). A similar calculation was made for intermediate and adult leaves, respectively.

3. Results

3.1. Continuous field survey (2008–2010)

3.1.1. *P. oceanica* compartmentalization

TE distribution (annual trends; Table 2A) between above- and below-ground tissues of *P. oceanica* sampled in front of the STARESO showed that V, Cr, Mn, Co, As, Mo, Cd and Pb were preferentially accumulated in leaves, while rhizomes concentrated more Al, Fe, Ni, Cu, Zn, Ag and Bi. Furthermore, adult leaves concentrated more Al, V, Cr, Mn, Co, Pb and Bi, while intermediate leaves concentrated more Cu, Ag and Cd (differences $> 10\%$). Both leaf types showed similar levels of Fe, Ni, Zn, As and Mo (differences $< 10\%$).

3.1.2. Trace elements in sediments

TE concentrations (annual means; Table 2B) in the 3 sediment size fractions and in unsieved sediments sampled in front of the STARESO decreased in the following order: Fe > Al > Mn > Pb, Zn > As, Cr, V, Cu > Ni > Bi > Co, Mo > Cd, Ag. Concentrations of most TEs were significantly higher ($p < 0.05$) in the $< 0.0625\ mm$ mud fraction than in the 2 other ones. Mud only represented 0.8% in average of superficial sediments $< 2\ mm$ at 10 m depth in the studied *P. oceanica* bed; TE profiles of unsieved sediments were therefore similar to the fine to medium or medium to coarse sand fractions (41.3% and 57.9% of sediment $< 2\ mm$, respectively).

3.2. Trace element in seawater and sediments of experimental setups

In seawater, mean concentrations of the 11 dissolved bioavailable TEs measurable with DGTs decreased in the following order (Table 1C): Zn > Mn, Fe > Ni > Cr > Al > Pb > Cu > Cd > Co > Ag in the canopy water of the *P. oceanica* control bed, Zn > Al > Mn > Ni > Pb > Cu > Fe > Cd > Co > Cr > Ag at moderate contamination levels, and Zn > Al > Mn > Cu > Ni > Pb > Fe > Cd > Co > Cr > Ag at acute contamination levels.

For V, As, Mo and Bi, concentrations in *P. oceanica* control bed and experimental setup water could not be calculated, as we do not know their diffusion coefficients through the diffusive gel (Zhang and Davison, 1995). Nevertheless, resins effectively accumulated V, Mo and Bi, and concentrations measured in resin eluates could therefore be compared. For As, strong interferences with ArCl recombinants during ICP-MS analysis did not permit to estimate contamination factors. DGT deployment time was too short in the *P. oceanica* control bed to detect low Bi background concentrations. Apart from these 2 cases, all TEs were above L_Q .

Table 2 (A) Mean annual trace element (TE) concentrations (mean \pm SD, in μggdw^{-1} ; number of replicates (n) = 166) in *P. oceanica* intermediate and adult leaves, in entire shoots and in rhizomes. (B) Mean annual TE concentrations (mean \pm SD, in μggdw^{-1} ; n = 6) in mud (<0.625 mm), in very fine to medium (0.625–0.5 mm), or medium to very coarse sands (0.5–2 mm). *P. oceanica* and sediment samples were collected seasonally between years 2008 and 2010 at 10 m depth in front of the oceanographic station STARESO, in the Calvi Bay (northwestern Corsica, France).

	As	Al	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	Mo	Ag	Cd	Pb	Bi
A. <i>P. oceanica</i>															
Inter. leaves	a 1.53 \pm 0.65	a 29 \pm 10	a 3.37 \pm 2.99	a 0.14 \pm 0.05	a 43 \pm 6	a 36.9 \pm 9.4	a 1.31 \pm 0.61	a 23.1 \pm 5.8	9.32 \pm 3.81	67 \pm 15	1.94 \pm 1.02	a 0.87 \pm 0.33	a 2.32 \pm 0.44	a 0.63 \pm 0.17	*a 0.0047 \pm 0.0015
Adult leaves	a 1.47 \pm 0.55	a 40 \pm 11	a 3.93 \pm 2.24	b 0.22 \pm 0.07	a 47 \pm 6	a 42.4 \pm 7.5	a 1.86 \pm 0.62	a 23.2 \pm 6.6	7.43 \pm 2.22	72 \pm 19	1.93 \pm 0.48	a 0.60 \pm 0.31	a 2.08 \pm 0.38	b 0.98 \pm 0.20	*b 0.0078 \pm 0.0017
Shoots	a 1.50 \pm 0.56	a 39 \pm 12	a 3.78 \pm 2.38	ab 0.19 \pm 0.06	a 45 \pm 4	a 41.0 \pm 6.8	a 1.67 \pm 0.52	a 23.1 \pm 6.0	7.92 \pm 2.42	70 \pm 16	1.96 \pm 0.62	a 0.69 \pm 0.29	a 2.18 \pm 0.41	b 0.87 \pm 0.18	*b 0.0070 \pm 0.0018
Rhizomes	b 0.84 \pm 0.29	b 121 \pm 78	b 0.63 \pm 0.29	ab 0.18 \pm 0.07	b 81 \pm 39	b 14 \pm 1.1	b 0.17 \pm 0.03	b 37.4 \pm 9.1	9.23 \pm 2.11	78 \pm 20	1.32 \pm 0.61	b 4.91 \pm 1.24	b 1.34 \pm 0.15	c 0.28 \pm 0.13	b 0.0105 \pm 0.0100
B. Sediment grain size (mm)															
<0.625	**ab 2.10 \pm 0.34	b 141 \pm 10	a 527 \pm 51	a 9.37 \pm 0.78	a 4.27 \pm 0.75	a 1055 \pm 98	a 32.5 \pm 2.6	a 0.352 \pm 0.029	a 4.25 \pm 0.51	a 7.36 \pm 0.85	a 20.75 \pm 1.02	a 0.387 \pm 0.161	a 0.192 \pm 0.046	a 1.163 \pm 0.244	
0.625–0.5	ab 1.76 \pm 1.40	b 122 \pm 15	b 1.52 \pm 0.15	ab 2.09 \pm 0.27	b 261 \pm 19	b 154 \pm 2.8	*b 0.79 \pm 0.12	*b 0.35 \pm 0.10	b 0.80 \pm 0.15	b 4.57 \pm 0.68	*b 0.052 \pm 0.003	ab 0.025 \pm 0.007	b 0.0112 \pm 0.003	b 0.34 \pm 0.08	
0.5–2	*ab 1.95 \pm 0.17	b 134 \pm 12	b 1.47 \pm 0.15	bc 240 \pm 17	c 115 \pm 2.0	*b 0.38 \pm 0.13	bc 0.70 \pm 0.11	bc 0.30 \pm 0.08	*b 0.381 \pm 0.13	bc 0.70 \pm 0.13	*b 0.041 \pm 0.008	*b 0.014 \pm 0.005	b 0.021 \pm 0.004	bc 0.34 \pm 0.40	ab 0.111 \pm 0.017

Letters represent significant differences between *P. oceanica* shoots and compartments or sediment grain size fractions. Concentrations bellow analytical limits are represented as followed: * For concentrations $< L_Q$, ** For concentrations $< L_C$.

Most TEs injected as acid multielemental solutions were little bioavailable under dissolved or colloidal forms once diluted in the basic seawater environment of experimental setups (Table 1D). Remaining bioavailable proportions of initially injected TE amounts ranged between 0.76% (Ag) and 31% (Mn) in the setup contaminated at moderate levels; they ranged between 0.24% (Ag) and 24% (Mn, Al) in the setup contaminated at acute levels. Contamination factors of water, i.e. the ratio between TE concentrations in experimental setups and TE concentrations in the *P. oceanica* control bed, ranged from 1.3 (Cr) to 55 (Ag) in the setup contaminated at moderate levels and from 8.7 (Cr) to 381 (Al) in the setup contaminated at acute levels (Table 1E).

Contrary to seawater, no contamination of superficial sediments was observed in experimental setups. The small quantities of dissolved TEs injected ($\mu\text{g L}^{-1}$ range) were indeed very low compared to the amounts present in sediments ($\mu\text{g g}^{-1}$ range).

3.3. *P. oceanica* trace element uptake kinetics

Models of TE uptake and loss kinetics of contaminated *P. oceanica* shoots, leaves and rhizomes are presented in Section 3.3 and the following Section 3.4 (corresponding equations and fitting parameters are given in Tables 3–5). Concentration data at the successive sampling times used in the modelling of TE kinetics in *P. oceanica* shoots and compartments are detailed in Appendix B. Contamination factors of *P. oceanica* shoots and leaves at the end of both experimental exposures and the amount of TEs trapped per m^2 of shoots or leaves are further given in Table 3. Only kinetics (and compartmentalizations) of some specifically selected TEs are graphically represented in order to give to the reader a comprehensible and synthesized overview of the different scenarios (see Tables 3–5) modelled in this work: Cu, Cr, Fe and Co for shoots and leaves; Zn, Cu and Ag for rhizomes. However, graphs of the uptake and loss kinetics of the 15 studied TEs in *P. oceanica* shoots, leaves and rhizomes, as well as TE compartmentalizations at the different sampling times of both experiments are given element by element in Appendix C.

3.3.1. Moderate contamination

P. oceanica shoots accumulated, when contaminated at moderate levels, As, Cu (Fig. 2A), Pb and Bi following an exponential model over the contamination time considered. Al, V, Cr (Fig. 2B), Ag and Cd were accumulated linearly. Only Fe (Fig. 2C), Co (Fig. 2D), and Zn tended to reach a steady state concentration at the end of the 5 contamination days, following a logarithmic model. Finally, Ni, Mo and Mn concentrations did not significantly increase ($p > 0.05$). Mean contamination factor between days C1 and C5 (Ni excluded) was 2.6 ± 1.5 , with a minimum for Fe, Mn and Co (1.2) and a maximum for Bi (6.1; Table 3).

Many TEs were differently accumulated in younger intermediate leaves and senescent adult ones. Cu (Fig. 2A), Cd, Pb, and Bi accumulations followed an exponential model for intermediate leaves, but a slower continuous linear model for adult leaves (not significant - $p > 0.05$ - for Cd). Slope of Cr (Fig. 2B), Al and Ag linear accumulations were higher for intermediate leaves than for adult leaves. Fe (Fig. 2C), linearly accumulated in intermediate leaves, tended to reach a plateau in adult leaves (logarithmic model). As, Mn and Mo (exponential increase) as well as V, Co (Fig. 2D) and Zn (linear increase) were significantly taken up ($p < 0.05$) in intermediate leaves only, while neither leaf type accumulated Ni. Mean contamination factor between days C1 and C5 (Ni excluded) was 3.0 ± 1.7 for intermediate leaves (min. = 1.2 for Fe; max. = 7.4 for Bi) and 2.0 ± 0.9 for adult leaves (min. = 0.95 for Cd; max. = 3.7 for Bi), as a result of the different uptake kinetics displayed in both leaf types (Table 3).

Table 3

Trace element (TE) uptake kinetic models and parameters of *P. oceanica* (A) shoots, (B) intermediate leaves and (C) adult leaves *in situ* contaminated at moderate and acute levels. c_1 = slope and $c_2 = y_0$ of linear (Lin) regressions and logarithmic (Log) functions. c_1 = plateau, $c_2 = y_0$ and c_3 = constant rate of exponential (Expo) and one-phase association (1-ph asso) functions. Fitting parameters are indicated (r^2 and p -levels). Contamination factors (c.f.) of shoots and intermediate or adult leaves, calculated as the ratio between their final and initial TE concentrations, and total amounts of TEs (in g) trapped per m² of *P. oceanica* shoots or leaves at the end of both contamination periods are given. n = number of data used in model building.

A. Shoots	Model	c_1	c_2	c_3	r^2	p	c.f.	TE (g m ⁻²)
<i>Moderate contamination (n = 16)</i>								
As	Expo	0.9003	0.03395	0.7588	0.8901	<0.001	2.4	1.3
Al	Lin	2.833	9.249	–	0.5385	<0.01	1.9	26.5
V	Lin	1.175	0.8549	–	0.4471	<0.05	2.9	3.9
Cr	Lin	0.1657	0.1765	–	0.7742	<0.001	2.8	0.20
Fe	Log	10.86	30.80	–	0.5635	<0.001	1.2	20.0
Mn	Lin	1.631	44.08	–	0.1876	n.s.	1.2	17.5
Co	Log	1.006	3.094	–	0.3820	<0.05	1.2	0.72
Ni	Lin	−0.6308	38.88	–	0.0873	n.s.	0.88	7.2
Cu	Expo	7.015	0.5512	0.6271	0.7316	<0.01	2.6	7.3
Zn	Log	77.47	140.4	–	0.6178	<0.001	1.4	34.7
Mo	Expo	1.363	0.000002923	2.638	0.7848	n.s.	2.2	1.5
Ag	Lin	1.841	−0.2676	–	0.7040	<0.01	4.6	1.1
Cd	Lin	0.3004	3.094	–	0.8089	<0.05	1.4	1.1
Pb	Expo	1.325	0.9725	0.4607	0.8200	<0.001	3.9	1.2
Bi	Expo	0.009070	0.04328	0.4622	0.8139	<0.001	6.1	0.015
<i>Acute contamination (n = 22, except for Pb: n = 21)</i>								
As	Log	467.1	−7.114	–	0.2810	<0.01	88.9	47.7
Al	Log	98.78	27.04	–	0.4016	<0.01	2.0	27.9
V	Log	92.60	8.307	–	0.2145	<0.01	9.1	12.3
Cr	Log	12.94	0.2186	–	0.8504	<0.001	15.5	1.1
Fe	Lin	18.83	26.74	–	0.3821	<0.01	1.3	21.4
Mn	Log	−21.43	50.01	–	0.1411	<0.05	0.86	12.5
Co	Lin	−0.1399	3.063	–	0.0269	n.s.	0.94	0.56
Ni	Lin	−4.541	37.54	–	0.2205	<0.05	0.85	7.0
Cu	Log	110.7	5.246	–	0.7611	<0.01	6.1	17.3
Zn	1-ph asso	140.1	−16913	5.842	0.7159	<0.001	1.5	37.0
Mo	Log	402.8	104.4	–	0.0327	n.s.	89.8	62.9
Ag	1-ph asso	10.06	−231.5	3.217	0.5589	<0.001	18.3	4.5
Cd	1-ph asso	4.016	−458.5	5.800	0.3446	<0.05	1.4	1.1
Pb	Log	123.0	8.942	–	0.2047	<0.05	39.1	12.1
Bi	Log	8.454	−0.07898	–	0.3463	<0.05	419.9	1.0
B. Intermediate leaves	Model	c_1	c_2	c_3	r^2	p	c.f.	TE (g m ⁻²)
<i>Moderate contamination (n = 16)</i>								
As	Expo	0.7721	0.01134	1.009	0.9128	<0.001	3.2	1.7
Al	Lin	2.994	2.321	–	0.5401	<0.01	2.5	26.2
V	Lin	1.405	0.4978	–	0.4505	<0.01	3.4	4.1
Cr	Lin	0.2070	0.09085	–	0.8632	<0.001	3.3	0.16
Fe	Lin	1.921	25.80	–	0.3629	<0.05	1.2	18.5
Mn	Expo	41.32	0.06347	1.076	0.7241	<0.001	1.3	16.9
Co	Lin	0.2735	2.448	–	0.5441	<0.01	1.3	0.59
Ni	Lin	−0.9112	39.72	–	0.1067	n.s.	0.79	6.5
Cu	Expo	9.163	0.5700	0.6567	0.7462	<0.001	2.5	8.4
Zn	Lin	28.01	96.68	–	0.8689	<0.001	1.8	43.1
Mo	Expo	1.245	0.00001779	2.285	0.9105	<0.05	2.2	1.5
Ag	Lin	2.416	−0.4468	–	0.7202	<0.01	4.8	1.5
Cd	Expo	3.008	0.3065	0.4712	0.8424	<0.01	1.8	1.5
Pb	Expo	1.595	1.056	0.5147	0.8496	<0.01	4.7	1.0
Bi	Expo	0.04298	0.03361	0.5853	0.8232	<0.01	7.4	0.012
<i>Acute contamination (n = 22)</i>								
As	Log	649.0	3.439	–	0.1649	<0.05	126.5	69.0
Al	Lin	27.55	−10.05	–	0.5125	<0.001	2.4	24.3
V	Log	130.8	6.601	–	0.2273	n.s.	10.6	12.8
Cr	Log	14.19	0.1083	–	0.8862	<0.001	21.4	1.1
Fe	Lin	21.48	18.18	–	0.4187	n.s.	1.5	22.6
Mn	Log	−8.485	43.50	–	0.0161	n.s.	0.92	12.1
Co	Log	0.3630	2.329	–	0.0128	n.s.	1.0	0.46
Ni	Lin	−0.2676	27.92	–	0.0006	n.s.	1.0	8.0
Cu	Log	137.1	4.547	–	0.7221	<0.001	7.3	24.3
Zn	1-ph asso	145.8	−6802	4.559	0.8058	<0.001	1.8	43.4
Mo	Log	567.7	128.3	–	0.0206	n.s.	116.4	80.5
Ag	1-ph asso	10.60	−596.2	4.020	0.5745	<0.001	14.2	4.4
Cd	1-ph asso	4.970	−1003	6.020	0.3413	<0.05	1.6	1.3
Pb	Log	210.7	3.039	–	0.3275	<0.05	73.0	16.3
Bi	Log	12.09	−0.06081	–	0.2834	<0.05	1685.5	2.8

Table 3 (continued)

C. Adult leaves	Model	c_1	c_2	c_3	r^2	p	c.f.	TE (g m^{-2})
<i>Moderate contamination (n = 16)</i>								
As	Lin	0.2750	0.6725	–	0.4157	n.s.	1.9	1.0
Al	Lin	2.955	13.15	–	0.2855	<0.05	1.7	25.3
V	Expo	3.180	0.00001502	2.522	0.5235	n.s.	2.4	3.4
Cr	Lin	0.1259	0.2572	–	0.4348	<0.01	2.3	0.18
Fe	Log	12.67	32.63	–	0.4004	<0.01	1.3	20.9
Mn	Log	3.748	49.22	–	0.0134	n.s.	1.1	16.7
Co	Log	0.7281	3.336	–	0.1370	n.s.	1.2	0.77
Ni	Lin	-0.4605	38.41	–	0.0414	n.s.	0.92	7.6
Cu	Lin	1.946	4.182	–	0.3686	<0.01	2.2	5.9
Zn	Log	18.13	156.1	–	0.0380	n.s.	1.1	26.9
Mo	Expo	1.410	0.000001279	2.776	0.4956	n.s.	2.1	1.4
Ag	Lin	1.209	0.4605	–	0.3831	<0.01	3.6	0.76
Cd	Lin	-0.02659	3.655	–	0.0162	n.s.	0.95	0.71
Pb	Lin	1.082	1.399	–	0.4323	<0.05	2.6	0.91
Bi	Lin	0.04636	0.01502	–	0.4455	<0.001	3.7	0.010
<i>Acute contamination (n = 22, except for Pb: n = 21)</i>								
As	Lin	98.19	-104.1	–	0.2318	<0.001	62.4	32.8
Al	Log	106.3	35.56	–	0.2538	<0.05	1.8	26.2
V	Log	67.15	10.29	–	0.1071	<0.05	8.3	11.6
Cr	Log	12.00	0.2949	–	0.7667	<0.001	12.3	1.0
Fe	Lin	17.20	33.06	–	0.2719	<0.05	1.2	20.4
Mn	Log	-32.34	55.12	–	0.2545	<0.05	0.80	12.1
Co	Lin	-0.3384	3.708	–	0.0786	n.s.	0.88	0.58
Ni	Lin	-7.978	45.14	–	0.3973	<0.01	0.76	6.3
Cu	Lin	26.57	-19.67	–	0.7090	<0.001	5.3	14.0
Zn	1-ph asso	135.8	-328977	9.290	0.3782	<0.05	1.3	32.2
Mo	Log	288.8	105.6	–	0.0165	n.s.	72.2	49.8
Ag	Log	25.70	1.902	–	0.3941	<0.01	25.4	5.4
Cd	Log	1.616	2.885	–	0.1016	n.s.	1.2	0.92
Pb	Log	59.57	13.26	–	0.0515	n.s.	22.5	7.9
Bi	Log	6.137	-0.04128	–	0.2094	n.s.	189.3	0.52

Rhizome response to moderate contamination was unexpected (Fig. 3). This below-ground tissue did not accumulate TEs; in contrast, rhizome TE concentrations tended to decrease (slopes significantly negative - $p < 0.05$ - for As, Co, Ni) during the contamination period, except for Cu, Ag and Bi (Table 4).

3.3.2. Acute contamination

P. oceanica shoots accumulated, when contaminated at acute levels, As, Al, V, Cu (Fig. 4A), Cr (Fig. 4B), Pb and Bi following a logarithmic model; the contamination period was generally not long enough for these elements to reach a steady state at contamination

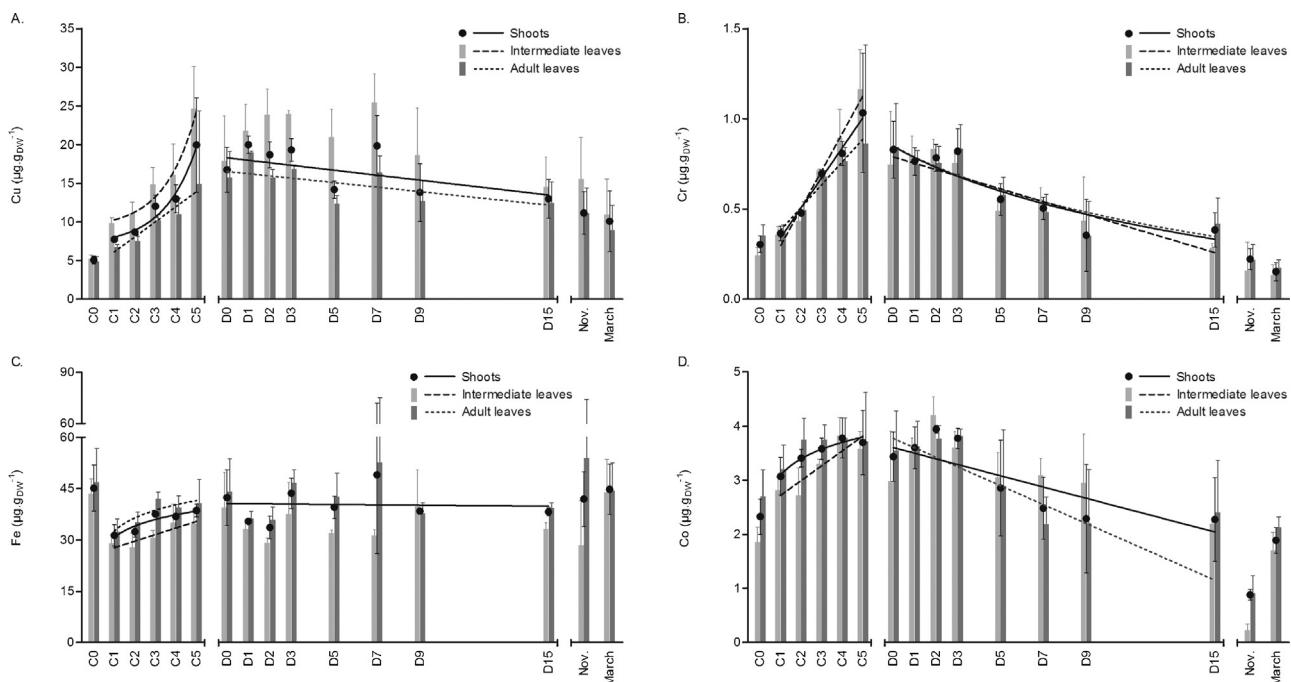


Fig. 2. Uptake and loss kinetic models of (A) Cu, (B) Cr, (C) Fe and (D) Co in *P. oceanica* shoots (full line), intermediate leaves (dashed line) and adult leaves (dotted line) in situ contaminated at moderate levels in June 2009. Kinetic parameters of regressions are given in Tables 3 and 5. Full black circles represent trace element (TE) mean concentrations in shoots and histogram bars represent TE mean concentrations in leaves (light grey for intermediate leaves; medium grey for adult leaves) at the different sampling times (number of replicates: 3–8). Post-controls sampled in November 2009 and March 2010 are also shown. TE concentrations are expressed in $\mu\text{g g}^{-1}$; error bars symbolize SD. On the temporal X-axis, contamination periods C0–C5 and decontamination periods D0–D15 are given in days.

Table 4

Parameters of linear regressions modelling the evolution of trace element (TE) concentrations in rhizomes of *P. oceanica* *in situ* contaminated at (A) moderate or (B) acute levels. Linear models were applied distinctly to both contamination and decontamination periods, and to entire experiments as well (i.e., contamination and decontamination periods together). c_1 = slope and c_2 = y0 of linear regressions. Fitting parameters are indicated (r^2 and p-levels). Decontamination day 15 was excluded from the modelling of Co evolution in the acute experiment, as well as contamination day 1 for Mo and contamination day 0 and decontamination day 1 for Bi. n = number of data used in linear regression building.

A. Moderate contamination	c_1	c_2	r^2	p
<i>Contamination period (n = 14, except for Pb: n = 13 and Bi: n = 13)</i>				
As	-0.0871	0.8666	0.3602	0.0233
Al	-6.0380	61.31	0.0757	0.3412
V	-0.0589	0.4895	0.0727	0.3512
Cr	-0.0127	0.1256	0.1057	0.2567
Fe	-5.5760	48.19	0.1800	0.1306
Mn	-0.1154	2.955	0.0690	0.3642
Co	-0.0093	0.1382	0.0026	0.0053
Ni	-2.7490	28.11	0.4502	0.0086
Cu	0.0950	5.434	0.0638	0.3837
Zn	-3.0310	63.11	0.1173	0.2307
Mo	-0.1346	1.013	0.2241	0.0873
Ag	0.1101	4.202	0.0149	0.6773
Cd	-0.0403	1.289	0.2263	0.0856
Pb	-0.0320	0.2443	0.2943	0.0554
Bi	0.0000	0.0024	0.0005	0.9403
<i>Decontamination period (n = 27, except for Fe: n = 26 and Bi: n = 25)</i>				
As	-0.0028	0.7145	0.0015	0.8469
Al	4.3000	45.35	0.0850	0.1401
V	0.0319	0.3283	0.0571	0.2300
Cr	0.0009	0.1205	0.0028	0.7922
Fe	1.5860	37.45	0.0340	0.3676
Mn	0.0746	2.781	0.1367	0.0577
Co	0.0000	0.1437	0.0000	0.9789
Ni	-0.0973	24.37	0.0027	0.7967
Cu	0.1130	6.561	0.0915	0.1252
Zn	1.0880	43.02	0.1270	0.0680
Mo	-0.0090	1.109	0.0025	0.8051
Ag	0.0513	4.568	0.0219	0.4613
Cd	0.0173	1.084	0.1839	0.0256
Pb	-0.0006	0.2353	0.0004	0.9214
Bi	0.0002	0.0024	0.1017	0.1203
<i>Entire experiment (n = 41, except for Fe: n = 40, Pb: n = 40 and Bi: n = 38)</i>				
As	0.0007	0.6535	0.0001	0.9430
Al	4.8740	35.71	0.1676	0.0079
V	0.0357	0.2607	0.1129	0.0317
Cr	0.0026	0.0951	0.0323	0.2607
Fe	2.0210	30.41	0.0883	0.0626
Mn	0.0919	2.508	0.2608	0.0006
Co	0.0016	0.1193	0.0468	0.1745
Ni	0.0690	21.62	0.0021	0.7766
Cu	0.1782	5.619	0.2974	0.0002
Zn	0.5530	50.18	0.0463	0.1767
Mo	0.0140	0.7600	0.0097	0.5401
Ag	0.0634	4.402	0.0482	0.1681
Cd	0.0140	1.124	0.1805	0.0056
Pb	0.0036	0.1718	0.0229	0.3513
Bi	0.0003	0.0019	0.2074	0.0041
B. Acute contamination	c_1	c_2	r^2	p
<i>Contamination period (n = 20, except for Mo: n = 14 and Bi: n = 17)</i>				
As	-0.0532	0.9268	0.0040	0.7916
Al	-17.580	127.1	0.0145	0.6133
V	-0.0279	0.5299	0.0012	0.8837
Cr	-0.0084	0.1534	0.0025	0.8340
Fe	-3.7840	65.25	0.0033	0.8090
Mn	-0.8232	5.009	0.0877	0.2048
Co	0.0009	0.1638	0.0001	0.9640
Ni	-1.5350	31.99	0.0032	0.8126
Cu	1.1700	7.059	0.0760	0.2393
Zn	5.4590	68.65	0.0130	0.6317
Mo	0.7508	0.4070	0.0435	0.4741
Ag	0.3264	4.962	0.0036	0.8011
Cd	-0.0752	1.595	0.0171	0.5822
Pb	0.0509	0.1782	0.0198	0.5538
Bi	-0.0005	0.0073	0.0047	0.7937
<i>Decontamination period (n = 27, except for Co: n = 24, Mo: n = 21 and Bi: n = 24)</i>				
As	0.0106	0.6687	0.0441	0.2931
Al	7.2130	89.35	0.0771	0.1607

Table 4 (continued)

B. Acute contamination	C_1	C_2	r^2	p
V	0.0071	0.4125	0.0128	0.5742
Cr	-0.0002	0.1489	0.0001	0.9719
Fe	3.5830	50.09	0.0804	0.1517
Mn	0.1270	3.081	0.1419	0.0528
Co	-0.0014	0.1671	0.0068	0.7008
Ni	0.8368	25.39	0.1254	0.0699
Cu	0.4860	8.532	0.2593	0.0067
Zn	4.0260	71.11	0.2136	0.0152
Mo	0.0234	0.9698	0.0275	0.4722
Ag	0.1700	5.433	0.0797	0.1536
Cd	0.0086	1.487	0.0267	0.4158
Pb	-0.0043	0.3083	0.0061	0.6990
Bi	0.0000	0.0060	0.0003	0.9318
<i>Entire experiment (n = 41, except for Co: n = 38, Mo: n = 35 and Bi: n = 35)</i>				
As	-0.0003	0.7866	0.0000	0.9756
Al	6.7760	93.98	0.0833	0.0672
V	0.0031	0.4557	0.0020	0.7805
Cr	0.0001	0.1458	0.0000	0.9732
Fe	3.2090	54.12	0.0783	0.0764
Mn	0.0842	3.534	0.0702	0.0941
Co	-0.0015	0.1677	0.0095	0.5610
Ni	0.6491	27.34	0.0709	0.0925
Cu	0.5212	8.160	0.3322	< 0.0001
Zn	4.0230	71.23	0.2573	0.0007
Mo	0.0020	1.206	0.0002	0.9349
Ag	0.1847	5.279	0.1047	0.0391
Cd	0.0076	1.499	0.0195	0.3834
Pb	-0.0008	0.2721	0.0003	0.9168
Bi	0.0000	0.0063	0.0006	0.8899

levels experimentally used, contrary to Zn, Cd and Ag (one-phase association uptake). The highly variable Mo uptake was poorly modelled with the logarithmic function. Only Fe (Fig. 4C) followed a linear kinetic uptake. Finally, Ni and Mn concentrations significantly decreased ($p < 0.05$) during the 24 h of acute contamination, contrary to Co (Fig. 4D). Contamination factors varied highly, with a mean (Ni, Mn and Co excluded) of 57.8 ± 118.5 , a minimum for Fe (1.3) and a maximum for Bi (419.9; Table 3).

Intermediate leaves accumulated Cr (Fig. 4B) and Bi following a logarithmic model, which was the case in adult leaves for Cr only. Zn accumulation reached a plateau following a one-phase association kinetic for both leaf types. The logarithmic uptake of As in intermediate leaves followed a continuous linear accumulation in adult leaves. The highly variable V, Cd and Pb uptakes could only be modelled either for intermediate or adult leaves, respectively, and neither leaf type significantly ($p > 0.05$) accumulated Mo. The linear uptake of Fe (Fig. 4C) was significant ($p < 0.05$) for adult leaves only. Al, Cu (Fig. 4A) and Ag uptake kinetic models differed between both leaf types. Finally, neither Ni nor Mn or Co (Fig. 4D) was accumulated in *P. oceanica* leaves; Mn and Ni level observed decreases were further significant ($p < 0.05$) in adult leaves. Mean contamination factor between days C0 and C1 (Mn, Ni and Co excluded) was 171.8 ± 478.8 for intermediate leaves (min. = 1.5 for Fe; max. = 1685.5 for Bi) and 33.6 ± 54.6 for adult leaves (min. = 1.2 for Fe and Cd; max. = 189.3 for Bi), as a result of the different uptake kinetics displayed in both leaf types (Table 3).

Rhizome accumulated none of the 15 TEs during the 24 h of the acute contamination (Table 4), and their concentrations remained mostly constant during this period, as shown for Zn, Cu and Ag in Fig. 3.

3.3.3. Trace element trapping

One m² of *P. oceanica* shoots naturally trapped 85 g of TEs in reference conditions, all 15 elements considered. In comparison, one m² of *P. oceanica* shoots trapped 124 g of TEs when contaminated at moderate level (min. = 0.015 g for Bi; max. = 34.7 g for Zn) and 266 g of TEs when contaminated at acute level (min. = 0.56 g for Co;

max. = 62.9 g for Mo). TEs, naturally more abundant in *P. oceanica* adult leaves (87 g m⁻² for adult leaves and 79 g m⁻² for intermediate leaves), displayed a higher total content in intermediate leaves at the end of both the moderate and acute contamination periods (132 and 324 g m⁻² for intermediate leaves and 113 and 222 g m⁻² for adult leaves, respectively).

3.4. *P. oceanica* trace element loss kinetics

3.4.1. Moderate contamination

Rapid initial loss kinetics of As, Mo and Pb as well as Cr (Fig. 2B) and Mn following the 5 days of moderate contamination were properly described with the exponential or the logarithmic model, respectively. Cu (Fig. 2A), Fe (Fig. 2C), Co (Fig. 2D), Ni, Zn, Ag, Cd and Bi decreased continuously in shoots, following a simple linear model. In the particular case of Fe, this linear decrease was very low. *P. oceanica* shoots eliminated TEs rapidly enough to reach back their initial C1 concentrations within 2 (V) to 15 (Cr, Zn) days, except for the essential micronutrients Fe and Cu, and for Ag and Bi. These last 2 TEs showed furthermore the highest contamination factors (4.6 and 6.1, respectively). Accumulated Al was not eliminated; V essentially decreased during the short time interval between sampling days C5 and D0, which explains the absence of a significant loss kinetic ($p > 0.05$) of this element during the 15 consecutive decontamination days (Table 5).

Intermediate leaves eliminated As, Mo and Pb more rapidly (exponential decrease) than adult leaves (logarithmic decrease). Linear Cr loss (Fig. 2B) in intermediate leaves was logarithmic in adult ones. Mn and Zn (logarithmic model) as well as Cu (Fig. 2A), Co (Fig. 2D), Ni, Cd and Bi (linear regression) levels significantly ($p < 0.05$) decreased in adult leaves, but not in intermediate ones (slopes of models were further always more elevated for adult leaves). Accumulated Fe (Fig. 2C) and Al were not significantly ($p > 0.05$) eliminated. The modelling of the Ag decrease was not significant ($p > 0.05$), and accumulated V was essentially lost during the short time interval between sampling days C5 and D0 (Table 5). The proportion of remaining TEs after 15 days of depuration (D15)

Table 5

Trace element (TE) loss kinetic models and parameters of *P. oceanica* (A) shoots, (B) intermediate leaves and (C) adult leaves *in situ* contaminated at moderate or acute levels. c_1 = slope and $c_2 = y_0$ of linear (Lin) regressions and logarithmic (Log) functions. c_1 = plateau, $c_2 = y_0$ and c_3 = constant rate of exponential (Expo) functions. Fitting parameters are indicated (r^2 and p -levels). Constraints applied to some TE loss kinetic models are detailed. n = number of data used in model building. D = decontamination, followed by the corresponding sampling day.

A. Shoots	Model	c_1	c_2	c_3	r^2	p	Constraints
<i>Moderate contamination (n = 30, except for V: n = 26 and Mo: n = 23)</i>							
As	Expo	0.8277	5.208	-0.3522	0.4009	<0.01	
Al	Log	-4.964	39.04	-	0.0036	n.s.	
V	Expo	2.280	23362	-1.648	0.2100	n.s.	x = D15 excluded
Cr	Log	-0.9486	1.587	-	0.6583	<0.001	
Fe	Lin	-0.05203	40.92	-	0.0009	<0.05	
Mn	Log	-29.73	68.29	-	0.3371	<0.001	
Co	Lin	-0.1040	4.225	-	0.4237	<0.001	
Ni	Lin	-0.6886	37.73	-	0.3463	<0.001	
Cu	Lin	-0.3206	20.21	-	0.2215	<0.01	
Zn	Lin	-4.740	234.4	-	0.2960	<0.01	
Mo	Expo	1.364	52.68	-0.6645	0.5447	<0.05	x = D7, D15 excluded
Ag	Lin	-0.1874	9.979	-	0.1553	<0.05	
Cd	Lin	-0.1332	5.528	-	0.4273	<0.001	
Pb	Expo	1.642	54.21	-0.2574	0.7017	<0.001	
Bi	Lin	-0.01746	0.5018	-	0.4388	<0.01	
<i>Acute contamination (n = 29, except for Mn: n = 26 and Pb: n = 28)</i>							
As	Expo	1.925	14287	-1.553	0.3106	n.s.	$c_1 = 1.925$
Al	Log	-23.15	66.61	-	0.0967	n.s.	
V	Expo	2.939	83767	-2.612	0.4807	n.s.	
Cr	Expo	-	6.293	-0.1910	0.6563	<0.01	Without plateau
Fe	Log	-17.94	73.15	-	0.1149	n.s.	
Mn	Log	-16.66	50.44	-	0.2611	n.s.	x = D15 excluded
Co	Lin	-0.03356	2.906	-	0.2370	<0.01	
Ni	Lin	-0.3394	31.83	-	0.1301	n.s.	
Cu	Expo	16.37	242.8	-0.7952	0.6568	<0.001	
Zn	Expo	118.1	454003	-3.385	0.3104	<0.01	
Mo	Expo	2.207	135281	-2.213	0.3790	n.s.	$c_1 = 2.207$
Ag	Lin	-0.4096	11.65	-	0.2993	<0.01	
Cd	Expo	2.995	34.33	-1.268	0.3370	<0.01	
Pb	Expo	2.222	1545	-1.204	0.4767	<0.05	$c_1 = 2.222$
Bi	Expo	0.07780	50.87	-1.011	0.3716	n.s.	$c_1 = 0.07780$
B. Intermediate leaves	Model	c_1	c_2	c_3	r^2	p	Constraints
<i>Moderate contamination (n = 26, except for V: n = 24 and Mo: n = 22)</i>							
As	Log	-1.042	2.099	-	0.1914	<0.001	
Al	Log	-13.46	37.26	-	0.0307	n.s.	
V	Log	-0.4176	2.635	-	0.0065	n.s.	x = D15 excluded
Cr	Lin	-0.03537	1.001	-	0.4193	<0.001	
Fe	Log	-7.890	42.92	-	0.0319	n.s.	
Mn	Lin	-0.5043	43.14	-	0.0522	n.s.	
Co	Lin	-0.06011	3.794	-	0.1209	n.s.	
Ni	Lin	-0.2873	35.60	-	0.0500	n.s.	
Cu	Lin	-0.1627	22.05	-	0.0204	n.s.	
Zn	Lin	-0.9044	245.9	-	0.0069	n.s.	
Mo	Log	-2.403	3.889	-	0.2258	<0.05	x = D7, D15 excluded
Ag	Log	1.125	8.805	-	0.0033	n.s.	
Cd	Lin	-0.08353	5.945	-	0.0754	n.s.	
Pb	Log	-25.95	35.64	-	0.3914	<0.001	
Bi	Lin	-0.01183	0.5775	-	0.0801	n.s.	
<i>Acute contamination (n = 29, except for Mn: n = 26)</i>							
As	Expo	1.638	515983	-2.636	0.2106	n.s.	$c_1 = 1.638$
Al	Log	-42.06	66.87	-	0.2706	n.s.	
V	Expo	2.784	5069000	-3.898	0.5273	n.s.	
Cr	Expo	-	7.216	-0.2273	0.7128	<0.001	Without plateau
Fe	Log	-29.10	74.59	-	0.2075	n.s.	
Mn	Lin	-1.508	45.21	-	0.2738	<0.01	x = D15 excluded
Co	Lin	-0.04366	2.646	-	0.1985	<0.05	
Ni	Lin	-0.3700	31.47	-	0.0933	n.s.	
Cu	Expo	20.99	430.4	-0.9560	0.5201	<0.05	
Zn	Expo	110.5	164677	-2.874	0.4005	<0.01	
Mo	Expo	2.068	512433	-2.576	0.4120	n.s.	$c_1 = 2.068$
Ag	Lin	-0.3496	12.39	-	0.1478	<0.05	
Cd	Expo	3.318	36.23	-1.150	0.2940	<0.05	
Pb	Expo	2.063	25476	-2.013	0.5072	<0.001	$c_1 = 2.063$
Bi	Expo	0.09540	548.2	-1.689	0.4109	n.s.	$c_1 = 0.09540$

Table 5 (continued)

C. Adult leaves	Model	c_1	c_2	c_3	r^2	p	Constrains
<i>Moderate contamination (n = 30, except for V: n = 26 and Mo: n = 23)</i>							
As	Expo	0.8213	6.367	-0.3777	0.3502	<0.01	
Al	Log	-13.72	52.20	-	0.0196	n.s.	
V	Expo	2.334	92208	-1.829	0.1901	n.s.	x = D15 excluded
Cr	Log	-0.9233	1.568	-	0.5310	<0.001	
Fe	Lin	-0.1084	43.34	-	0.0034	n.s.	
Mn	Log	-33.93	73.33	-	0.3303	<0.001	
Co	Lin	-0.1747	4.820	-	0.4130	<0.001	
Ni	Lin	-0.7150	37.56	-	0.3237	<0.01	
Cu	Lin	-0.2909	18.28	-	0.2366	<0.01	
Zn	Log	-107.6	267.6	-	0.3456	<0.001	
Mo	Expo	1.422	120.1	-0.7927	0.5590	<0.001	x = D7, D15 excluded
Ag	Lin	-0.1451	8.832	-	0.0567	n.s.	
Cd	Lin	-0.1143	4.847	-	0.5128	<0.001	
Pb	Expo	1.929	92.44	-0.3871	0.7968	<0.001	
Bi	Lin	-0.01461	0.4044	-	0.5555	<0.001	
<i>Acute contamination (n = 29, except for Mn: n = 26 and Pb: n = 28)</i>							
As	Expo	2.099	1914	-0.9877	0.2360	n.s.	$c_1 = 2.099$
Al	Log	-13.16	69.99	-	0.0204	n.s.	
V	Expo	3.132	7699	-1.882	0.3325	n.s.	
Cr	Expo	-	5.697	-0.1654	0.5203	<0.05	Without plateau
Fe	Log	-13.17	74.98	-	0.0552	n.s.	
Mn	Log	-15.28	51.06	-	0.1992	n.s.	x = D15 excluded
Co	Lin	-0.03504	3.191	-	0.1339	<0.05	
Ni	Lin	-0.3574	32.49	-	0.1226	<0.001	
Cu	Expo	13.54	243.0	-0.8291	0.5692	<0.001	
Zn	Lin	-1.065	134.2	-	0.1105	n.s.	
Mo	Expo	0.7837	38177	-1.855	0.3145	<0.05	
Ag	Log	-7.301	13.30	-	0.2577	<0.01	
Cd	Expo	2.716	1351	-2.591	0.3927	<0.01	
Pb	Expo	2.989	312.8	-0.7923	0.3690	n.s.	
Bi	Expo	0.06900	14.33	-0.6802	0.2279	n.s.	$c_1 = 0.06900$

was 10% higher for adult leaves ($65 \pm 23\%$, ranging from 27% for Pb to 100% for Al) than for intermediate leaves ($55 \pm 27\%$, ranging from 14% for Pb to 100% for Al) when compared to their respective levels recorded at the end (C5) of the exposure period.

Rhizome TE concentrations did not change significantly ($p > 0.05$) during the decontamination period, except for Cd. However, results of the linear modelling of the rhizome TE concentration change during the 21 days of the entire experiment (i.e. both contamination and decontamination periods together) showed a significant ($p < 0.05$) small increase of Al, V, Mn, Cu (Fig. 3B), Cd and Bi levels over time; Fe level increase was further very close to be significant ($p = 0.06$; Table 4).

3.4.2. Acute contamination

P. oceanica TE loss kinetic following the acute contamination was linear for Ag, but followed a rapid initial exponential decrease for Cu (Fig. 4A), Cr (Fig. 4B), Zn, Cd and Pb; for Cu, the reached plateau concentration was about 2.6 times higher than the initial (C0) concentration. *P. oceanica* shoots excreted Cd so rapidly that they reached back their initial content within 2 decontamination days, while it took 15 days for Pb. *P. oceanica* Co (Fig. 4D) content significantly ($p < 0.05$) decreased (linear regression) contrary to Mn and Ni. Finally, accumulated Al and Fe (Fig. 4C) were not significantly ($p > 0.05$) lost during the decontamination period, and the highly variable exponential decreases of As, V, Mo and Bi were not significant ($p > 0.05$; Table 5).

Intermediate and adult leaves eliminated Cu (Fig. 4A), Cr (Fig. 4B) and Cd following a very rapid initial exponential decrease. Accumulated Fe (Fig. 4C) and Al were not significantly ($p > 0.05$) eliminated. The continuous linear decrease of Ag in intermediate leaves was initially more rapid (logarithmic model) in adult leaves. The highly variable exponential decreases of As, V and Bi were not significant ($p > 0.05$) for both leaf types, while Zn, Mo and Pb decreases were significant ($p < 0.05$) either for intermediate or adult leaves, respectively. Finally, leaves that did not accumulate Mn, Co (Fig. 4D) and Ni

during the contamination period significantly ($p < 0.05$) eliminated Mn (intermediate leaves), Co (both leaf types) and Ni (adult leaves) during the decontamination period (Table 5). The proportion of remaining TEs after 15 days of depuration in uncontaminated seawater was 7% higher for adult leaves ($39 \pm 38\%$, ranging from 2% for As and Mo to 100% for Al) than for intermediate leaves ($32 \pm 33\%$, ranging from 1% for As and Mo to 84% for Fe) when compared to their respective levels recorded at the end (C1) of the exposure period.

Rhizome TE concentrations did not evolve significantly ($p > 0.05$) during the decontamination period, except for Zn (Fig. 3A) and Cu (Fig. 3B). Concentrations of Cu, Zn and Ag (Fig. 3C) also significantly ($p < 0.05$) increased during the 16 days of the entire experiment (i.e. both contamination and decontamination periods together). Al, Fe, Mn and Ni level increases were further very close to be significant ($0.07 \leq p \leq 0.09$; Table 4).

3.5. Trace element uptakes in epiphytes

Before contaminations (at T0), mean TE concentrations in epiphytes decreased in the following order: Al > Fe > Zn > Mn > V, Ni > As > Cu, Pb > Cd > Cr, Mo > Co > Ag > Bi (Table 6). At the end of the contamination periods, the 15 TEs presented different patterns of accumulation (compared to T0), depending on contamination levels. Al, Fe, As, Mo (significant – $p < 0.05$) and V (non-significant – $p > 0.05$) were accumulated at acute contamination levels only. Cr, Cu, Pb, Bi (significant – $p < 0.05$) and Cd (non-significant – $p > 0.05$) were accumulated at both contamination levels, and epiphyte concentrations tended to be the highest at acute levels. Co, Ni and Zn were more accumulated (significant – $p < 0.05$) at moderate contamination levels than at acute levels. Finally, Ag concentrations were equally concentrated (significant – $p < 0.05$) in epiphytes contaminated at both experimental levels, as for Mn (non-significant – $p > 0.05$).

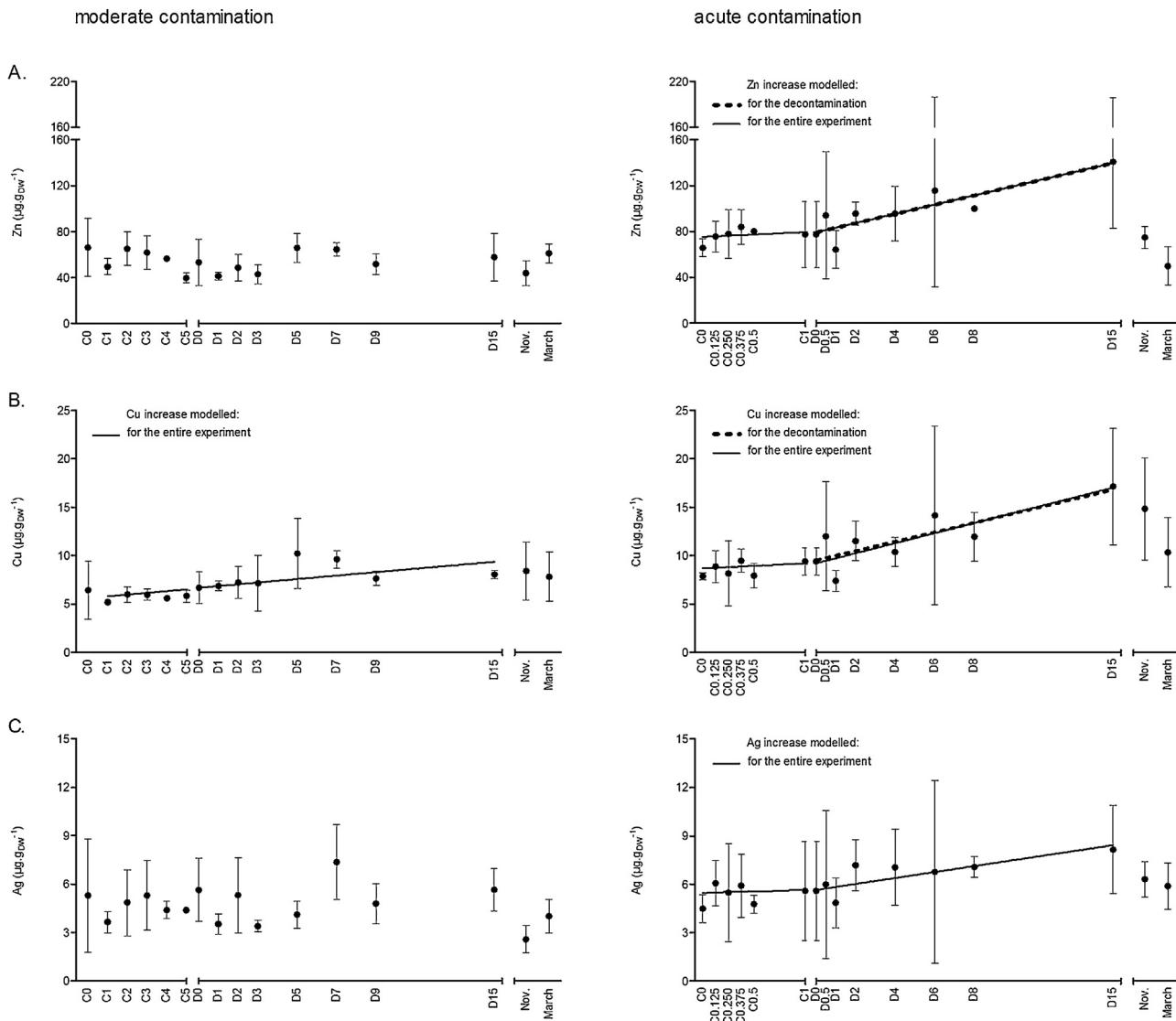


Fig. 3. Parallel comparison of kinetics of (A) Zn, (B) Cu and (C) Ag in rhizomes of *P. oceanica* contaminated at moderate (left) or acute (right) levels. Dotted thick lines represent distinct linear kinetics modelling contamination and/or decontamination periods, respectively; continuous thin lines represent the linear increase of rhizome trace element (TE) concentrations during entire experiments (i.e. contamination and decontamination periods together). Kinetic parameters of linear regressions are given in Table 4. Full black circles represent TE mean concentrations in rhizomes at the different sampling times (number of replicates: 3–8). Post-controls sampled in November 2009 and March 2010 are also shown. TE concentrations are expressed in $\mu\text{g g}^{-1}$; error bars symbolize SD. Y-axis scales are, for each element, similar for both experimental contamination levels in order to facilitate their visual intercomparison. On the temporal X-axis, contamination periods C0–C5 (moderate level) or C0–C1 (acute level) and decontamination periods D0–D15 (both levels) are given in days.

Table 6

Trace element concentrations (mean \pm SD, in $\mu\text{g g}^{-1}$) in epiphytes scraped from uncontaminated control *P. oceanica* shoots (number of replicates (n) = 22) sampled in June 2009 at 10 m depth in front of the oceanographic station STARESO (Calvi, northwestern Corsica, France) and from shoots sampled at the end of the moderate (n = 4) and acute (n = 4) contamination periods.

	Control	Moderate contamination	Acute contamination
As	a 6.59 ± 1.27	a 4.46 ± 1.15	b 11.19 ± 5.38
Al	a 778 ± 359	a 668 ± 181	b 1616 ± 365
V	a 10.41 ± 3.59	8.03 ± 1.84	18.84 ± 15.62
Cr	a 1.25 ± 0.21	b 2.84 ± 0.53	b 11.00 ± 6.16
Fe	a 420 ± 74	b 264 ± 48	a 630 ± 141
Mn	a 29.4 ± 6.0	34.2 ± 4.1	34.6 ± 1.5
Co	a 0.67 ± 0.11	b 1.01 ± 0.12	a 0.78 ± 0.05
Ni	a 10.1 ± 1.5	b 14.0 ± 2.8	a 10.7 ± 0.6
Cu	a 3.5 ± 1.4	b 12.6 ± 6.2	b 18.1 ± 6.7
Zn	a 110 ± 15	b 219 ± 12	c 167 ± 20
Mo	a 1.23 ± 0.36	a 1.24 ± 0.27	b 79.07 ± 120.55
Ag	a 0.07 ± 0.02	b 7.34 ± 1.67	b 9.30 ± 2.38
Cd	a 2.16 ± 0.41	2.80 ± 0.92	3.03 ± 0.49
Pb	a 3.82 ± 0.80	b 37.61 ± 21.47	b 46.14 ± 31.39
Bi	a 0.045 ± 0.021	b 0.349 ± 0.070	b 1.854 ± 2.044

Letters represent significant differences between control and contaminated epiphytes.

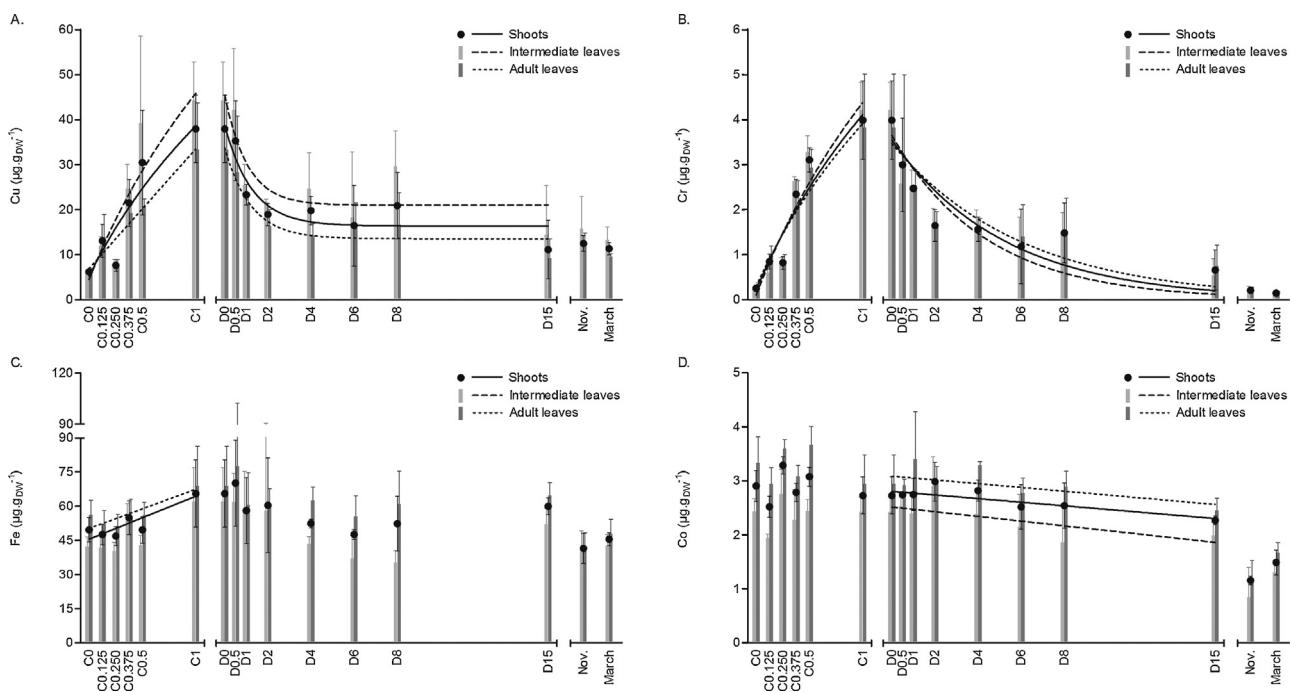


Fig. 4. Uptake and loss kinetic models of (A) Cu, (B) Cr, (C) Fe and (D) Co in *P. oceanica* shoots (full line), intermediate leaves (dashed line) and adult leaves (dotted line) in situ contaminated at acute levels in June 2009. Kinetic parameters of regressions are given in Tables 3 and 5. Full black circles represent trace element (TE) mean concentrations in shoots and histogram bars represent TE mean concentrations in leaves (light grey for intermediate leaves; medium grey for adult leaves) at the different sampling times (number of replicates: 3–7). Post-controls sampled in November 2009 and March 2010 are also shown. TE concentrations are expressed in $\mu\text{g g}^{-1}$; error bars symbolize SD. On the temporal X-axis, contamination periods C0–C1 and decontamination periods D0–D15 are given in days.

4. Discussion

4.1. The Calvi Bay as a reference for the Northwestern Mediterranean

Mean concentrations of the dissolved bioavailable TEs measurable with DGTs are low to very low in the control *P. oceanica* bed (Table 1C) in comparison to other Mediterranean coastal areas (Appendix A Table A-1). The water column of the Calvi Bay deserves the status of reference body of water for the northwestern Mediterranean. This finding corroborates observations made by Luy et al. (2012) who monitored the TE contamination along French Mediterranean coasts using *P. oceanica* as bioindicator: shoot TE levels in the Calvi Bay were always among the lowest when compared to the other studied sites.

The seagrass meadow in front of the oceanographic station STARESO presents furthermore an overall good ecological status, with a low anthropization index of its water body (index defined as the sum of 7 impact factors affecting the seawater quality and/or the biotope quality: fish farming, industrial development, agriculture, tourism, fishing, commercial ports and urbanization; Gobert et al., 2009). This *P. oceanica* bed also presents criteria of "a good reference monitoring site", as defined in the SeagrassNet Monitoring Manual (Short et al., 2002): the meadow is representative of the location and is relatively homogeneous; it is located in a place which you can come back to and monitor again at regular intervals, and is removed from any large obvious impact (e.g. a marina, a dredge channel or a sewage outfall).

TE concentrations in STARESO superficial sediments (Table 2B) are similar to other uncontaminated *P. oceanica* meadows from the northwestern Mediterranean and are in the lowest range of values recorded for this basin (Schlacher-Hoenlinger and Schlacher, 1998; Sanchiz et al., 2000; Tranchina et al., 2005; Lafabrie et al., 2007). As for its water column, STARESO sediments can be considered as a reference for numerous bioavailable trace metals (Cu,

Zn, Pb, Cd, Ni) classically investigated in monitoring surveys within the Mediterranean. Co, Cu, Zn, Pb, Cd, Ni, Co, As, Cr and Ag concentrations in *P. oceanica* vegetated sediments are also low to very low when compared to the wide range of concentrations reported for other seagrass bed sediments (Lewis et al., 2007; Lewis and Devereux, 2009).

4.2. *P. oceanica* natural compartmentalization

Luy et al. (2012; ecotoxicological survey) and Sanz-Lázaro et al. (2012; TE cycling by *P. oceanica*) recently enlarged notoriously the number of TE measured in *P. oceanica* to Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb, Bi, Ba, Cs, Ga, Li, Ni, Rb, Sr and Tl. These TE, sometimes essential to the plant, may also be toxic and have been barely studied or not at all, contrary to Cd, Hg, Pb, Cu, Zn, Cr, Fe and Ni. The majority of these TE are efficiently accumulated in *P. oceanica* (Luy et al., 2012; Sanz-Lázaro et al., 2012), and the plant may play a relevant role in their cycling in the Mediterranean (Sanz-Lázaro et al., 2012). The spatial variation of recorded levels along the Mediterranean coasts results either from specific anthropogenic pressures or from a natural heterogeneity of the environmental facies (Luy et al., 2012; Sanz-Lázaro et al., 2012).

In the reference *P. oceanica* bed in front of the STARESO, V, Cr, Mn, Co, As, Mo, Cd and Pb are preferentially accumulated in *P. oceanica* leaves, while rhizomes concentrate more Al, Fe, Ni, Cu, Zn, Ag and Bi (mean annual trends; Table 2A). This accumulation pattern within plant compartments can, however, vary. For instance, the TE compartmentalization near the STARESO is consistent with results obtained by Sanz-Lázaro et al. (2012) in the reference *P. oceanica* meadow of Sounion, Greece, for Cr, Mn, Co, Cd in leaves or Fe, Ag and Bi in rhizomes, but not for V, As, Pb (> in rhizomes), Cu, Zn (> in leaves) or Ni (~ in both tissues). In 5 sites located along the little anthropized coast of Favigna Island, Sicily (Italy), Campanella et al. (2001) systematically measured higher Cd and Zn levels in leaves and higher Cr and Pb levels in rhizomes, while similar Cu

concentrations between tissues could sometimes be higher in leaves (3 sites) or rhizomes (2 sites), depending on sites. Finally, Sanchiz et al. (2000) who monitored 17 sites along the Mediterranean Spanish coast measured higher Cd and Zn concentrations in leaves, but lower to higher Pb levels in leaves compared to rhizomes. TE distribution in *P. oceanica* above- and below-ground compartments can thus be inconsistent, even between or within uncontaminated areas, as illustrated above. Such differences in TE accumulation among plant compartments are mostly due to differences in the relative bioavailability of TEs in the water column and sediments (Malea et al., 2008).

4.3. Trace element uptake kinetics in *P. oceanica* shoots and leaves

Bioavailable TE levels in the 2 experimental setups (Table 1C) were realistic when compared to the literature (Appendix A Table A-1), and even concentrations measured in the setup contaminated at acute levels were similar to highly polluted coastal areas colonized by seagrasses (e.g. Tokyo Bay – Japan – in Akagi et al., 1985; Huelva Estuary – Spain – in Morillo and Usero, 2008). In our experimental design, with the injected amounts of TEs used, the principal route of uptake was through the water column, as no contamination of superficial sediments was observed. *P. oceanica* *in situ* contaminated responded specifically and quantitatively to the added TEs; most dissolved TEs were accumulated efficiently in shoots (and epiphytes), except for Mn and Ni at moderate levels and Mn, Co and Ni at acute levels (Table 3, Figs. 2 and 4, Appendix C). The advantage in using low added contaminant levels is that several TEs may be studied simultaneously. This approach further diminishes the biological variability occurring when separate experiments are driven for each TE and enhance the comparability of their response (Warnau et al., 1996). It also reduces the number of required experimental setups and facilitates their deployment *in situ*, which permits to contaminate and to monitor seagrasses in their natural habitat.

Some unquantified antagonistic interactions between TEs with respect to their uptake in *P. oceanica* shoots occurred, even though multielement exposure levels remained realistic. So, Co, accumulated at moderate levels, was no further accumulated at acute levels (Figs. 2D and 4D). Noraho and Gaur (1995) reported that 100 μM of interacting cations and metals (Ca, Mg, K, Na, Cu, Fe, Ni and Zn) lowered the Cd accumulation efficiency of the duckweed *Lemna polyrhiza*, and Dhir and Srivastava (2011) observed that the capacity of trace metal removal by the free-floating weed *Salvinia natans* was influenced by the combination of metals present in the medium and by their initial concentration. The total concentration of TEs dissolved in experimental setups contaminated at moderate or acute levels were 24.0 and 193.4 μg L⁻¹, respectively, compared to the 4.4 μg L⁻¹ in the control *P. oceanica* bed (V, As, Mo and Bi excluded, because not quantifiable with the DGT technique). These experimented multielement levels are low, when compared to most toxicological studies focusing on 1 or a few metals. These multielement levels are, however, similar to pollution levels recorded in some coastal areas colonized by seagrasses (Appendix A Table A-1). Antagonistic interactions on TE bioaccumulation efficiencies are then expected to occur when seagrasses are sampled from such polluted meadows. Contamination status of coastal areas exposed to multielement pollution sources might therefore be underestimated when biomonitoring with seagrasses alone, without any direct measurement of their levels in abiotic compartments (water and sediments).

The significant incorporation of TEs at even low environmental levels was previously reported by Warnau et al. (1996). They contaminated *P. oceanica* shoots in 10 L laboratory tanks during 15 days with very low levels of radiolabelled Zn, Ag, Cd, Cs and Am. They observed a continuous linear uptake of Zn and Cd, while

Ag followed a one-phase association uptake model. Ledent et al. (1992) contaminated *P. oceanica* *in situ* under Plexiglas bell with Cd at 6 concentrations (1, 5, 20, 50, 100 and 200 μg L⁻¹); contaminations were performed during 5 days (20 days for the 5 μg L⁻¹ concentration). Except for the concentration 1 μg L⁻¹, Cd levels used in their experiments were very high and unrealistic compared to natural seawater concentrations (Table 1A). However, *P. oceanica* leaves and epiphytes successfully accumulated Cd linearly even at the highest contamination levels, and the leaf-epiphyte complex reached a maximal concentration of 130 μg gDW⁻¹ after 5 days of exposure at 200 μg L⁻¹.

For the moderate experimental design, the length of the contamination period was not long enough to reach a plateau, as most TEs displayed a linear or an exponential uptake. Only Fe (Fig. 2C), Co (Fig. 2D) and Zn accumulation slowed down at the end of the contamination period to reach a plateau. In contrast, *P. oceanica* contaminated at acute levels accumulated TEs following a one phase association or a logarithmic model, except for Fe (exponential uptake, Fig. 4C), Mn, Co (Fig. 4D) and Ni (no uptake). The fact that mostly the one phase association or the logarithmic model were relevant follows from the experimental design of TE injections (4 injections the first 12 h, the 5th injection lasting 12 more hours). When TE uptake kinetics were modelled for the first 12 h only (TEs injected regularly every 3 h), most TEs were then accumulated linearly, except for As, V, Mo and Pb (results – not shown – of supplementary AICc analysis). So, even at acute levels, TE concentrations were mostly not saturating during the first 12 h of contamination (from C0 to C0.5; see Fig. 4); that confirms the ability of that species to accumulate TE proportionally to their environmental pollution levels (excluding synergistic interactions between elements), even in highly contaminated meadows.

Several studies have been carried out concerning the use of a wide diversity of magnoliophytes as bioindicators of TE contamination (reviews in Pergent-Martini and Pergent, 2000; Lewis and Devereux, 2009; Luy et al., 2012). However, according to Rainbow and Phillips (1993), if these species integrate the quality of their environment, they generally respond belatedly, thus limiting their utilization for biomonitoring short-term (daily to monthly) environmental variations. *P. oceanica* seems to be an exception to this rule. Indeed, experiences on TE incorporation kinetics in that species demonstrate the inverse, at least for TEs, as plants immediately and proportionally accumulated pollutants present in their environment within hours, from very low to high levels (present study, Ledent et al., 1992; Warnau et al., 1996).

TE uptake kinetics differed between tissues. Contamination factors were always higher for intermediate leaves than for adult leaves (Table 3), except for Fe or Ag for the moderate or acute contamination, respectively (higher c.f. in adult leaves), and corroborate results of previous studies for Cd, Zn, Ag, Cs and Am (Ledent et al., 1992; Warnau et al., 1996). The higher metabolic rate of intermediate leaves compared to adult leaves may explain this difference in accumulation efficiency, as suggested by Ledent et al. (1992). However, contamination factors of TEs (Co, Cu, Fe, Mn, Mo, Zn) essential and beneficial for the development of plants (Kapustka et al., 2004; Romero et al., 2006) differed little between intermediate leaves and adult leaves, mainly when they were added at low levels for a longer period (*i.e.* moderate contamination design). In contrast, TEs with high contamination factors (e.g. As, Cr, Ag, Pb, Bi) are mostly non-essential, which suggests less regulation of their accumulation compared to the previous.

4.4. Trace element loss kinetics in *P. oceanica* shoots and leaves

Within 2–15 days, the major part of most TEs taken up in *P. oceanica* shoots during contamination periods of both experiments were eliminated (Table 5, Figs. 2 and 4, Appendix C), which is

consistent with the rapid TE loss kinetics reported by Ledent et al. (1992) and Warnau et al. (1996). This is particularly true for toxic non-essential TEs, which were not trapped in *P. oceanica* leaves, contrary to essential TEs (mainly Cu (Figs. 2A and 4A), Fe (Figs. 2C and 4C) and Zn). Slower loss kinetics exhibited in leaves for essential micronutrients involves the existence of specific and efficient sequestration mechanisms within plant tissues. The proportion of remaining TEs after 15 days of depuration in uncontaminated seawater was higher in adult leaves than in intermediate leaves, when compared to their respective levels recorded at the end of the exposure periods. This appears to be due to the generally higher retention capacity of adult leaves compared with that of intermediate leaves (Warnau et al., 1996). So, TE overall kinetics in older adult leaves were slower than in younger intermediate ones; their proportionally higher retention capacity of accumulated TEs should therefore be considered more as a consequence of a lower detoxifying capacity instead of an active retention process. Finally, *P. oceanica* post-control shoots sampled in November 2009 and March 2010 had recovered their TE levels of June 2009 (i) because younger growing leaves could decontaminate for several months and mostly (ii) because contaminated deciduous older leaves had been replaced by newly formed tissues; the seasonal variability still observed was correlated to the plant natural physiological cycle (Richir, unpub. data).

When the non-contaminating environment was restored, the concentration of some TEs still increased a little during 12 h to 4 days (Co (Fig. 2D), Cd and Bi in the moderate contamination; Fe (Fig. 4C), Ag and Al in the acute contamination). Ledent et al. (1992) also observed this phenomenon in Cd contaminated *P. oceanica* leaves. According to previous studies on Cd and ¹⁴C transfers between seagrass above- and below-ground tissues (Brinkhuis et al., 1980; Libes and Boudouresque, 1987), Ledent et al. (1992) suggested that the little Cd quantities accumulated in contaminated rhizomes could supply leaves by translocation processes during a limited time. We did not observe any decrease of Co, Cd, Bi, Fe, Ag (Fig. 3C) or Al concentrations in rhizomes during the decontamination period of experiments that could reveal their basipetal translocation to leaves; furthermore, Bi, Cd, Ag, Fe and Al levels increased in rhizomes during overall experiments (Table 4), meaning that basipetal translocation processes from leaves to rhizome dominated. Following these observations, assumptions made by Ledent et al. (1992) are not satisfying for the present study and another process must take place, as explained below.

TEs biosorb on *P. oceanica* fibres, as shown for Cr by Ncibi et al. (2008) and Krika et al. (2012). Biosorption, even if more efficient at acidic pH, could also be relevant for many metals at natural seawater pH, as shown for the seaweed *Sargassum* spp. or *Ulva fasciata* when exposed to Cd and Pb (Kumar and Kaladharan, 2006; Nessim et al., 2011). TEs biosorbed on external leaf surface, proportionally to the pollution level experimented, could later be absorbed within leaf tissues once the non-contaminating environment was restored, resulting in the little transitory TE concentration increase sometimes observed. Numbers of phytoremediation studies further focus on this sorption ability of macrophytes fibres for the removal of pollutants from the environment (Lytle and Lytle, 2001; Gardea-Torresdey, 2003; Rai, 2009).

4.5. Trace element kinetics in *P. oceanica* rhizomes

Ledent et al. (1992) observed an increase of Cd levels in rhizomes after 5 days of *in situ* contamination, at Cd levels of 100 and 200 µg L⁻¹ only, equivalent to 4 and 7 times their initial natural Cd concentration (0.5 µg g_{DW}⁻¹), a contamination factor much lower than for leaves or epiphytes. These authors suggested that differences between plant compartment responses towards Cd exposure were in relation with their respective physiological activity, as

leaves and epiphytes productivity is considerably higher than rhizomes (1000 to 10 000 kg ha⁻¹ year⁻¹ against 250 kg ha⁻¹ year⁻¹, respectively; Ledent et al., 1992). However, they did not follow Cd kinetics in rhizomes during the 2 decontamination days of their experiment, and they did not consider the basipetal translocation of TEs from leaves to rhizomes. Our results show that there was no significant evolution of TE concentrations in rhizomes during contamination periods of both experiments. However, Cu, Zn and Cd increased significantly ($p < 0.05$) during the 15 decontamination days following the moderate or acute contamination, and Al, V, Mn, Cu, Zn, Ag, Cd and Bi increased significantly ($p < 0.05$) when considering the overall moderate or acute contamination experiment (Fig. 3, Table 4, Appendix C).

TE levels that increased in rhizomes during decontamination periods of experiments are either essential: Cu, Zn and Mn, or are naturally preferentially accumulated in this tissue: Al, Ag and Bi (Table 2A). Rhizomes are key organs for nutrient storage; this storage occurs during periods of high availability and low demand (winter). Stored nutrients then supply plant demands during the moment of maximum leaf growth (late spring; Alcoverro et al., 2000; Invers et al., 2002; Romero et al., 2006). We experimentally supplied *P. oceanica* with essential micronutrients, and shoots responded by translocating part of leaf accumulated TEs to rhizomes. It is more the imbalance between below- and above-ground tissues concentrations than dissolved TE levels in the water column that regulate this acropetal translocation, as rhizome TE concentrations increased continuously during decontamination periods.

4.6. Trace element levels in epiphytes

Contrary to *P. oceanica* leaves and rhizomes, data on TE levels in epiphytes are scarce. Concentrations varied highly, ranging from 0.045 µg g_{DW}⁻¹ for Bi to 778 µg g_{DW}⁻¹ for Al (Table 6). Levels of V, Cr, Fe, Zn, As, Pb and Bi were 4.5 ± 2.6 times higher in epiphytes than in *P. oceanica* leaves, and till 18.4 times higher for Al. A contrario, *P. oceanica* leaves concentrated more Mn, Co, Ni, Cu, Mo (mean ratio = 2.2 ± 0.7) and mainly Ag (ratio = 6.4) than epiphytes and Cd levels were similar between shoots and epiphytes. These observations are consistent with Sanz-Lázaro et al. (2012) for V, Cr, Fe, As, Pb and Ni but not for Zn (higher levels in leaves), Mn, Co and Cu (higher levels in epiphytes), and with Schlacher-Hoenlinger and Schlacher (1998) for Cu and Pb but not for Zn and Cd (higher level in leaves).

P. oceanica epiphytes, analyzed at the end of both contaminations periods (Table 6), accumulated Cr, Mn, Cu, Zn, Mo, Cd and Pb with a relative efficiency similar to shoots, as observed by comparing their respective concentration factors (c.f. of *P. oceanica* shoots are listed in Table 3; c.f. of epiphytes – not shown – were calculated from concentrations reported in Table 6). Furthermore, significantly ($p < 0.05$) lower Co, Ni and Zn accumulations measured in epiphytes exposed to the acute treatment when compared to the moderate exposure suggest that some antagonistic interactions occurred between TEs, as reported earlier for Co incorporation in *P. oceanica* shoots.

For As, Al, V, Fe, Ag and Bi, specific responses of *P. oceanica* shoots and epiphytes to TE exposures rely on their natural TE contents. As, V and mainly Al and Fe are naturally more abundant in epiphytes than in *P. oceanica* shoots (Tables 2 and 6; Sanz-Lázaro et al., 2012). Low amounts of these 4 TEs injected in the setup contaminated at moderate levels (Table 1B) were probably insufficient to observe any supplementary incorporation in epiphytes (Table 6), while they were significantly ($p < 0.05$) accumulated in *P. oceanica* shoots (Table 3); it further explains the lower incorporation efficiency of As and V in epiphytes at acute levels, compared to *P. oceanica* shoots. In the same way, the relative incorporation of Ag is more efficient in epiphytes (Warnau et al., 1996), this element being

naturally 9 times less concentrated in epiphytes than in *P. oceanica* shoots; in contrast, the relative incorporation of Bi is more efficient in *P. oceanica* shoots, this element being naturally 6 times less concentrated in *P. oceanica* shoots than in epiphytes. These observations therefore suggest a passive incorporation of most TEs linked to the external/internal concentration gradient. Also, in ecotoxicological surveys, it is not so much the absolute pollutant concentration in a bioindicator, but rather the relative difference between pollutant levels (*i.e.* contamination factors between stations, compared to reference conditions) that should condition the election of one specific species or tissue for a particular pollutant.

5. Conclusions

P. oceanica contaminated *in situ* with a mix of 15 TEs (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Pb and Bi) at realistic environmental concentration levels successfully accumulate dissolved TEs from the water column. Depending on the plant compartment where TEs are mainly accumulated and on their incorporation and loss dynamics, *P. oceanica* could act as a sink or a source of TEs and could play a key role in the cycling of TEs in Mediterranean coastal environments. The very rapid and proportional response of *P. oceanica* leaves to environmental changes of TE levels makes it undoubtedly an excellent short-term biomonitor and suggests their routine use in regularly scheduled monitoring programmes. Nevertheless, since *P. oceanica* shoots detoxify rapidly and because this plant is deciduous, long-term accumulation recordings would also necessitate the analysis of the roots-rhizomes system. The experimental exposure of shoots to the multielement solution showed that antagonistic and synergistic interactions between TEs can already occur at levels reported in contaminated areas of the Mediterranean. Such interactions, as well as toxicity tests, should be further investigated to enhance our knowledge on *P. oceanica* ecology and ecotoxicology.

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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.aquatox.2013.05.018>.

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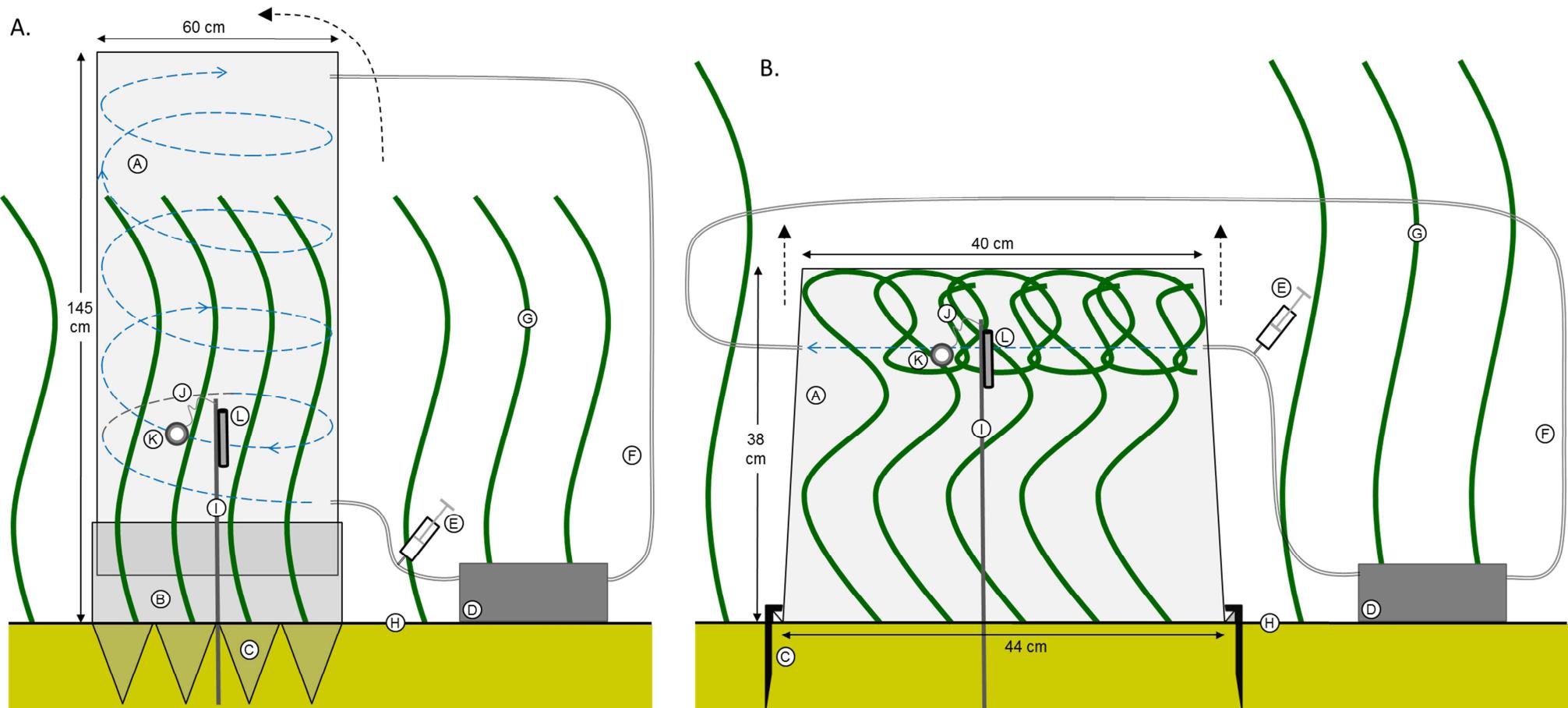
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Appendix A.

Figure A-1:

Schematic designs of experimental setups used for the moderate (A: polyvinyl chloride – PVC – cylinder) and acute (B: polypropylene – PP – trapezoidal box) contaminations. Circled letters correspond to: a) movable PVC cylinder or PP box; b) fixed circular basis of PVC cylinder; c) teeth of PVC cylinder circular basis or stainless steel moorings of PP box buried in sediments; d) submersible pump; e) syringe for multielement injections; f) recirculating pipe; g) *P. oceanica* shoots; h) sedimentary interface; i) plastic stage holding l) the Minilog (temperature logger) and the k) diffusive gradient in thin films (DGT) device, fixed to the stage with j) a nylon fishing line. Experimental setup dimensions are annotated on schemas. Black dotted arrows symbolise the setup opening for DGT replacement and shoot sampling during contamination periods. Blue dotted arrows symbolize the water circulation within experimental setups. The 2 taut nylon boots tightly fixed to 4 cross-shaped stainless steel moorings anchoring setup A on the sea-bottom are not shown.



Appendix A.

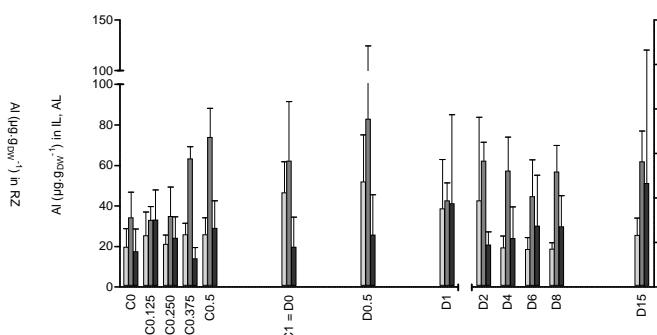
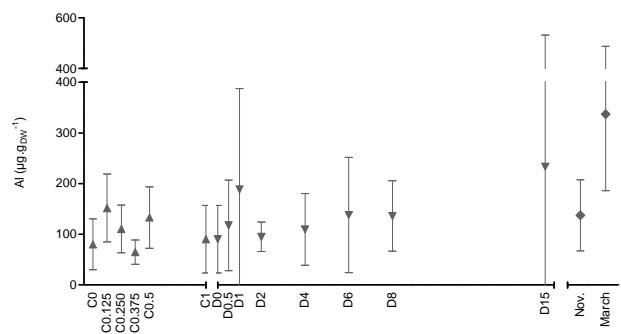
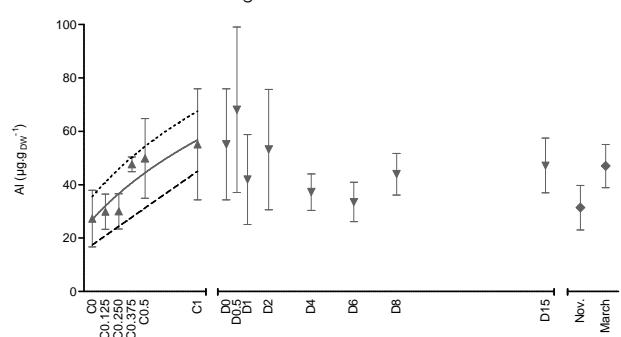
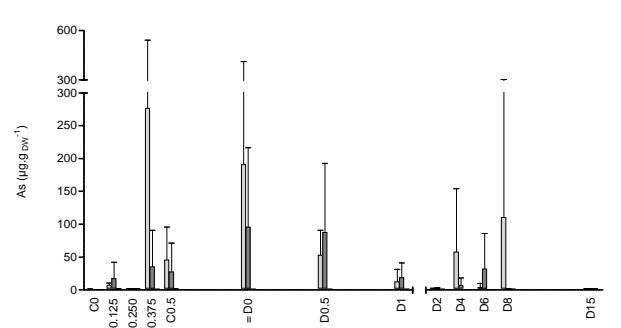
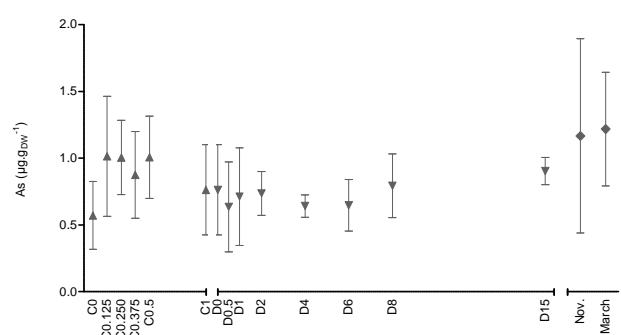
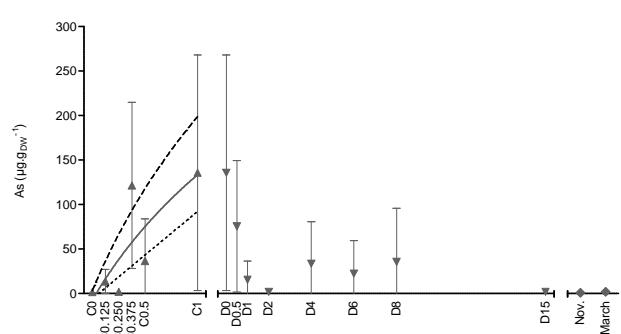
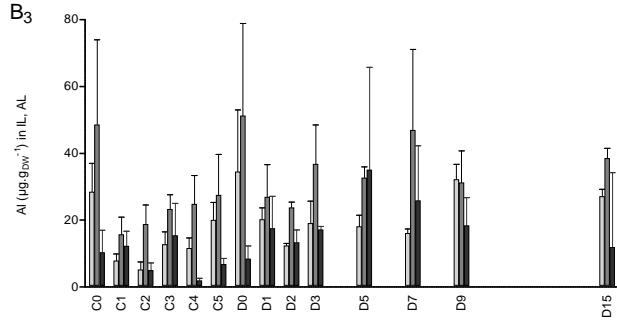
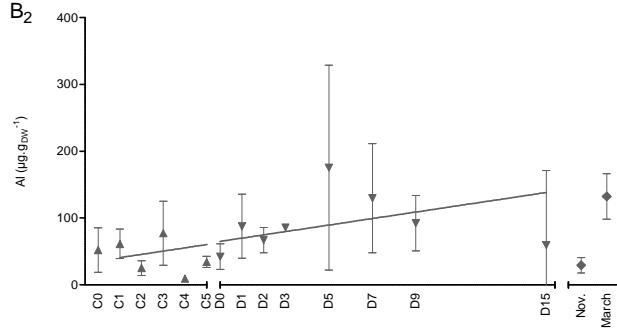
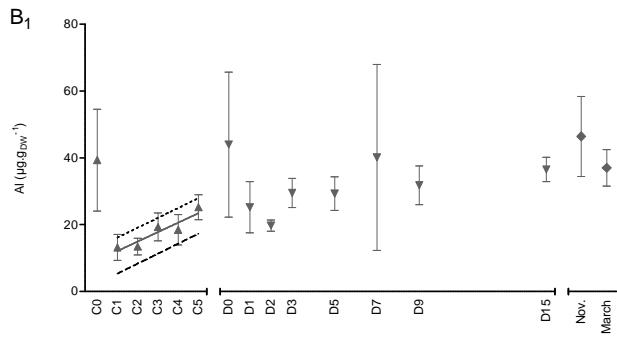
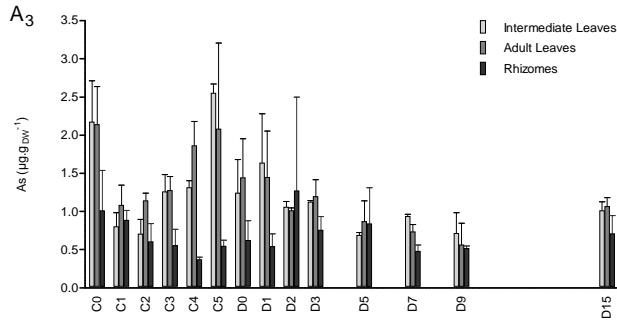
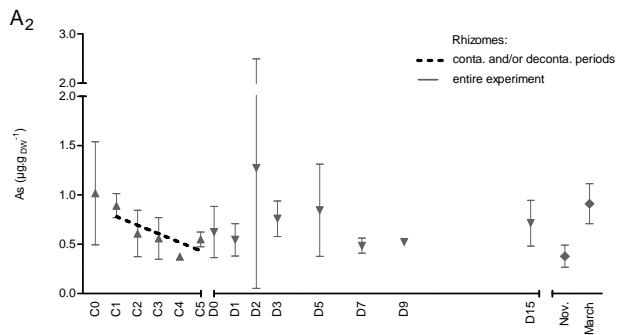
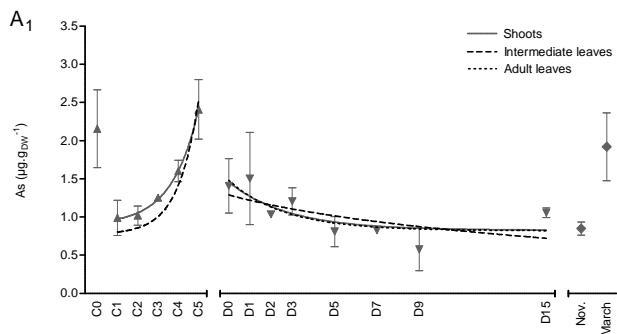
Table A-2:

Evaluation of analytical accuracy through the analysis of certified reference materials (CRMs). Italic values represent indicative values of CRMs. PACS-2 CRM values are from Townsend et al. (2007; Mar. Pollut. Bull. 54(2), 236-239). Certified (and most indicative) and measured CRM trace element concentrations are given as mean \pm SD, in $\mu\text{g.g}_{\text{DW}}^{-1}$; n = number of replicates analysed.

	As	Al	V	Cr	Fe	Mn	Co	Ni
CRM values								
BCR 60	8	<i>4180 \pm 120</i>	<i>6</i>	<i>26</i>	<i>2380</i>	<i>1760 \pm 60</i>	<i>4</i>	<i>40</i>
BCR 62	0.2	<i>448 \pm 18</i>	<i>1</i>	<i>2</i>	<i>280</i>	<i>57.0 \pm 2.4</i>	<i>0.2</i>	<i>8</i>
GBW 07603	<i>1.25 \pm 0.15</i>	<i>2000 \pm 300</i>	<i>2.40 \pm 0.40</i>	<i>2.6 \pm 0.2</i>	<i>1070 \pm 57</i>	<i>61 \pm 5</i>	<i>0.41 \pm 0.05</i>	<i>1.7 \pm 0.3</i>
V463		<i>172 \pm 13</i>		<i>3.37 \pm 0.61</i>	<i>366 \pm 25</i>	<i>24.87 \pm 0.78</i>	<i>0.18 \pm 0.06</i>	<i>3.37 \pm 0.18</i>
PACS-2	<i>14 \pm 2</i>	<i>3400 \pm 330</i>	<i>38 \pm 5</i>	<i>12 \pm 1</i>	<i>8260 \pm 570</i>	<i>53 \pm 4</i>	<i>2.8 \pm 0.2</i>	<i>9 \pm 0.9</i>
Our values								
BCR 60 (n=11)	<i>6.4 \pm 0.2</i>	<i>1823 \pm 443</i>	<i>4.4 \pm 0.3</i>	<i>17.5 \pm 2.5</i>	<i>1913 \pm 88</i>	<i>1571 \pm 126</i>	<i>4.2 \pm 0.1</i>	<i>45.2 \pm 2.7</i>
BCR 62 (n=11)	<i>0.20 \pm 0.02</i>	<i>370 \pm 29</i>	<i>0.82 \pm 0.05</i>	<i>1.8 \pm 0.5</i>	<i>302 \pm 21</i>	<i>55 \pm 3</i>	<i>0.34 \pm 0.07</i>	<i>7.5 \pm 1.9</i>
GBW 07603 (n=18)	<i>1.33 \pm 0.10</i>	<i>1189 \pm 224</i>	<i>2.0 \pm 0.2</i>	<i>1.8 \pm 0.1</i>	<i>963 \pm 34</i>	<i>65 \pm 2</i>	<i>0.59 \pm 0.09</i>	<i>5.7 \pm 2.1</i>
V463 (n=13)	<i>0.11 \pm 0.02</i>	<i>129 \pm 21</i>	<i>0.27 \pm 0.03</i>	<i>1.63 \pm 0.34</i>	<i>348 \pm 23</i>	<i>24 \pm 1</i>	<i>0.14 \pm 0.02</i>	<i>3.1 \pm 1.0</i>
PACS-2 (n=12)	<i>8.6 \pm 3.0</i>	<i>3710 \pm 230</i>	<i>35 \pm 1</i>	<i>12.7 \pm 0.5</i>	<i>9685 \pm 464</i>	<i>64 \pm 4</i>	<i>2.6 \pm 0.1</i>	<i>8.4 \pm 0.4</i>
	Cu	Zn	Mo	Ag	Cd	Pb	Bi	
CRM values								
BCR 60	<i>51.2 \pm 1.9</i>	<i>313 \pm 8</i>	<i>2</i>	<i>0.2</i>	<i>2.20 \pm 0.10</i>	<i>64 \pm 4</i>		
BCR 62	<i>46.6 \pm 1.8</i>	<i>16.0 \pm 0.7</i>	<i>0.2</i>	<i>0.2</i>	<i>0.10 \pm 0.02</i>	<i>25.0 \pm 1.5</i>		
GBW 07603	<i>6.6 \pm 0.8</i>	<i>55 \pm 4</i>	<i>0.28 \pm 0.05</i>	<i>0.049 \pm 0.007</i>	<i>0.38</i>	<i>47 \pm 3</i>	<i>0.023 \pm 0.005</i>	
V463	<i>4.72 \pm 0.54</i>	<i>61.19 \pm 1.39</i>	<i>0.83 \pm 0.08</i>		<i>1.66 \pm 0.32</i>			
PACS-2	<i>215 \pm 20</i>	<i>293 \pm 18</i>	<i>1.9 \pm 0.2</i>		<i>1.9 \pm 0.2</i>	<i>141 \pm 13</i>		
Our values								
BCR 60 (n=11)	<i>52.1 \pm 5.5</i>	<i>299 \pm 13</i>	<i>0.89 \pm 0.04</i>	<i>0.279 \pm 0.025</i>	<i>2.10 \pm 0.06</i>	<i>62 \pm 1</i>	<i>0.440 \pm 0.046</i>	
BCR 62 (n=11)	<i>44.6 \pm 3.6</i>	<i>15.5 \pm 0.9</i>	<i>0.20 \pm 0.04</i>	<i>0.026 \pm 0.011</i>	<i>0.09 \pm 0.01</i>	<i>25 \pm 1</i>	<i>0.046 \pm 0.013</i>	
GBW 07603 (n=18)	<i>9.3 \pm 3.9</i>	<i>56 \pm 2</i>	<i>0.35 \pm 0.04</i>	<i>0.057 \pm 0.004</i>	<i>0.80 \pm 0.08</i>	<i>50 \pm 1</i>	<i>0.031 \pm 0.007</i>	
V463 (n=13)	<i>4.9 \pm 0.9</i>	<i>55 \pm 3</i>	<i>0.48 \pm 0.06</i>	<i>0.029 \pm 0.003</i>	<i>1.50 \pm 0.14</i>	<i>13 \pm 9</i>	<i>0.123 \pm 0.034</i>	
PACS-2 (n=12)	<i>169 \pm 13</i>	<i>273 \pm 7</i>	<i>1.67 \pm 0.38</i>	<i>0.23 \pm 0.11</i>	<i>1.84 \pm 0.11</i>	<i>155 \pm 5</i>	<i>0.219 \pm 0.025</i>	

moderate contamination

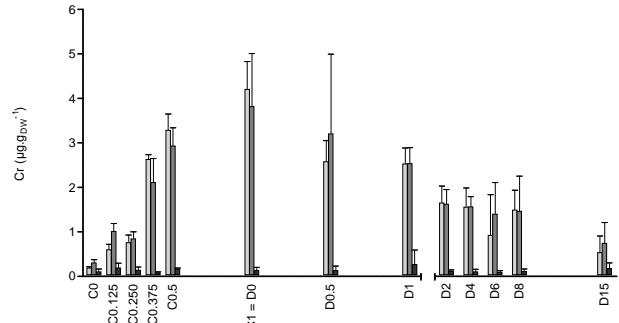
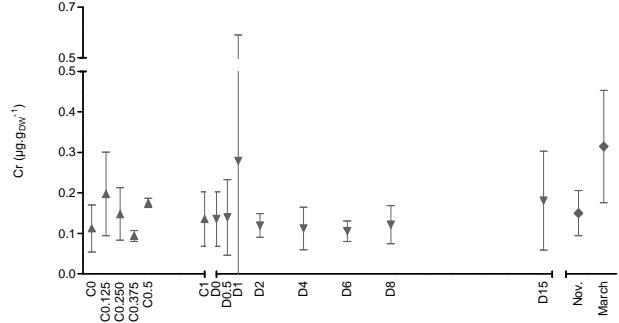
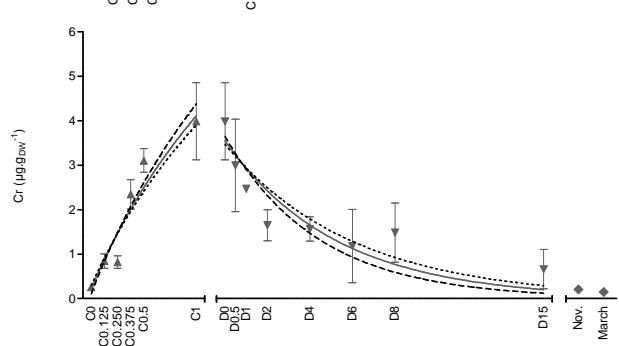
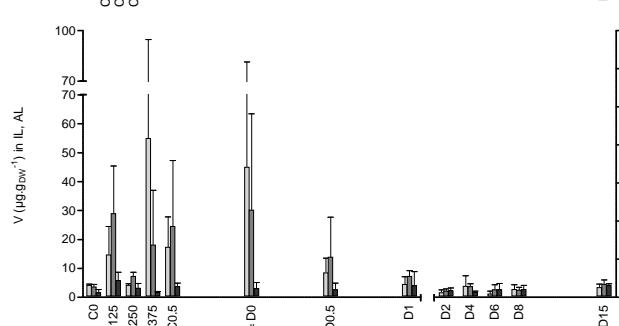
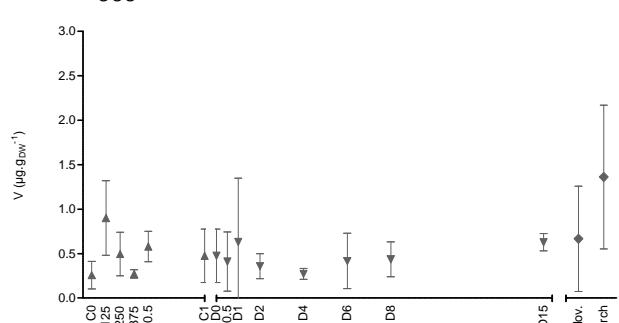
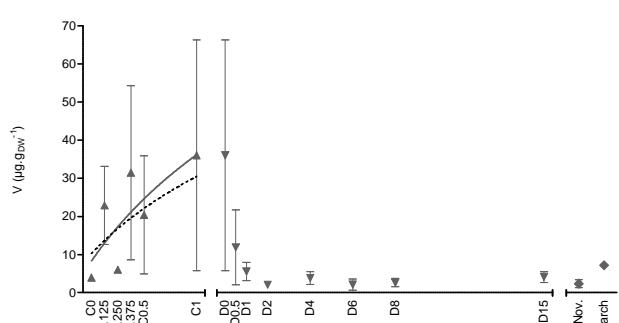
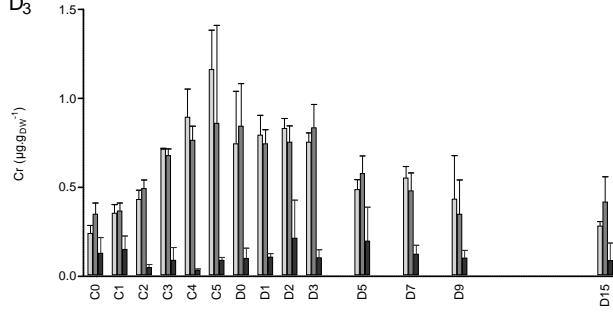
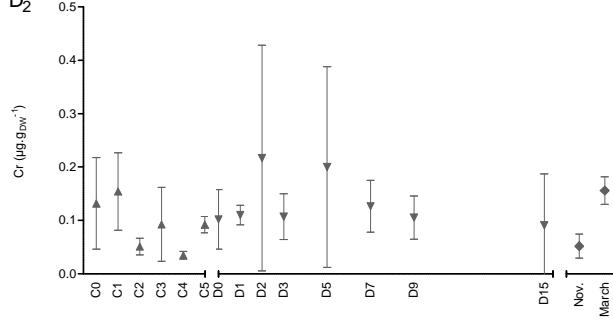
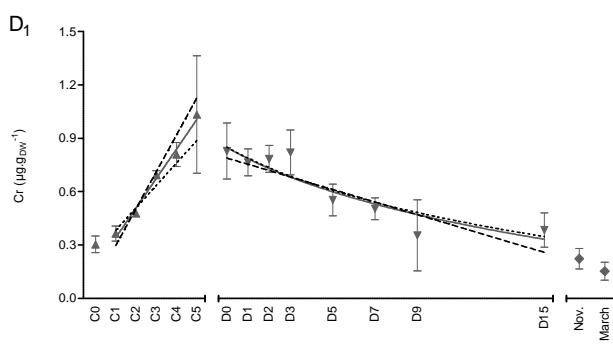
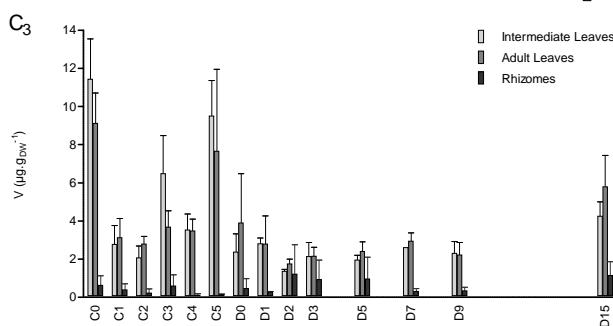
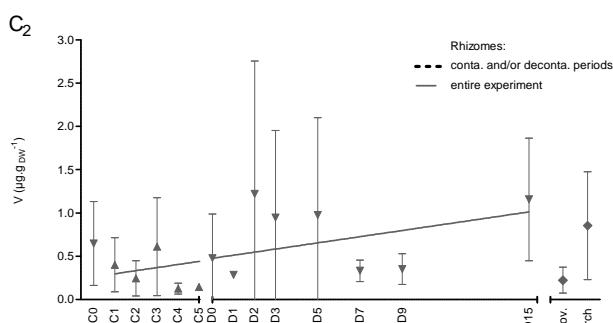
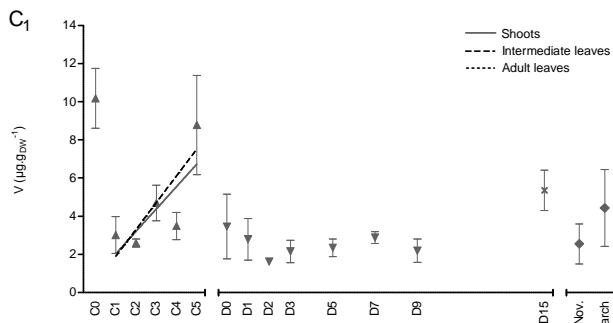
acute contamination



Al ($\mu\text{g g}^{-1}$) in RL

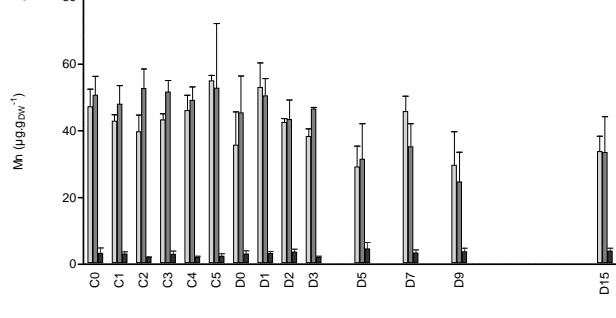
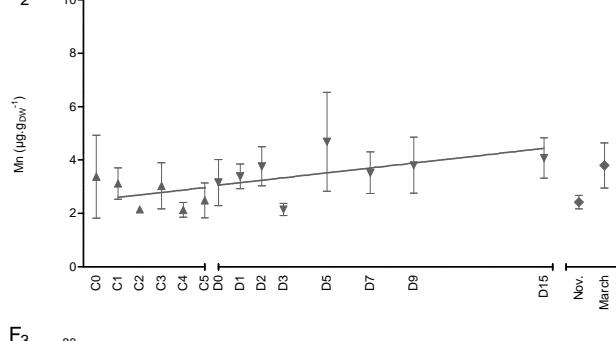
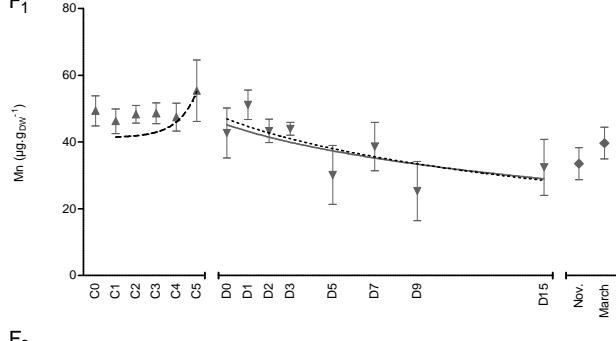
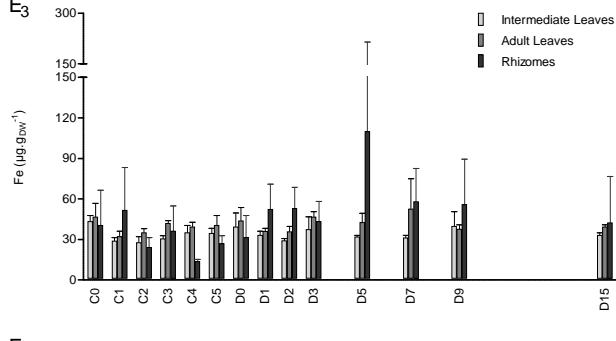
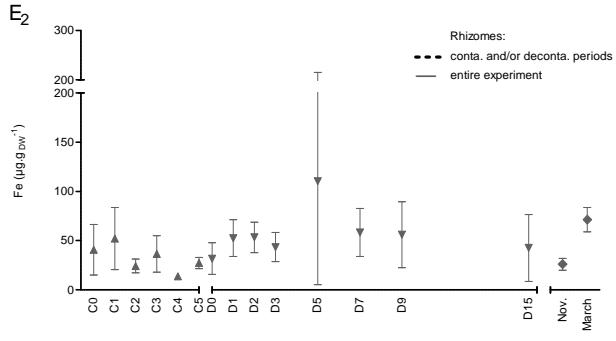
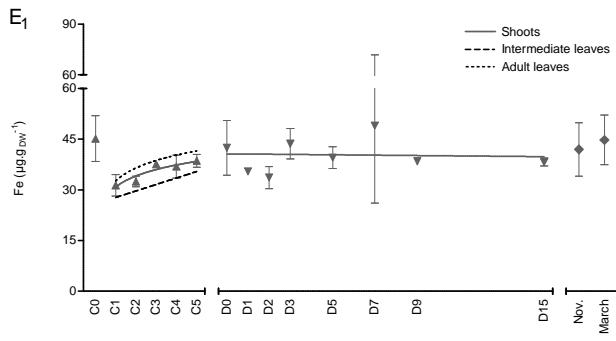
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acute contamination

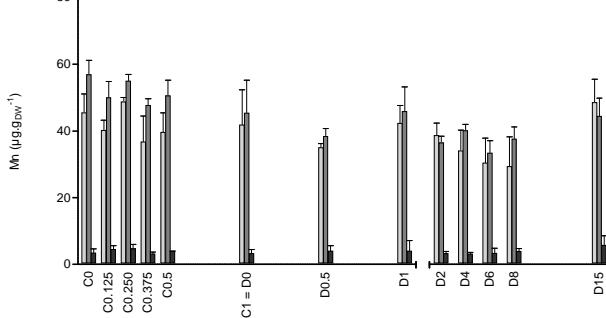
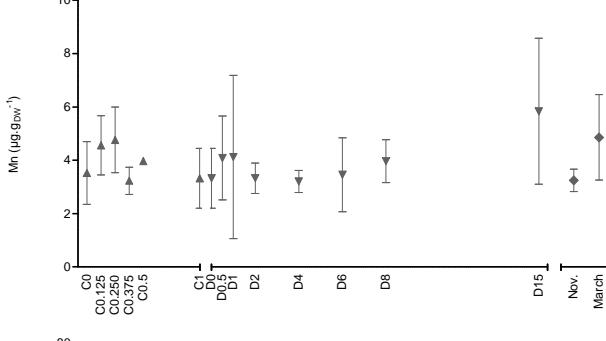
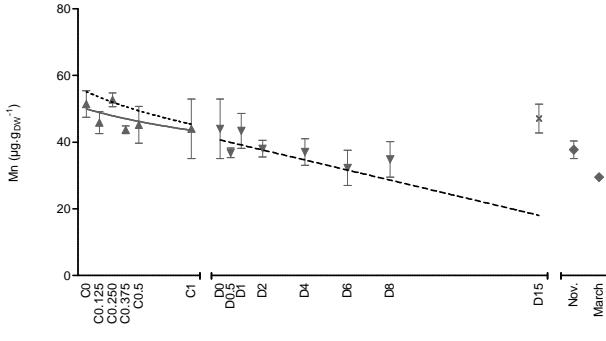
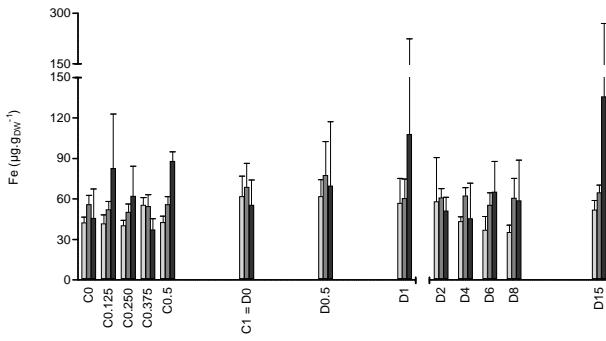
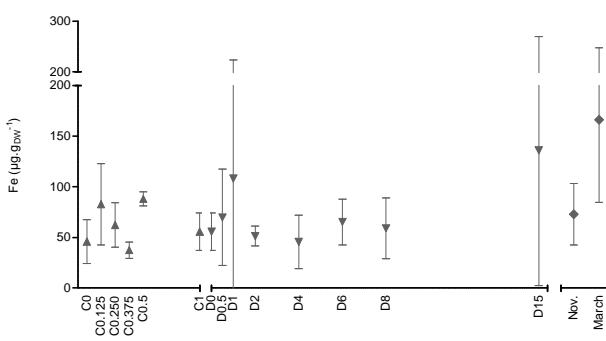
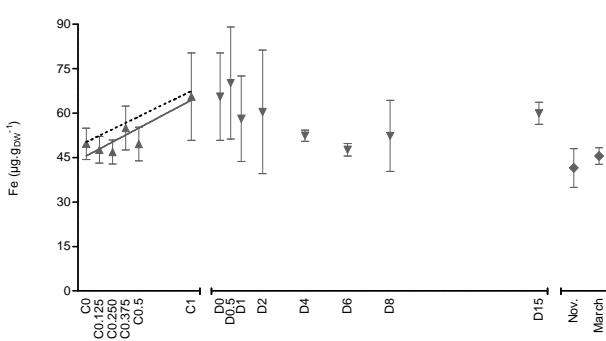


V (µg g⁻¹)
Cr (µg g⁻¹)

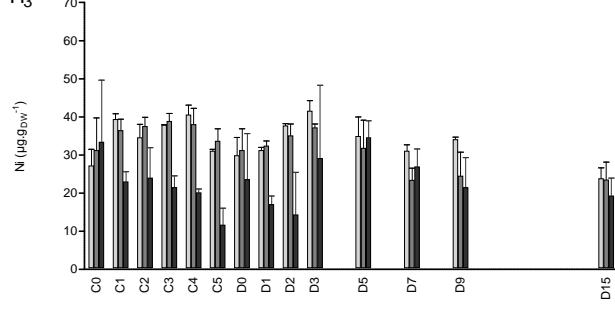
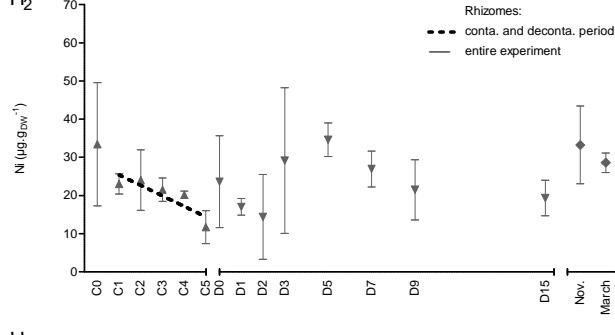
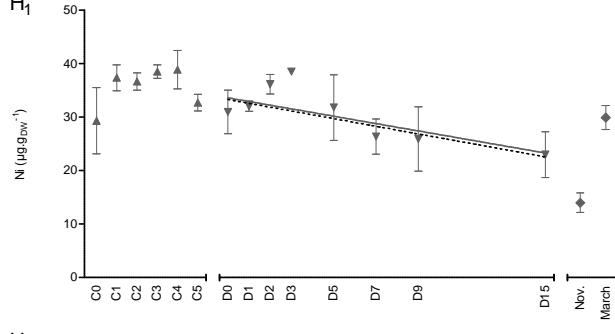
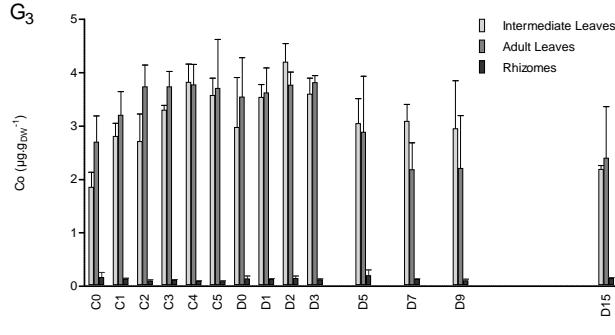
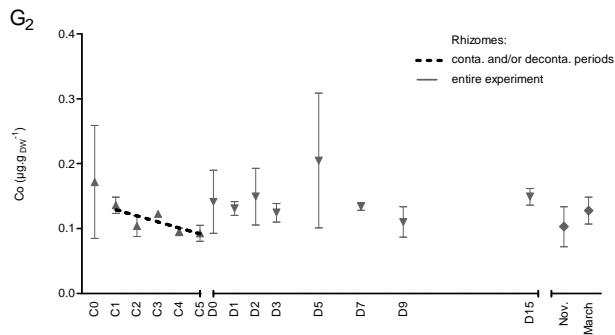
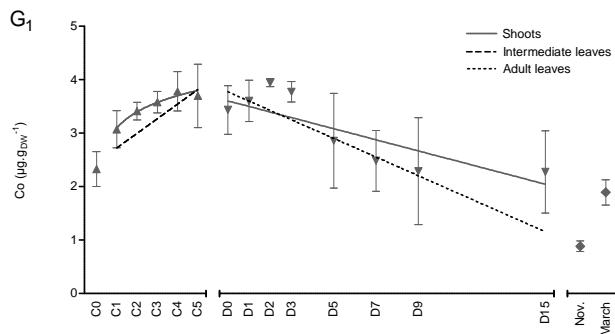
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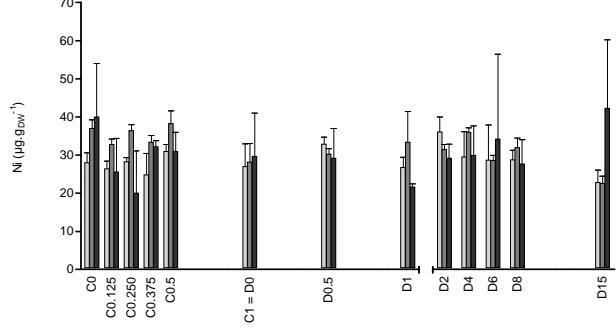
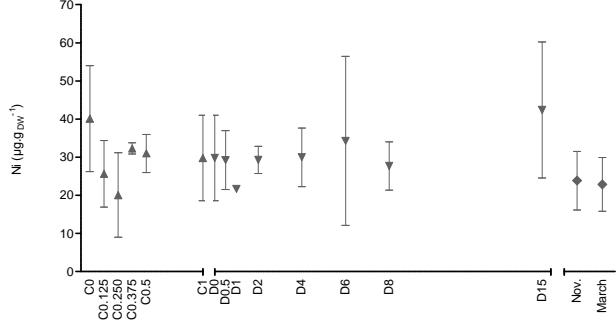
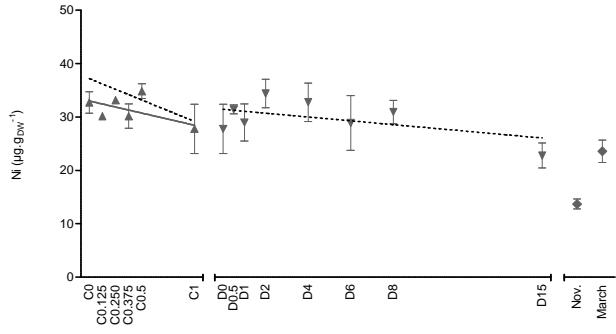
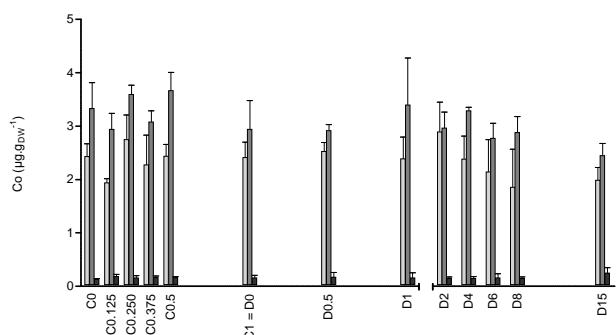
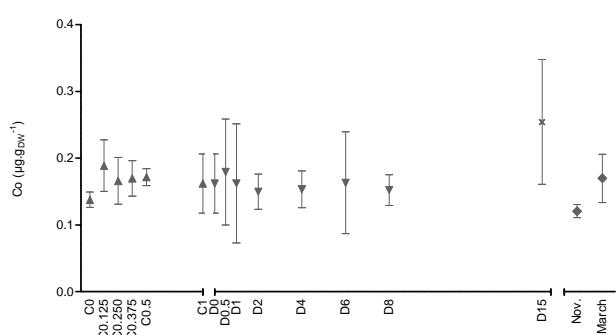
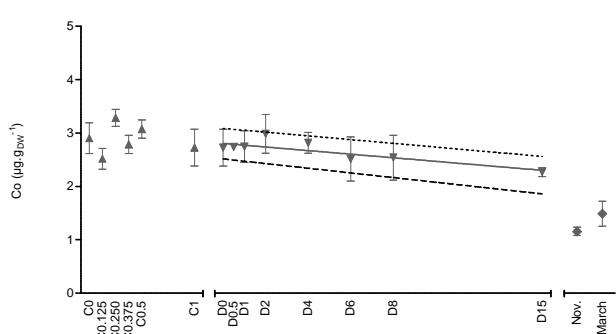
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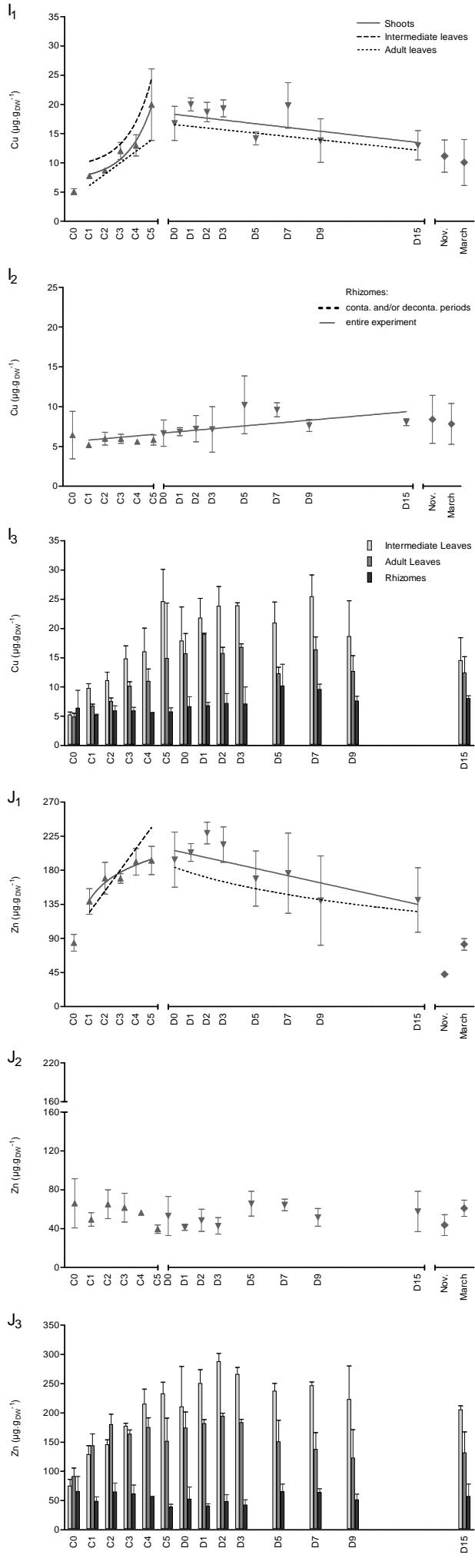
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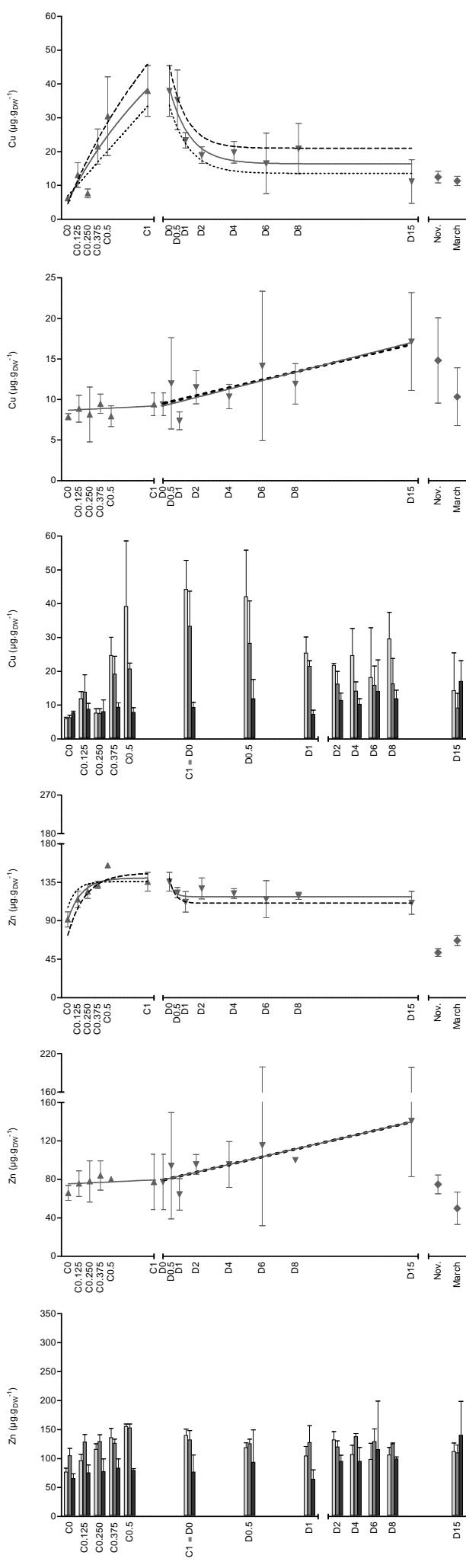
acute contamination



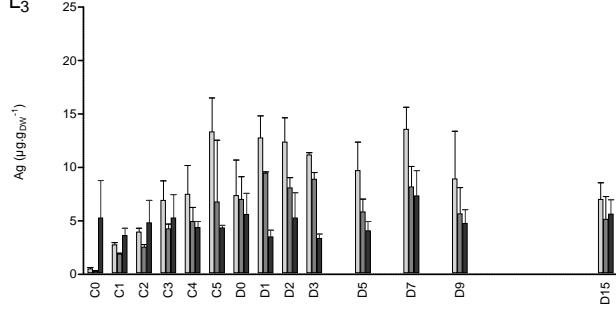
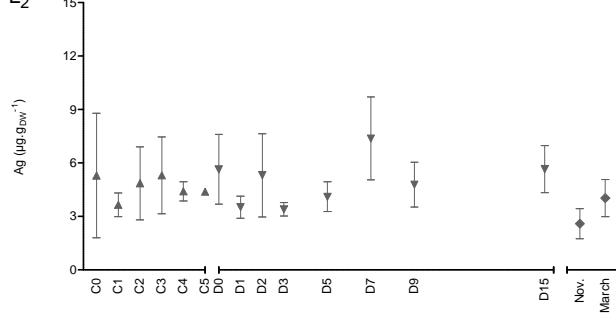
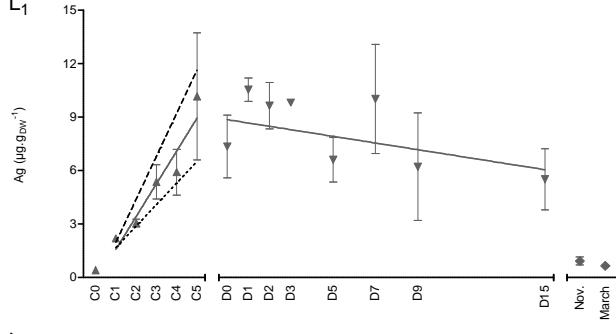
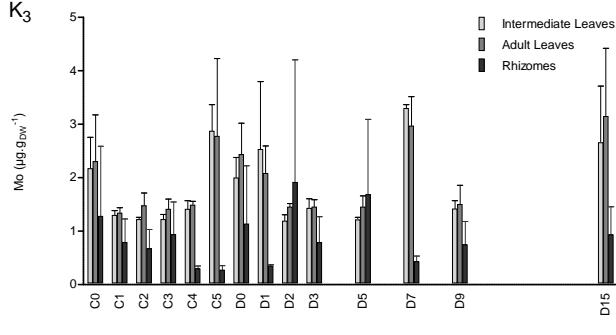
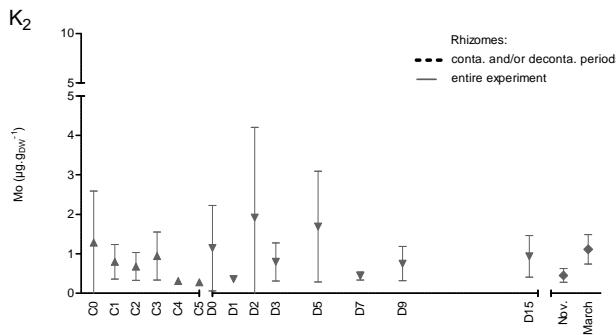
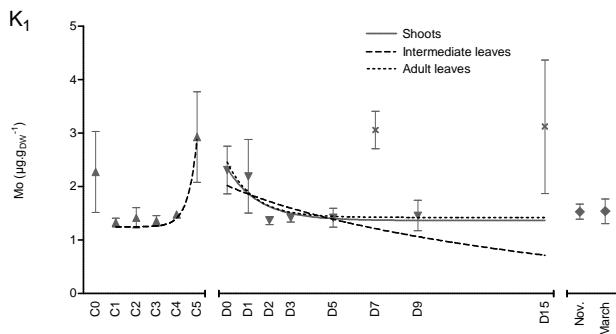
moderate contamination



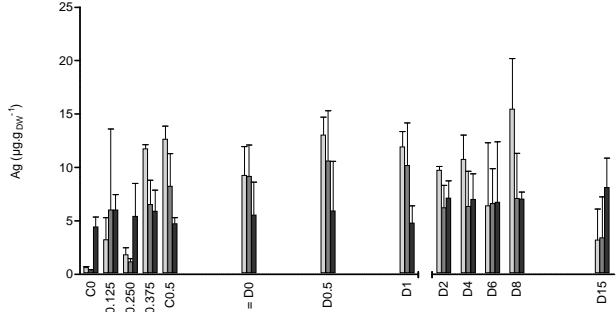
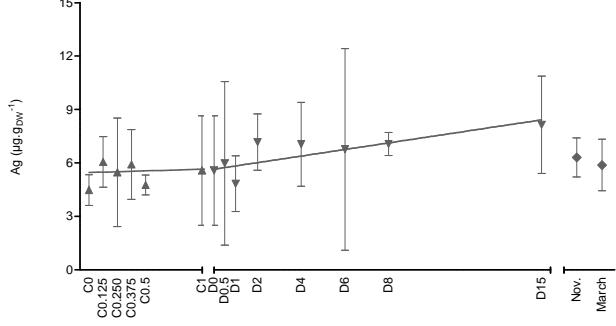
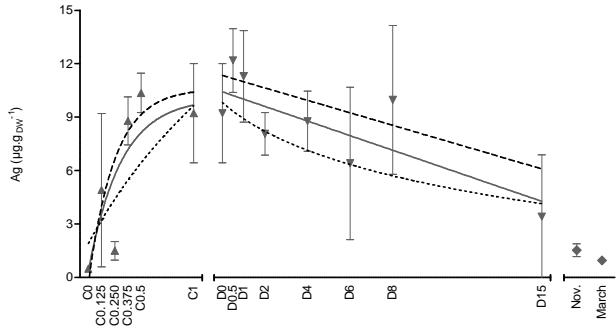
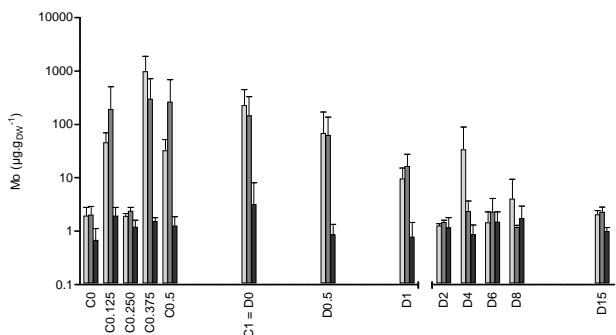
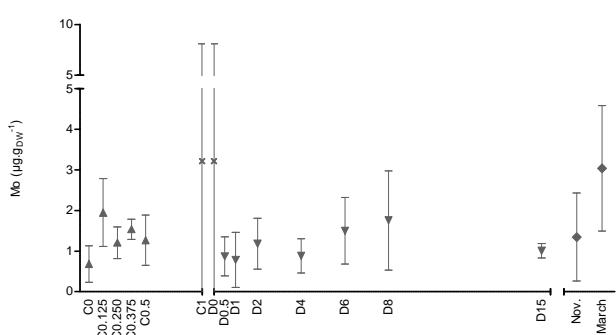
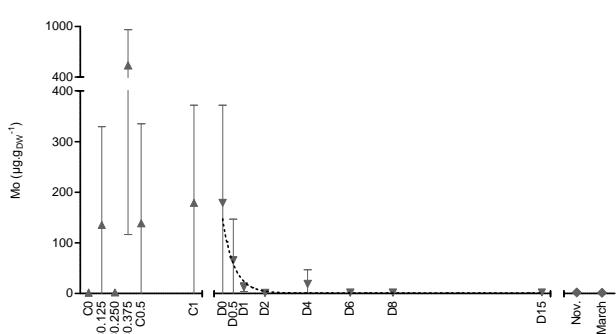
acute contamination



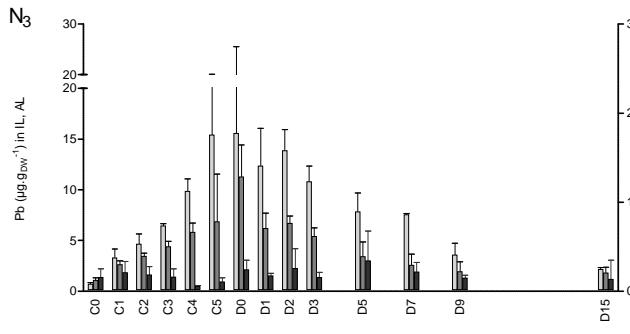
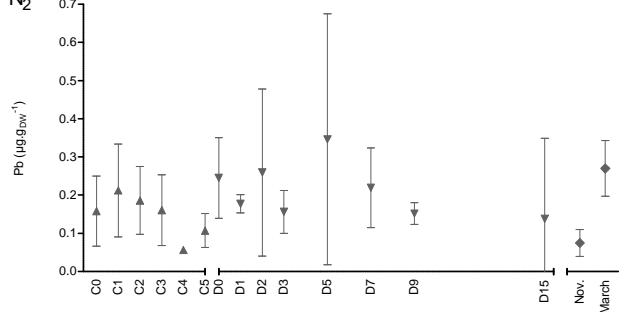
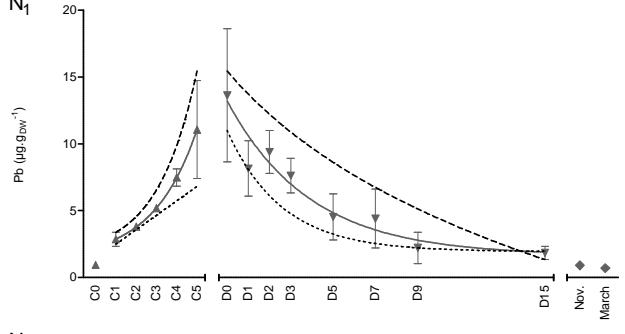
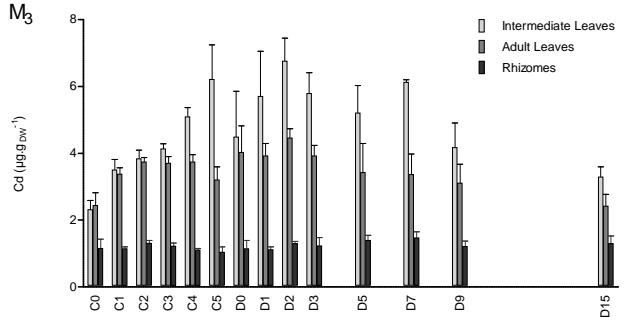
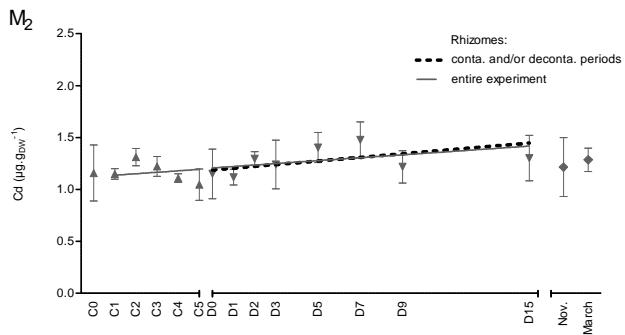
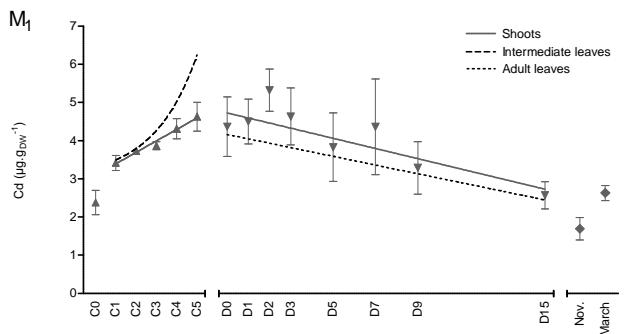
moderate contamination



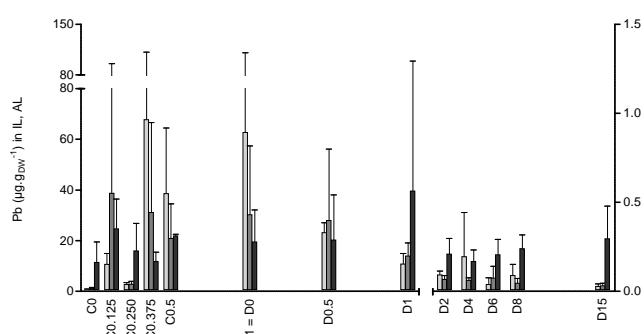
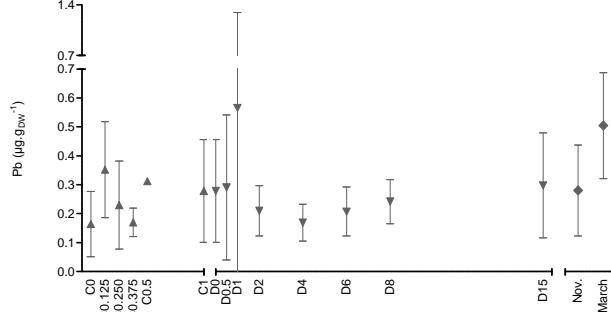
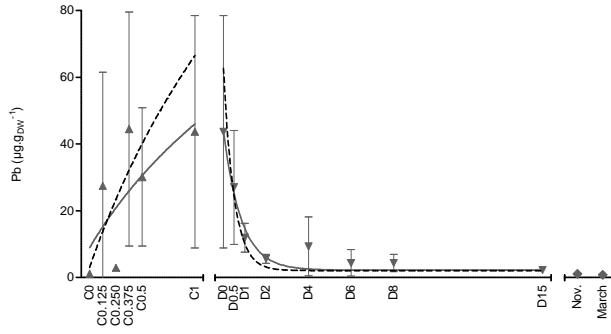
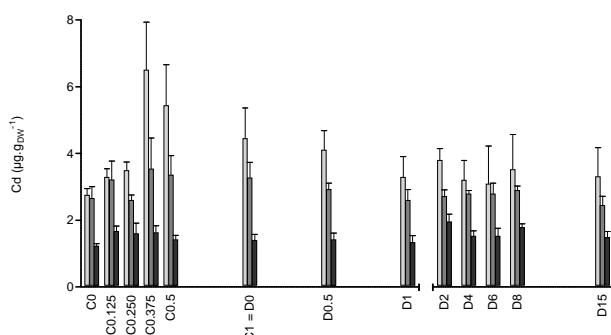
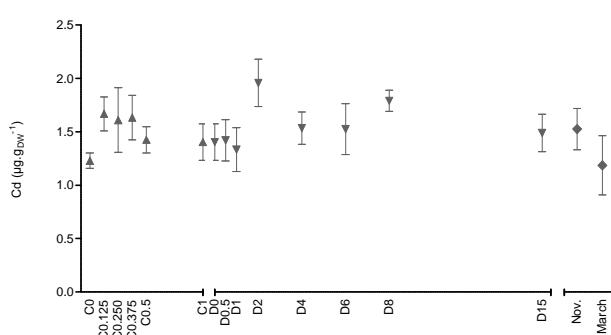
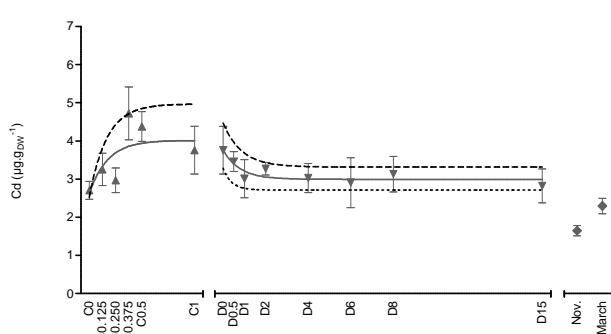
acute contamination



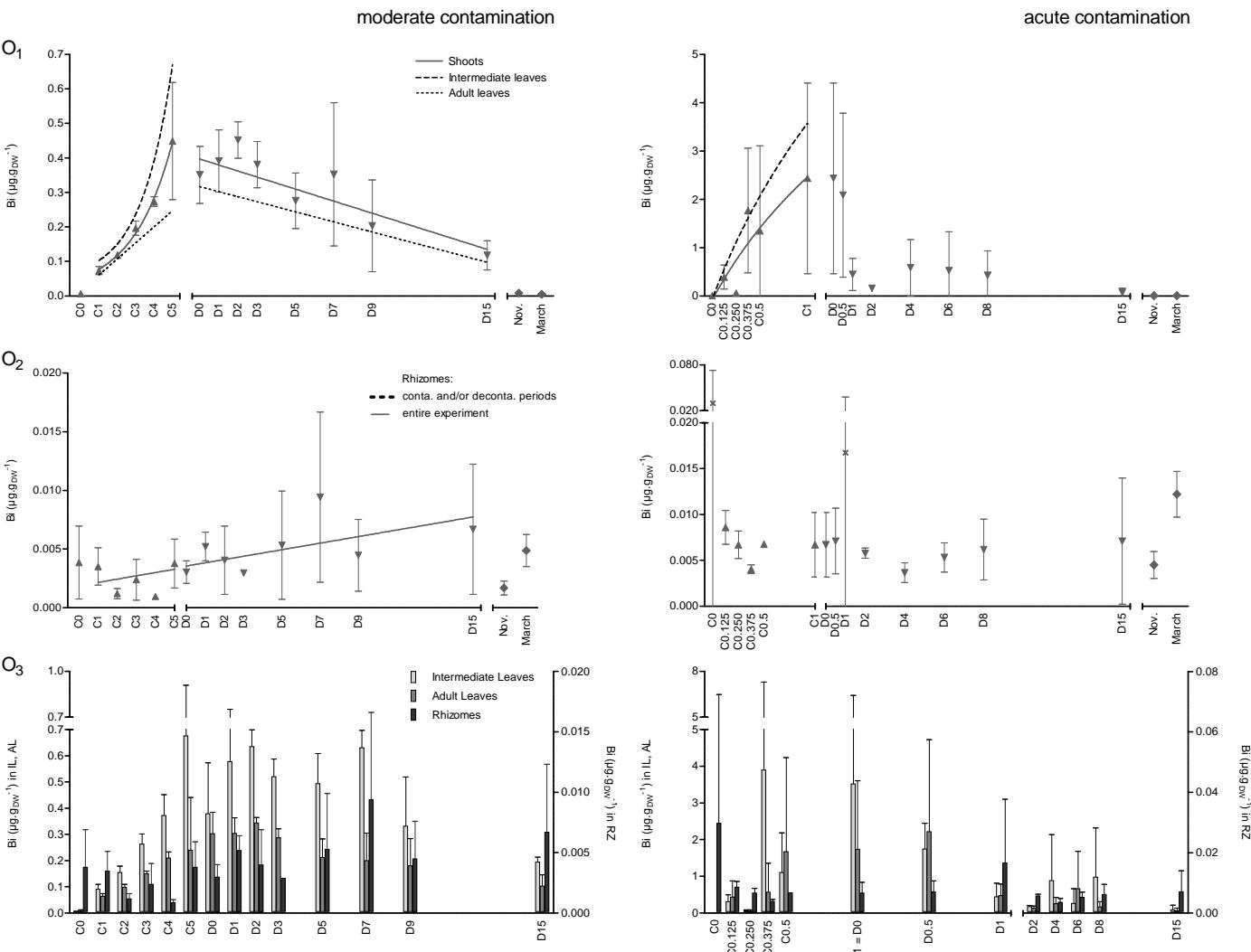
moderate contamination



acute contamination



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Appendix C.

Uptake and loss kinetic models of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A₁ to O₁) in *P. oceanica* shoots (full grey line), intermediate leaves (dashed black line) and adult leaves (dotted black line) contaminated at moderate (left) or acute (right) levels. For clarity purpose, only mean concentrations of shoots are indicated on kinetic graphs. Symbol significance: upward triangle = uptake; downward triangle = loss; trapezium = November and March post-controls; cross = excluded from analysis.

Kinetics of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A₂ to O₂) in rhizomes of *P. oceanica* contaminated at moderate (left) or acute (right) levels. Dotted thick black lines represent distinct linear kinetics modelling contamination and/or decontamination periods, respectively; continuous grey thin lines model linear kinetics of the evolution of trace element concentrations in rhizomes during entire experiments (i.e. contamination and decontamination periods together). Symbol significance: upward triangle = uptake; downward triangle = loss; trapezium = November and March post-controls; cross = excluded from analysis.

Histograms of As, Al, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Mo, Ag, Cd, Pb and Bi (A₃ to O₃) compartmentalizations between intermediate leaves (IL; light grey bar), adult leaves (AL; medium grey bar) and rhizomes (Rz; dark grey bar) during the contamination and decontamination periods at moderate (left) or acute (right) levels.

On the temporal X-axis, contamination periods C0 to C5 (moderate level) or C0 to C1 (acute level) and decontamination periods D0 to D15 (both experiments) are given in days. Trace element mean concentrations are expressed in µg.g_{DW}⁻¹. Error bars symbolize standard deviations. For clarity purpose, legends of kinetics and tissue compartmentalizations are only given on the 3 upper left graphs (moderate contamination) and are the same for the 9 other graphs of every sheet.