Potential of *Ranunculus acris* L. for biomonitoring trace element contamination of riverbank soils: photosystem II activity and phenotypic responses for two soil series

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Abstract Foliar ionome, photosystem II activity, and leaf growth parameters of *Ranunculus acris* L., a potential biomonitor of trace element (TE) contamination and phytoavailability, were assessed using two riverbank soil series. *R. acris* was cultivated on two potted soil series obtained by mixing a TE (Cd, Cu, Pb, and Zn)-contaminated technosol with either an uncontaminated sandy riverbank soil (A) or a silty clay one slightly contaminated by TE (B). Trace elements concentrations in the soil-pore water and the leaves, leaf dry weight (DW) yield, total leaf area (TLA), specific leaf area (SLA), and photosystem II activity were measured for both soil series after a 50-day growth period. As soil contamination increased, changes in soluble TE concentrations depended on soil texture. Increase in total soil TE did not affect the leaf DW yield, the TLA, the SLA, and the photosystem II activity of *R. acris* over the 50-day exposure. The foliar ionome did not reflect the total and soluble TE concentrations in both soil series. Foliar ionome of *R. acris* was only effective to biomonitor total and soluble soil Na concentrations in both soil series and total and soluble soil Mo concentrations in the soil series B.

Responsible editor: Elena Maestri

Highlight Gradual increase in total trace element (TE) concentrations in a sandy and a silty clay soil series was generally not mirrored by changes in TE concentrations of the soil-pore water and leaves of *Ranunculus acris*.

Electronic supplementary material The online version of this article (doi:10.1007/s11356-015-4646-9) contains supplementary material, which is available to authorized users.

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Abbreviations

b-CA	b-Carbonic anhydrase
CEC	Cationic exchange capacity
DGT	Diffusive gradients in thin-film
DOC	Dissolved organic carbon
DW	Dry weight
EC	Electric conductivity
EEA	European Environmental Agency
EXAFS	Extended x-ray absorption fine structure
F_0	Minimal fluorescence level in the dark adapted state
F_m	Maximum fluorescence
F_v	$F_m - F_0$
GACGC	German Advisory Council on Global Change
ISO	International Organization of Standardization
Kd	Partition coefficient
NRAMP	Natural resistance-associated macrophage protein
OM	Organic matter
PCA	Principal component analysis
PSII	Photosystem II
SLA	Specific leaf area
TE	Trace element

TLA	Total leaf area
VDLUFA	Association of German Agricultural Analytic and Research Institutes
WHC	Water holding capacity
XANES	X-ray absorption near edge structure

Introduction

Worldwide, some 22 million hectares of land have been degraded by contamination, and case studies arise everywhere from increasing industrialization, urbanization, and lax environmental regulations or their lack of respect (GACGC 1995; Evangelou et al. 2012). In the European Union, on average, about 4.2 potentially contaminating sites are estimated to exist per 1,000 inhabitants and about 5.7 contaminated sites per 10, 000 inhabitants (EEA 2014). A tentative extrapolation to cover 39 European countries results in an estimated 2.5 million potentially contaminated sites of which about 14 % (340 000 sites) is estimated to be contaminated and in need of remediation measures. Trace elements (TE) are of concern at 35 % of these sites (EEA 2014). Increases in TE (here, essential and non-essential metal(loid)s with common concentrations in plant shoots below 100 mg kg^{-1} dry weight, DW; Adriano 2001) in soils over time is mainly due to anthropogenic activities in both urban and agricultural soils (Burt et al. 2014; Capra et al. 2014; Washa et al. 2014). Cadmium and Zinc often deserve special emphasis due to their mobility in soils and their presence in many pollutant linkages (Defra 2012); indeed, there are many sources and pathways that lead to an increase of Cd and Zn in soils, which can result in risks for water resources or human health (Beesley et al. 2010; Bearup et al. 2014; Li et al. 2014; Rashti et al. 2014). Briefly, Cd inputs to agricultural soils are predominantly through aerial depositions, and applications of P-fertilizers, soil amendments, manure, and sewage sludges (McLaughlin and Singh 1999). Anthropogenic soil Zn contamination mainly results from short- and long-range industrial and urban emissions to the atmosphere (e.g., burning of coal and oil, waste incineration, industrial processes including non-ferrous metal smelting), and (long-term) applications of P- and Zn-fertilizers, sewage sludges, some livestock manures, agrochemicals, and industrial waste products (Alloway 2008; Belon et al. 2012). Consequently, many topsoils display higher TE (Cd, Zn, etc.) concentrations than background levels for their soil type, e.g., median values range from 9 to 131 mg Zn and from 0.06 to 0.77 mg Cd kg^{-1} soil DW in French agricultural soil series (Baize et al. 2007; GIS sol 2009). Floodplain and riverbank soils are among sites for dynamic biogeochemical research as they largely control pollutant storage and release, having interfaces with the atmosphere, soils, ground-, and surface waters (Barth et al. 2009). Riverbank soils often showed water and soil TE contaminations downstream efflux

of water treatment plants (Vystavna et al. 2012; Marchand et al. 2014).

Physico-chemical analysis does not always provide evidence of the biological actions of soil TE contamination (Zhou et al. 2008). In addition, while providing relevant information on geochemistry and fractionation of TE in the soil profile, soil manipulation can disturb soil structure, enhances the surface area exposed to extractants, and may distance the data interpretation from field conditions (Beesley et al. 2010; Moreno-Jiménez et al. 2011). Biomonitoring and bioassays, notably with plants and stress enzyme activities (e.g., enzyme activities involved in plant responses to stress caused by exposure to TE in excess), complement the physico-chemical analysis of environmental matrices, accounting for the subtle biological changes in organisms affected by exogenous contaminants (Mench et al. 2000; Markert 2007; Marchand et al. 2011; Kolbas et al. 2013; Kumpiene et al. 2014). Plant morphological traits are generally considered poorly sensitive to fully assess potential phytotoxicity in moderately TE-contaminated soils (Meers et al. 2006), while one additional method would be to measure the photosynthetic performance (Cambrollé et al. 2012). Measuring the yield of photosystem II (PSII) chlorophyll fluorescence provides insights into the extent to which environmental stresses have damaged the photosynthetic apparatus of a plant (Maxwell and Johnson 2000).

The use of root-emerged macrophytes to biomonitor total and soluble TE concentrations in riverbank soils is under investigations at several sites such as the Jalle d'Eysines River, a Garonne tributary, SW of France (Bonanno 2013; Marchand et al. 2014; Delmail 2014). Total metal concentrations in the Jalle d'Eysines riverbank soil series were monitored by foliar metal concentrations: for Mo in *Phragmites australis* (Cav.) Trin. Ex Steud and *Phalaris arundinacea* L., for Cd in *P. australis* and *Carex riparia* Ehrh. (Marchand et al. 2014). Copper and Mo concentrations in the soil-pore water can be monitored by respectively *P. arundinacea* and *P. australis*.

Ranunculus acris L. (*R. acris* or meadow buttercup, Ranunculaceae) is a weed of old pasture and hay meadows, widely distributed throughout Europe and considered as a common plant species of the agricultural landscape (Schmitz et al. 2013; Bourdot et al. 2013). It is one of the most common species along the Jalle d'Eysines River course, where it was used to monitor total Ni concentration in soils, showing also a site-dependent foliar ionome (Marchand et al. 2014). It was not clear why *R. acris* was biomonitoring Ni and not Cd and Zn, which have a speciation in the soil controlled by similar factors, i.e., pH, content, and type of soluble organic matter, hydrous metal oxides and clay, presence of organic and inorganic ligands, and competition from other metal ions.

In addition to the Jalle d'Eysines river course, *R. acris* was present at the TE-contaminated Fresnes sur Escaut site, north of France. Therefore, this pot experiment aimed at appraising the photosystem II activity and phenotypic responses of the

meadow buttercup to increasing soil TE contamination, with special attention to Zn and Cd, in two soil series differing by their soil texture. They were made using the fading technique with either a sandy loam (A) or silty clay (B) riverbank soil from the Jalle d'Eysines River, SW France, and a TE-contaminated technosol (F) developed over dredged sediments of the Escaut River channel, N France. Morphological traits, foliar ionome, and photosystem II activity of *R. acris* were compared on both soil series. In the general context of TE transfer in the soil-plant continuum, the relationships between total element concentration in the riverbank soil, the soluble element concentration, the foliar ionome of a rooted macrophyte, in this case *R. acris*, and its trait responses were monitored. Based on such relationships, the use of *R. acris* for biomonitoring TE concentrations in riverbank soils and their soil-pore water was questioned.

Material and methods

Soil preparation and analysis

The TE-contaminated technosol F (FAO World Reference Base for Soil Resources, thereafter referred to soil F) was sampled (0–25 cm soil layer) at the PHYTOSED Scale 1 platform (1 ha, Fresnes-sur-Escaut, France) in February 2012. It has developed over dredged sediments of the Escaut River channel deposited at this landfill site over the last century (Bert et al. 2012). Pseudo-total element concentrations in the soil F (aqua regia extraction) are reported in Table 1. For the soil F, emergence of *Brassica rapa* L. (ISO 11269–2:2005) was

reduced to 80 % and its growth by 10 % after 18 days, compared to an uncontaminated VDLUFA (Association of German Agricultural Analytic and Research Institutes) soil. In the ISO 17512-1 (2008) test with *Eisenia fetida*, earthworms were, however, more attracted (18 %) by the soil F compared to the ISO soil. In the ISO 10872:2010 test with *Caenorhabditis elegans* Maupas, the nematode reproduction was inhibited by 71.5 % compared to the VDLUFA soil (Bert et al. 2012).

Riverbank soils used to make the soil series, displaying lower total soil TE concentrations (e.g., 16–274 mg Zn and 0.09–1.6 mg Cd kg^{-1} DW soil) were sampled (0–25 cm soil layer) in February 2012 at two sites, i.e., A and B corresponding to the sandy soil #2 and clay soil #4 in Marchand et al. (2014), along the Jalle d'Eysines River, a tributary of the Garonne River located in southwest France (44° 53′ 36″ N, 00° 40′ 40″ O), north of Bordeaux. From its source to its confluence with the Garonne River, the Jalle d'Eysines River is 32-km long. Water depth varies from 0.8 to 2.5 m annually and average flow volume is 3 m^3 s^{-1}. Sites A and B were, respectively, located at 20 and 30 km from the river's source. Soil texture is sandy at site A with low clay proportion while the soil B contains more clay than sand (Table 2, Marchand et al. 2014).

Details on the geographic position of Escault and Jalle d'Eysines Rivers and the location of sampling points (A, B, and F) are presented in supplementary material (S1).

Soils A, B, and F were air-dried and sieved at 5 mm prior to their use with the fading technique. It consists in mixing contaminated and uncontaminated soils, preferably of the same type, in various proportions to obtain a realistic contaminated

Table 1 Total element concentrations in the riverbank soils ($n=6$) at the sites A and B along the Jalle d'Eysines River and in the soil F at the PHYTOSED Scale 1 platform, Fresne sur Escaut

Site	Total element concentration in soils (mg kg^{-1} DW)									
	Cu	Zn	Cr	Ni	Co	Pb	Cd	Mn	Mo	As
A	8.6±6.7 a	16.1±5.9 a	17.4±7.1 a	3.9±2.3 a	1.6±0.8 a	11.5±1.8 a	0.09±0.03 a	84.4±5a	0.27±0.21a	–
B	39.8±4.4 b	274.2±52.8b	85.3±9.1 b	39.1±1.6 b	16.1±0.4 b	70.1±5.1 b	1.6±0.54 b	767±107b	0.95±0.1b	–
F	*110±7.2*	*6089±825*	99.7±4.2	42.9±3.5	17±1.6	*956±88.6*	*9.4±0.9*	976±126	4±0.4	42.9±3.5
French guideline values for dredged sediment management	100	300	150	50	–	100	2	–	–	30
Inquiry threshold values for French soils	35	150	100	70	30	60	0.7	–	–	–
	Al (g kg^{-1})	Fe (g kg^{-1})	Ca (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Na (g kg^{-1})	P (g kg^{-1})			
A	10.5±5.3 a	4.6±2.5 a	2.9±1.3 a	3.2±1.1 a	0.4±0.1 a	0.6±0.1a	0.08±0.03a			
B	82±5.1 b	43±1.7 b	16±4.5 b	22.5±1.6 b	10.5±1.3 b	4.5±0.6b	0.18±0.06b			
F	17±2	86±14	31±1.5	3.8±0.4	3±0.2	0.2±0.04	3.05			

Values are means±SD. Soils A and B: total element concentrations after wet-digestion in hydrofluoric acid (NF X 31147) and analysis by ICP-AES. Soil F: pseudo-total element concentration after aqua regia extraction and ICP-AES analysis. The different letters stand for statistical significance between the sites A and B at the 0.05 level with Student's *T* test. Values in italics exceeded both the French guideline values for dredged-sediment management (http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000423497) and the inquiry threshold values for French soils (Baize et al. 2007). Values for the soil F (Kidd et al. 2014). P Olsen extraction

Table 2 Main characteristics of soils (*n*=6) and soil-pore waters (*n*=4) at the sites A and B along the Jalle d'Eysines River and at the site F of the PHYTOSED Scale 1 platform, Fresnes sur Escaut

Soil parameters

Soils	Clays (<2 μm) (g kg^{-1})	Silts (2–62 μm) (g kg^{-1})	Coarse sand (200–2000 μm) (g kg^{-1})	C (g kg^{-1})	N (g kg^{-1})	OM (g kg^{-1})	C/N
A	81±19 a	42±31 a	779.3±24.9 a	11.3±2.9 a	0.93±0.22 a	19.5±5.1 a	12.0±0.6 a
B	383±28 b	451±47 b	58.3±48.3 b	26.3±6.8 b	2.4±0.5 b	45.5±11.7 b	10.7±0.8 b
F	134	661	151	152	4.9	30.6	31

Soil parameters

Site	CEC (cmol kg^{-1})	pH	EC (mS cm^{-1})	WHC (%)	Soil-pore water Eh (mV)	pH	EC (mS cm^{-1})
A	5.2±1.7 a	7.5±0.3 a	0.15±0.06 a	20.1±0.7 a	288±11.7 a	7.6±0.3 a	0.77±0.3 a
B	26.7±4.6 b	7.9±0.3 a	0.47±0.03 b	24.8±1 b	264±2.1 b	7.9±0.2 a	1.06±0.2 a
F	23.5	7.2±0.3	–	–	–	–	–

Values are means±SD. The different letters stand for statistical significance between sites A and B at the 0.05 level with Student's *T* test. Values for the soil F (Kidd et al. 2014

EC electrical conductivity, *WHC* water holding capacity, *OM* organic matter

soil series. Here, the soil series displayed a wide range of metal (Cd, Cu, Pb, and Zn) exposures, well distributed, allowing to gain dose-effect relationships, upper critical TE concentrations for plant parameters, and effective TE concentrations in the soil-pore water. This technique allows to better model the field soil conditions than hydroponics (Japenga et al. 2007). The A soil series was made by carefully mixing (run-over-run) the soil F with the soil A in a ratio from 0:100 to 100:0 % with a 20 % gradual step. Soil samples (1 kg DW) were placed in plastic pots (1.3 L) to prepare six treatments (four pots/treatment) labeled from A (0 %) to F (100 %), the percentage between brackets being the soil F proportion. Similarly, the B soil series was made by mixing soils F and B. Soils were watered twice a week between February and April 2012 with deionized water and maintained at 70 % of field capacity (water holding capacity, 10 % of air-dried soil mass). In week 10, one Rhizon MOM moisture sampler (Eijkelkamp, The Netherlands) was inserted with a 45° angle into each potted soil. For all soils, soil-pore waters were collected (three times 10 mL during weeks 16 and 17 to make a 30-mL sample) and kept at 4 °C prior to TE analysis by ICP-AES (Varian liberty 200, Germany) and ICP-MS (Thermo X series 200, USA) at the INRA USRAVE laboratory (Villenave d'Ornon, France). Trace element concentrations in soil-pore water along the Jalle d'Eysines River and at the PHYTOSED Scale 1 platform were detailed by Marchand et al. (2014) and Kidd et al. (2014), respectively. The soil-soil-pore water partition coefficients [Kd$_{TE}$ (L kg^{-1})=total soil TE concentration (mg kg^{-1})/TE concentration in the soil-pore water (mg L^{-1})] were calculated for three (Zn, Cd, and Cu) out of the four TE which exceeded both the French guideline values for dredged-sediment management and the inquiry threshold values for French soils on the soil series A and B. The Kd values were not calculated for

Pb since its concentrations in the soil-pore water remained below the detection limit (<0.8 μg L^{-1}).

Plant material

In June 2011, 50 individuals of meadow buttercup (*R. acris* L.) were sampled along the Jalle d'Eysines River. Plant samples were kept separate in buckets, standardized (similar belowground and aboveground volumes), and then placed in water in a greenhouse at the Centre INRA-Bordeaux Aquitaine (Villenave d'Ornon, France). Individuals were thereafter grown in separate polyethylene containers (volume, 60×40× 15 cm^3) containing perlite soaked with a quarter-strength Hoagland nutrient solution (HNS, Hoagland and Arnon, 1950): KNO$_3$ (1.62 mM), Ca(NO$_3$)$_2$ (0.69 mM), NH$_4$H$_2$PO$_4$ (0.25 mM), MgSO$_4$ (0.5 mM), H$_3$BO$_3$ (11.53 μM), MnCl$_2$ (2.29 μM), CuSO$_4$·5H$_2$O (0.08 μM), (NH$_4$)$_6$Mo$_7$O$_{24}$ (0.13 μM), ZnSO$_4$·7H$_2$O (0.19 μM) and FeSO$_4$ (48.6 μM). Water volume was maintained constant by adding tap water. Water was renewed and nutrients were added every month during the growing season and every 2 months during winter to avoid anoxia and nutrient depletion in the growth medium. In March 2012 (week 10), one standardized plant (3–5 leaves) was transplanted into each potted soil. All pots were randomly placed on a bench in the same greenhouse during 7 weeks [day (9–21 h) 1911±1232 μM photons m^{-2} s^{-1}, 28±5 °C, night (21–9 h) 19±3 °C].

Chlorophyll fluorescence

Chlorophyll fluorescence was measured once a week for all plants in a fully developed and randomly selected leaf (*n*=4 replicates for all soils) using a portable modulated

fluorometer (Pam-2500 Waltz, Germany) during the 7-week growth period.

In the morning, in a dark chamber, the minimal fluorescence level in the dark-adapted state (F_0) was measured using a modulated pulse (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) which was too small to induce significant physiological changes in the plant. Maximal fluorescence in this state (F_m) was measured after applying a saturating actinic light pulse of 15, 000 μmol m^{-2} s^{-1} for 0.7 s. The value of F_m was recorded as the highest average of two consecutive points. Values of variable fluorescence ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII photochemistry (F_v/F_m) were calculated from F_0 and F_m. This ratio of variable to maximal fluorescence correlates with the number of functional PSII reaction centers, and dark-adapted values of F_v/F_m can be used to quantify photoinhibition (Cambrollé et al. 2012).

Growth parameters

In week 17, after measuring the survival rate (S2), plants were harvested. The total leaf area (TLA, cm^{2}plant^{-1}) of each individual was immediately measured by digital image analysis (Epson Expression 10000XL, WINFOLIA software, Regent Instrument, Canada). Leaf samples were then carefully washed with tap water, rinsed with deionized water, blotted with filter paper, placed in paper bags, and oven-dried for 48 h at 55 °C to a constant weight. In week 18, the leaf dry weight (DW) yield (g plant^{-1}) was measured [$n=4$ replicates for all soils except for B (0 %): $n=1$ survivor plant and B (80 %): $n=2$ survivor plants, due to powdery mildew]. The specific leaf area (SLA, cm^2 g^{-1}) was calculated as the ratio of the total leaf area (TLA) over the leaf DW yield.

Mineral analysis

Dried leaf samples were ground (<1.0-mm particle size, Retsch MM200) and stored in plastic containers (100 mL) at room temperature, in dark conditions. For all soil treatments, four leaf aliquots (0.5 g DW) from individual replicates were wet digested under microwaves (180 °C, CEM Marsexpress, USA) with 5 mL supra-pure 14 M HNO$_3$, 2 mL 30 % (v/v) H$_2$O$_2$ not stabilized by phosphates, and 1 mL Milli-Q water. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Macroelements and TE concentrations in solutions were then determined by ICP-AES (Varian liberty 200) and ICP-MS (Thermo X series 200) at the INRA USRAVE laboratory [$n=3$ out of four replicates, randomly selected, except $n=2$ for B (80 %)]. All elements were recovered (>95 %) according to the standard values and the standard deviation for replicates ($n=3$) was <5 %. All element concentrations in plant and soil samples are presented on DW basis.

Statistical analysis

The influence of sampling sites A and B on the main soil characteristics and the total TE concentrations in riverbank soils were tested using a Student's T test. The dilution rate effect on the foliar ionome of *R. acris* was assessed with a one-way ANOVA for both the soil series A and B. A principal component analysis (PCA) was conducted on soil-pore waters and a second one on the foliar ionome of *R. acris* after a 50-day exposure to the soil series A and B. Pearson correlation coefficients between soil TE concentrations, soil-pore water TE concentrations, and foliar ionome of *R. acris* grown on the soil series A and B were calculated. The x,y data sets were curve-fitted by the Excel software (Microsoft, USA) for the Kd values of Zn, Cd, and Cu vs. the dilution rate for the soil series A and B. The maximum efficiency of PSII (F_v/F_m) in leaves of *R. acris* depending on the soil series A and B, dilution rate (%), and exposure time (weeks) were represented using a surface response analysis. Finally, the crossed effects of the soil series and the dilution rates after a 50-day exposure were tested on the leaf DW yield, the TLA, and the SLA using a two-way ANOVA. Normality and homoscedasticity of residuals were met for all tests. Differences were considered statistically significant at $p<0.05$. All statistical analyses were performed using the R software (version 3.0.1, R Foundation for Statistical Computing, Vienna, Austria).

Results

Physico-chemical analyses of soils and soil-pore waters

Total soil Cu, Cd, Fe, Mo, Pb, and Zn varied in the decreasing order: F>B>A, while total soil Cr, Co, Ni, and Mn ranked as F≈B>A (Table 1). All TE concentrations in the soil A were lower than both the inquiry threshold value for French soils (Baize et al. 2007; Table 1) and the French guideline values for dredged-sediment management (Arrêté du 9 août 2006, Table 1). Total soil Zn was within its common range (20–200 mg Zn kg^{-1} DW, Blum et al. 2012) for both soils A and B. Total soil Cd and Pb were within their common ranges (i.e., 0.05–1 mg Cd and 10–30 mg Pb kg^{-1} DW) in the soil A, but were slightly higher in the soil B. Total Cu, Zn, Pb, and Cd concentrations in the soil B exceeded the inquiry threshold values for French soils, i.e., 2-fold for Zn and Cd, but were below French guideline values for dredged-sediment management (Table 1). Total soil concentrations of the other TE were within the common ranges and below both the French guideline values for dredged sediment management and the inquiry threshold values for French soils. Higher total soil Al, K, Mg and Na, and extractable P in the soil B likely resulted from its higher clay and OM contents (Tables 1 and 2). For the soil F, total soil Cd, Cu, Pb, and Zn largely surpassed both the inquiry

threshold values for French soils and French guideline values for dredged-sediment management, i.e., 20-, 10-, and 5-fold for Zn, Pb, and Cd, respectively (Table 1), and total soil As as well for these guidelines.

Soil physico-chemical parameters at site A differed from those at site B (Table 2). Soil texture was sandy at site A with a low clay content while the soil B contained more clay than sand. Similarly the soil A had lower C, N, and OM contents and lower CEC, EC, and WHC values than the soil B. Mean soil pH (7.5–7.9) did not significantly differ between soils A and B and was similar to pH value for the soil F (7.2). The soil F texture was silty loam with an intermediate OM content between the soil A and B values. The CEC values of soils F and B were similar and higher than for the soil A.

Total element concentrations in the soil-pore water did not follow the gradient of total element concentrations in soils (Table 3, Fig. 1). For convenience, we referred to element concentration in the soil-pore water as soluble element concentration throughout the paper.

Soil series A Mean values of soluble Ni, Fe, B, P, K, and As concentrations peaked for the soil A (0 %), soluble Cu and Mo ones for the soil A (20 %) but their values were similar compared to A (0 %), and soluble Cr, Cd, and Zn concentrations for the soil A (60 %) (Table 3). As the percentage of soil F increased, soluble concentrations decreased for Na, K, P, Ni, Mo, Mg, Fe, and As; remained steady for Al, B, Cu, Mn, and Pb; and rapidly raised at A (20 %) to reach a plateau for Zn (39 µg L^{-1}) and Cd (0.3 µg L^{-1}).

Soil series B The soil B displayed the highest soluble Na and Mg concentrations, and they decreased thereafter as the soil F percentage raised (Table 3). Lowest soluble concentrations were for As, Cd, Mo, K, and Zn in the soil B (0 %) and for Cu and Cr, respectively, in the soil B (40 %) and the soil B (60 %). Soluble P and Fe concentrations were low at all dilution rates. Soluble Zn and Cd concentrations were the lowest in soil B (0 and 20 %) and the highest in soil F (Table 3, Fig. 1). Soluble Zn and Cd concentrations displayed maximum values around 22 µg Zn and 0.2 µg Cd L^{-1} in soil F (Table 3). Soluble Ni concentration was the lowest for the soil F, and soluble Al, Mn, and Pb concentrations remained below detection limits for all soils.

PCA on the composition of the soil-pore water The first axis (PC1, 33.4 %) corresponded to the dilution rate of the soil series A, while the second axis (PC2, 21.3 %) matched with the dilution rate of the soil series B. Accordingly, the soil-pore water can be divided in three groups. Group 1 (A 0% and A 20%) included soil-pore water with the highest B, Fe, Mn, P, K, As, Mo, and Cu concentrations. Group 2 (B 0 to 40 %) gathered soil-pore water with the highest Ca, Mg, and Na concentrations, but with the lowest Cd and Zn ones. Group

Table 3 Total element concentrations in the soil-pore waters of the soil series A and B and of the soil F (Fresne sur Escault)

	Al µg L^{-1}	As µg L^{-1}	B µg L^{-1}	Cd µg L^{-1}	Ca mg L^{-1}	Cr µg L^{-1}	Cu µg L^{-1}	Fe µg L^{-1}	Mg mg L^{-1}	Mn µg L^{-1}	Mo µg L^{-1}	Ni µg L^{-1}	P mg L^{-1}	Pb µg L^{-1}	K mg L^{-1}	Na mg L^{-1}	Zn µg L^{-1}
A (0 %)	<50	23.3±6.3	231.1±36.4	0.1±0.01	71±35	3.2±0.5	19.8±6.9	98.8±17.1	10.9±4.6	<20	41.0±22.1	15.9±3.8	1.4±0.2	<0.8	132.1±1.1	130.3±26.9	12.3±1.3
A (20 %)	<50	16.1±2.9	205.1±0.08	0.3±0.01	52±32	1.9±0.1	31.2±5.7	57.2±10.6	6.9±5	<20	48.4±24.5	7.7±2.6	0.5±0.4	<0.8	93.0±68.4	117.4±67.4	32.6±4.5
A (40 %)	<50	10.3±1.2	213.6±0.01	0.2±0.01	73±5.6	1.4±0.01	20.1±3.9	22.2±4.1	6.7±0.5	<20	39.1±14.5	4.6±0.4	0.3±0.1	<0.8	75.4±10.7	74.8±10.3	38.2±6.1
A (60 %)	<50	11.5±2.8	195.5±0.03	0.3±0.01	60±1.7	38.9±26.1	18.9±3.2	15.1±1.5	4.7±0.2	<20	33.7±24.1	5.7±0.9	<0.2	<0.8	39.2±16.8	35.6±7.4	39.4±5.4
A (80 %)	<50	7.7±2.1	210.7±0.01	0.2±0.03	78±9.2	9.3±10.5	16.1±0.8	<20	6.2±1.4	<20	25.0±4.5	4.8±1.3	<0.2	<0.8	52.9±5.5	44.6±6.8	37.7±14.3
B (0 %)	<50	4.4±1.3	175.7±109.1	<0.1	107±114	1.4±0.6	9.2±5.6	<20	19.4±20.1	<20	8.8±8.1	3.6±2.7	<0.2	<0.8	15.1±11.5	134.2±138.1	<7
B (20 %)	<50	5.6±0.7	159.7±60.3	<0.1	78±40	1.2±0.01	12.8±3.9	20.8±10.6	13.1±6.6	<20	10.5±6.1	3.6±1.1	<0.2	<0.8	16.6±6.5	102.0±55.6	<7
B (40 %)	<50	5.4±0.1	188.6±27.3	0.1±0.01	111±27	1.1±0.2	7.8±0.5	24.6±0.9	15.8±4.1	<20	10.9±0.01	3.2±0.01	<0.2	<0.8	23.5±5.7	112.5±11.5	10.5±4.5
B (60 %)	<50	5.4±0.3	157.9±28.1	0.2±0.04	76±29	1.0±0.01	11.1±1.9	<20	9.2±2.9	<20	12.8±1.6	4.1±1.6	<0.2	<0.8	24.4±5.4	68.2±27.1	17.7±5.1
B (80 %)	<50	6.9±0.02	170.7±4.1	0.2±0.03	67±0.9	1.1±0.01	15.2±2.1	20.9±0.8	6.5±0.1	<20	15.7±0.4	3.3±0.4	<0.2	<0.8	26.7±0.1	47.8±0.7	22.7±1.7
F (100 %)	<50	7.1±1.5	171.4±25.1	0.2±0.02	72±20	3.5±4.1	11.1±6	<20	4.6±1.5	<20	14.8±4.4	2.9±0.4	<0.2	<0.8	39.8±11.7	32.9±11.7	27.1±18.3

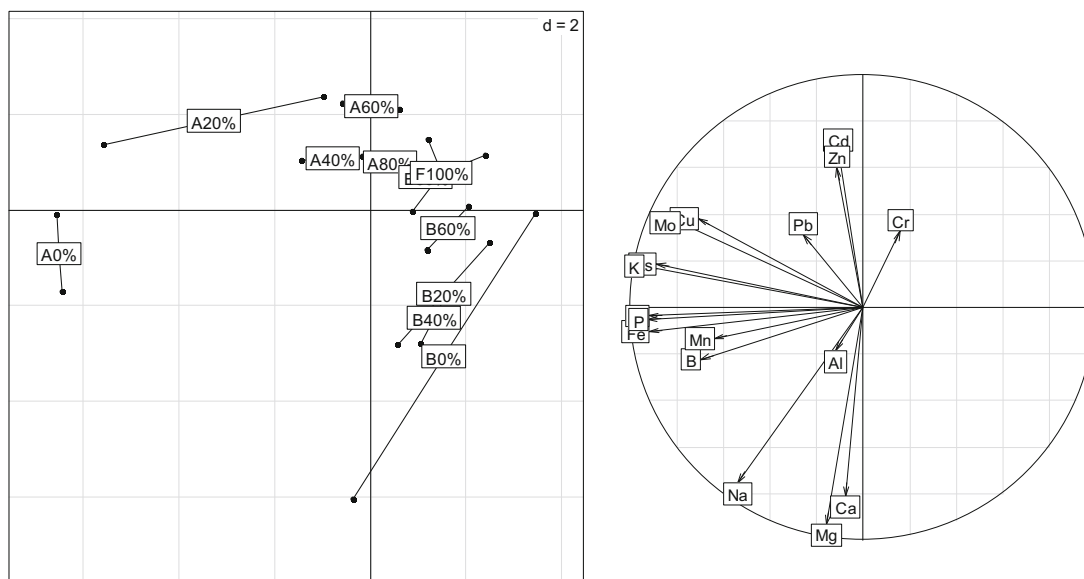The percentage between brackets is the proportion of the soil F. Values are means ($n=2$)±SD

Fig. 1 Principal component analysis (PCA) of the soil series A and B and of the soil F accounting for Al, As, B, Cd, Ca, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, K, Na, and Zn concentrations in the soil-pore water (PC1, 33.4 %; PC2, 21.3 %)

3 (A 40 to 80 %, B 60, and 80 %, F) had the highest soluble Cr, Zn, and Cd concentrations (Fig. 1).

Partition coefficients The Log Kd_{Zn} values ranged from 2.8 (soil A) and 4.6 (soil B) to 5.6 L kg^{-1} (soil F). The Log Kd_{Cd} values varied from 2.9 (soil A) and 4.2 (soil B) to 4.7 L kg^{-1} (soil F). Values of the Log Kd_{Cu} increased from 2.5 (soil A) and 3.5 (soil B) to 4.4 (soil F). For Cd, Cu, and Zn, the distribution of Log Kd values vs. the dilution rate was best curve-fitted with a quartic polynomial equation for both the soil series A and B (Fig. 2).

Foliar ionome of *R. acris*

No symptoms of element toxicity such as spotted necrosis and chlorosis were visible on any *R. acris* leaves but four plants on the soil B (0 %) and two plants on the soil B (80 %) suffered from a powdery mildew attack leading to the death of three plants for the soil B (0 %) and two for the soil B (80 %).

The foliar element concentrations did not follow the patterns of total element concentrations in the soils and the soil-pore waters (Fig. 5; Table 4). The ranges of foliar concentrations (in mg kg^{-1} DW) were: Cd 0.1–0.3, Cu 6.5–12.7, Cr 0.4–0.8, Ni 0.4–0.7, and Pb 1.9–3.5 (Table 4). Foliar Zn concentrations peaked for plants cultivated on the soils A (80 %), B (80 %), and F, i.e., 124, 115, and 112 mg kg^{-1}, respectively. In both soil series, foliar Al, Ca, Cu, Mg, P, K, and Na concentrations did not significantly depend on the dilution rate. Foliar Mo, Pb, and Zn did not vary with the dilution rate in the soil series A, as well as foliar Cd concentration in the soil series B. Conversely, foliar As, B, Cd, Cr, Fe, Mn, and Zn concentrations, respectively, peaked at 0–20, 20, 80, 80, 20–

80, 0–20–80, and 80 % in the soil series A and at 80 % for all elements in the soil series B except for As (40 %) and Cd (no variation with the dilution rate).

The PCA based on the foliar ionome discriminated two groups. The first one (B 60 %, A 40 %, and A 60 %) displayed lower foliar Ni, Mn, Mg, Fe, Cu, Cr, and B concentrations compared to the second group (A 0%, A 20%, A 80 %, B 0 %, B 20 %, B 40 %, B 80 %, and F) (Fig. 5). Several foliar element concentrations were correlated: Cr/As/Na/Fe/Zn, P/K/B/Cd/Cu, and Mn/Mg/Ca/Mo.

Relationships between soil TE concentrations, soil-pore water TE concentrations, and the foliar ionome of *R. acris*

The Pearson coefficient (r) between the total concentration in the soil and the soluble concentration was significant ($p<0.05$) for As, Cu, Fe, Mo, Ni, K, and Na for the soil series A and for As, Cd, Mg, Mo, K, Na, and Zn for the soil series B. This Pearson coefficient calculated between the total concentration in the soil and the foliar ionome of *R. acris* was only significant for Na in the soil series A and for Mg, Mn, Mo, and Na in the soil series B. Finally, the Pearson coefficient between the soluble element concentrations and the foliar ionome of *R. acris* was significant for Mo and Na in both the soil series A and B (Table 5).

Chlorophyll fluorescence and growth parameters

Leaves of *R. acris* displayed steady maximum quantum efficiency of PSII (F_v/F_m) at dawn over a 50-day exposure period for both soil series A and B (F_v/F_m ranged between 0.65 and 0.8) (Fig. 3). The leaf DW yield ranged between 0.24–0.78
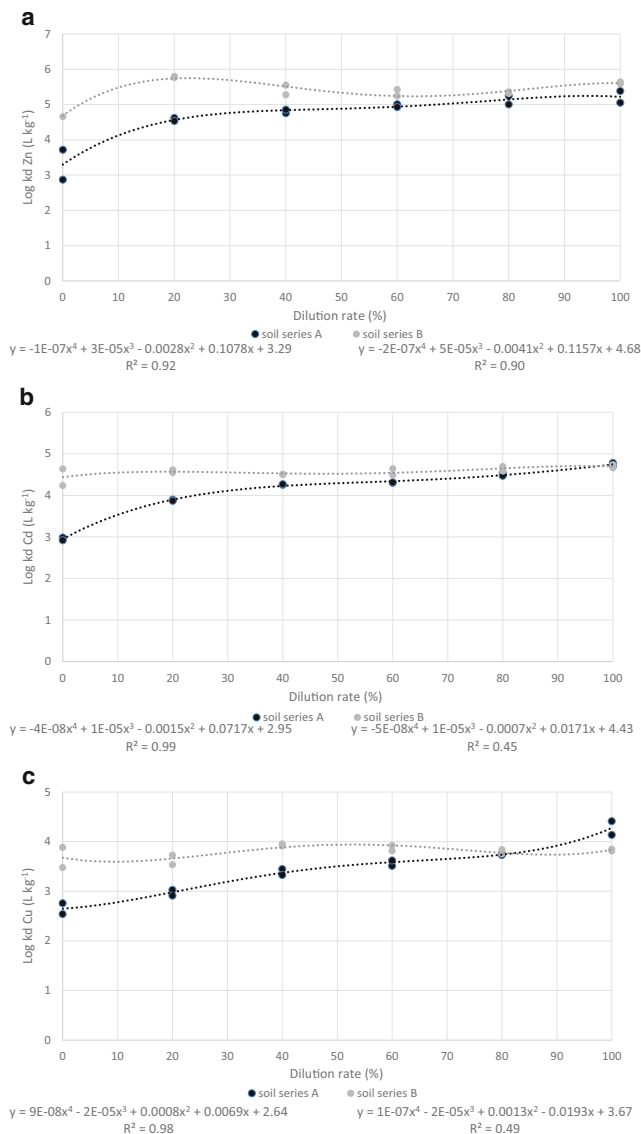
y = -1E-07x⁴ + 3E-05x³ - 0.0028x² + 0.1078x + 3.29
R² = 0.92

y = -2E-07x⁴ + 5E-05x³ - 0.0041x² + 0.1157x + 4.68
R² = 0.90

y = -4E-08x⁴ + 1E-05x³ - 0.0015x² + 0.0717x + 2.95
R² = 0.99

y = -5E-08x⁴ + 1E-05x³ - 0.0007x² + 0.0171x + 4.43
R² = 0.45

y = 9E-08x⁴ - 2E-05x³ + 0.0008x² + 0.0069x + 2.64
R² = 0.98

y = 1E-07x⁴ - 2E-05x³ + 0.0013x² - 0.0193x + 3.67
R² = 0.49

Fig. 2 Calculated soil-soil-pore water partition coefficients (Log Kd) for **a** Zn, **b** Cd, and **c** Cu as a function of the dilution rate on the soil series A and B

and 0.01–0.4 g plant^{-1} for the soil series A and B, respectively (Fig. 4). However, neither the influence of the soils A and B, nor the dilution rate, nor their interaction was significant on this leaf DW yield ($p > 0.05$, Table 5). Substantial variations in leaf DW yield between replicates at each dilution rate and for both soil series were noted. Similarly, the mean TLA values ranged between 40–110 and 10–80 cm^{-2}plant^{-1} for both soil series A and B, with relatively large differences between replicates and no significant differences for the TLA according to the soil series, the dilution rate and their interaction (Table 6, Fig. 4, $p > 0.05$). Conversely, the SLA was significantly lower for the soil series A (80–120 cm^{-2} g^{-1} plant^{-1}) compared to the soil series B (110–220 cm^{-2} g^{-1} plant^{-1}) (Table 6, Fig. 4, $p < 0.05$).

Discussion

Relationships between total and soluble TE concentrations in the soil series

The fading technique, previously used by Japenga et al. (2007) and Kolbas et al. (2013), allowed to obtain the soil series A and B, with a realistic gradual soil TE contamination but differing by their soil texture. Trace element concentrations of soil-pore water in the soil series A and B slightly exceeded the As and Cu values, but not the Zn one, for an uncontaminated soil with similar pH but slightly lower OM content (in μg L^{-1}: As [1–3]; Cu [2–10]; and Zn [<1–360], Beesley and Dickinson 2010). Soluble Cr concentrations were however similar to previous findings in uncontaminated conditions (Burbridge et al., 2012; Marchand et al. 2014). Our soluble TE concentrations are generally lower than values reported in the soil-pore water of contaminated soils (in μg L^{-1}): Cu [370–1780] (Bes et al. 2010); Cu [1050] and Pb [80] (Karami et al. 2011); Cu [168–270], Zn [57–610], and Pb [1.25–14.9] (Kabala et al. 2014) and Moreno-Jiménez et al. (2011). Unlike the gradient of total concentrations created by fading the soil F with either the soil A or soil B, the soil-pore water in both soil series generally did not display gradual increases in soluble element concentrations in line with the percentage of TE-contaminated soil F. Generally, soil TE availability depends on the characteristics of particle surfaces, e.g., organic matter (OM), oxyhydroxides of Fe, Al, and Mn (FeMnOx), phyllosilicate minerals, carbonates, and sulphides, their sorption properties and/or reactions with the solution in contact. Such reactions are driven by soil physico-chemical parameters such as pH, CEC, and the redox potential and dissolution-precipitation reactions (Violante et al. 2008; Moreno-Jiménez et al. 2011; Manzano et al. 2014). The clay content, total OM content, and the CEC in our soils were in the increasing order B>F>A, while the coarse sand content was in the decreasing order B<F<A. The Fe/Mn (hydr)oxide concentrations were the highest in the soil F and the lowest in the soil. Consequently, the CEC, total OM, clay, and Fe/Mn (hydr)oxide contents increased in the soil series A, while they decreased, except for Fe/Mn (hydr)oxides, in the soil series B.

The slight increase in soluble Zn/Cd in both soil series was mainly related to the increment of total soil Zn/Cd, but only correlated in the soil series B. The soil texture of the soil series indeed influenced markedly the soluble Zn/Cd concentrations. Their values were higher in the soil series A than in the B one, even though the slight decrease of pH in the soil series B from 7.9 to 7.2 may in theory favor metal desorption from Fe/Mn (hydr)oxides. This was reflected by differences in the Kd$_{Zn}$ and Kd$_{Cd}$ modeling soluble vs. total soil Zn/Cd. Both Kd$_{Zn}$ and Kd$_{Cd}$, and also Kd$_{Cu}$ were higher in the soil series B, notably at dilution rates between 0 and 20 %, suggesting that the OM and clay contents and the hydrous Fe/Mn oxides

Table 4 Foliar ionome of *Ranunculus acris* (n=3, except for the soil B (80 %) n=2) for the soil series A and B after a 50-day exposure period

	Al mg kg⁻¹	As mg kg⁻¹	B mg kg⁻¹	Cd mg kg⁻¹	Ca g kg⁻¹	Cr mg kg⁻¹	Cu mg kg⁻¹	Fe mg kg⁻¹	Mg g kg⁻¹
A (0 %)	81.3±29.1a	0.4±0.1a	74.3±5.8ab	0.1±0.1b	15.8±17.4a	0.7±0.1a	7.6±0.8a	150.3±37.6ab	2.3±2.1a
A (20 %)	111.8±65.3a	0.4±0.1a	88.6±13.2a	0.2±0.1ab	18.7±18.4a	0.7±0.3ab	8.3±0.3a	237.4±107.7ab	2.0±2.1a
A (40 %)	47.3±32.3a	0.2±0.1b	46.5±28.2b	0.2±0.1ab	18.0±15.4a	0.4±0.1b	6.5±3.6a	121.4±49.8b	2.2±2.1a
A (60 %)	50.3±13.7a	0.3±0.1ab	58.5±14.2b	0.2±0.1ab	11.2±7.7a	0.5±0.1b	8.6±3.4a	168.0±87.9ab	1.4±1.1a
A (80 %)	85.9±22.2a	0.3±0.1ab	56.1±20.7ab	0.3±0.1a	8.9±12.3a	0.8±0.3a	9.6±2.0a	237.3±79.5a	1.0±1.3a
B (0 %)	NA	NA	NA	NA	NA	NA	NA	NA	NA
B (20 %)	113.3±68.8a	0.3±0.1ab	47.3±3.0b	0.2±0.1a	12.3±12.2a	0.6±0.3ab	11.6±1.8a	224.1±126.5ab	2.5±2.3a
B (40 %)	262.0±158.0a	0.4±0.2ab	58.2±4.8ab	0.2±0.1a	11.6±11.5a	0.7±0.3ab	*12.7±1.8a*	170.2±30.1b	2.2±2.0a
B (60 %)	55.7±12.2a	0.2±0.1b	44.8±15.2ab	0.2±0.1a	11.8±10.7a	0.4±0.1b	9.5±3.6a	142.2±24.1b	1.9±1.7a
B (80 %)	129.8±8.5a	0.4±0.1a	61.3±1.8a	0.2±0.1a	10.1±9.7a	0.8±0.2a	11.6±0.1a	371.0±38.2a	1.7±1.8a
F (100 %)	77.7±31.1	0.3±0.1	58.3±4.2	0.2±0.1	11.9±12.7	0.6±0.1	9.4±0.9	275.3±118.5	2.0±1.9
Typical foliar ionome[a]	–	0.1–0.5	3–90	0.05–0.5	–	0.1–0.5	3–12	50–200	–
Foliar ionome of *R. acris* along the Jalle d'Eysines River (France)[b]	–	0.1–0.4	–	0.1–0.3	9.4–12.4	0.7–1.3	15.8–16.9	95–389	1.6–2.7

	Mn mg kg⁻¹	Mo mg kg⁻¹	Ni mg kg⁻¹	P g kg⁻¹	Pb mg kg⁻¹	K g kg⁻¹	Na mg kg⁻¹	Zn mg kg⁻¹
A (0 %)	22.0±0.2a	2.3±0.9a	0.4±0.1a	1.7±1.8a	2.3±1.1a	15.0±16.8a	13.2±12.8a	85.7±36.3a
A (20 %)	24.5±3.7a	2.2±0.5a	0.5±0.1a	2.2±2.1a	2.6±1.0a	20.4±20.9a	10.4±10.4a	77.1±18.6a
A (40 %)	12.5±5.3b	2.0±1.4a	0.4±0.1a	1.9±1.9a	1.9±1.0a	22.2±23.4a	10.8±9.4a	77.7±50.3a
A (60 %)	16.4±5.2ab	3.2±2.3a	0.4±0.2a	1.5±1.5a	2.1±0.4a	20.2±16.7a	5.1±3.5a	75.1±16.9a
A (80 %)	24.9±5.0a	3.7±1.8a	0.5±0.1a	1.2±2.0a	2.4±0.8a	12.5±18.0a	4.3±5.0a	*124.9±44.4a*
B (0 %)	NA	NA	NA	NA	NA	NA	NA	NA
B (20 %)	32.4±12.3a	1.8±0.7b	0.6±0.1ab	2.7±2.7a	2.0±1.1b	19.5±21.6a	10.6±8.7a	76.7±38.5b
B (40 %)	26.3±8.2a	2.6±0.9ab	0.7±0.2a	2.6±2.6a	2.5±1.5ab	22.3±23.3a	8.8±7.1a	80.4±18.7b
B (60 %)	17.5±7.5a	2.7±1.5ab	0.4±0.1b	2.6±1.9a	3.5±3.9ab	23.3±16.9a	6.0±5.7a	67.7±23.7b
B (80 %)	20.9±3.9a	3.3±0.8a	0.6±0.1ab	2.3±2.1a	3.5±0.6a	18.5±17.4a	5.5±6.7a	*115.2±5.7a*
F (100 %)	18.3±1.6	3.3±1.1	0.5±0.0	2.6±2.2	2.8±0.9	22.4±21.6	7.0±5.7	*112.3±23.5*
Typical foliar ionome[a]	20–400	–	0.2–2	–	0.1–0.5	–	–	20–100
Foliar ionome of *R. acris* along the Jalle d'Eysines River (France)[b]	29–83	1.2–3.4	1–2.1	2.7–4.5	1–1.4	29.2–32.3	–	37.5–54.9

NA not available

Values are means±SD. The different letters stand for statistical significance in both the A and B soil series at the 0.05 level with ANOVA. Values in italics excess the typical foliar ionome (Blum et al. 2012)

[a] Data from Blum et al. (2012)

[b] Data from Marchand et al. (2014)

Table 5 Pearson correlation coefficients (r) between soil TE concentrations, soil-pore water (SPW) TE concentrations and foliar ionome of *R. acris* grown on the soil series A and B

Soil series	Total concentration in the soil/concentration in the SPW		Total concentration in the soil/foliar ionome		Concentration in the SPW/foliar ionome	
	A	B	A	B	A	B
Al	0.33	0.26	0.12	−0.31	−0.1	0.38
As	−0.85*	0.72	−0.24	0.18	0.43	0.25
B	−0.5	−0.02	−0.42	0.37	−0.08	−0.04
Cd	0.19	0.84*	0.38	−0.05	0.44	0.06
Ca	0.18	−0.31	−0.3	0.19	−0.19	−0.34
Cr	0.32	0.36	−0.05	0.04	−0.28	0.03
Cu	−0.67*	0.19	0.35	−0.39	−0.25	0.02
Fe	−0.85*	−0.2	0.37	0.37	−0.22	0.05
Mg	−0.61	0.65*	−0.34	0.59*	0.37	0.49
Mn	−0.54	−0.25	−0.12	−0.57*	0.18	−0.25
Mo	−0.67*	0.55*	0.38	0.51*	−0.58*	0.54*
Ni	−0.81*	−0.21	0.31	−0.27	−0.35	−0.32
P	–	–	–	–	−0.05	0.04
Pb	−0.29	0.2	0.11	0.2	0.07	0.1
K	−0.78*	0.76*	0.33	0.01	−0.24	0.04
Na	0.83*	0.66*	0.73*	0.62*	0.75*	0.64*
Zn	0.21	0.74*	0.39	0.52	0.23	0.26

The values in italics are statistically significant (*$p < 0.05$)

would more buffer soluble Zn, Cd, and Cu concentrations in this soil series despite a significant supply of total Zn, Cd, and Cu. Such results confirmed that the kinetically labile solid-phase pool of metal would be key factor for plant Zn and Cd uptake along with the labile metal in soil solution (Nolan et al. 2005). The soil and SPW pH remained roughly steady (7.2–7.9) in both soil series, likely explaining low soluble Al, Mn, and Pb concentrations, as these elements likely (co)precipitate as (hydr)oxides and with phosphates and carbonates and react

with clay and OM. Soluble Ca and B concentrations remained steady in both soil series despite contrasting soil textures and increase in total soil Ca in line with the soil F percentage.

In the soil series A, soluble As, Fe, Ni, and Mg concentrations progressively decreased despite the gradual increase of their total soil concentrations. This occurred also for soluble Cu and Mo concentrations but at high soil F addition rates. Such decreases may be driven in theory by increases in soil CEC, hydrous Fe/Mn oxides, clay, and OM contents in line

Fig. 3 Response surface for the maximum efficiency of PSII (F_v/F_m) in the leaves of *R. acris* depending on the soil series A and B (between brackets), the dilution rate (%), and exposure time (weeks). ($n=4$)
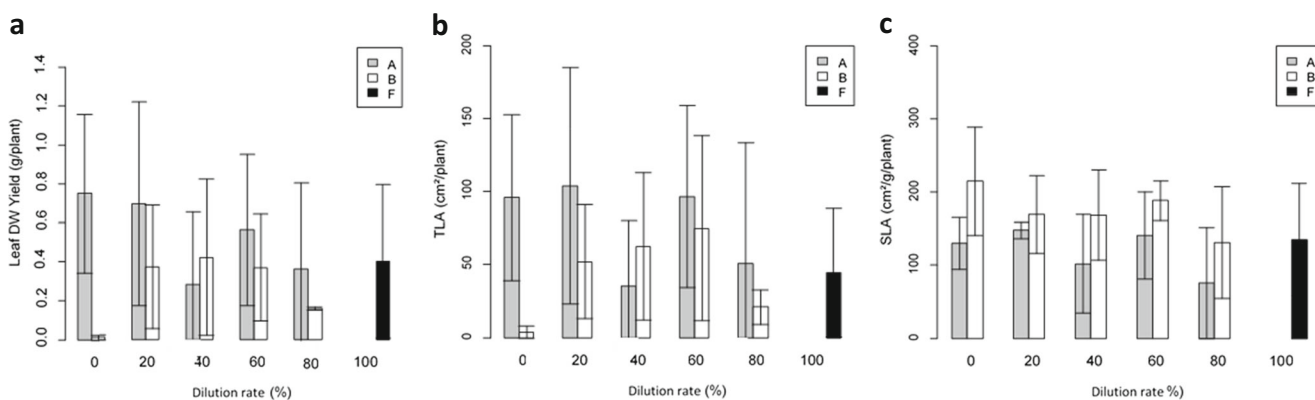

Fv/Fm (A)

Fv/Fm (B)
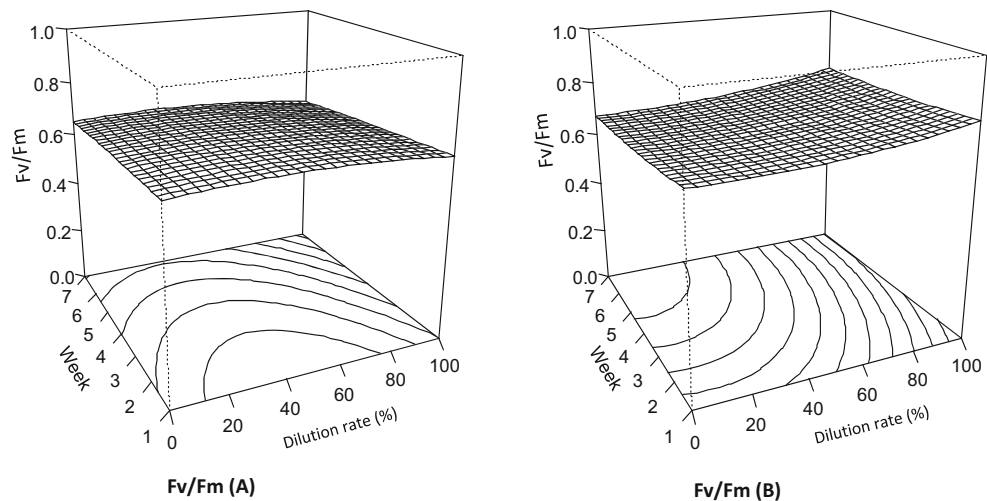
Fig. 4 **a** Leaf DW yield (g), **b** total leaf area (cm^2 plant^{-1}), and **c** specific leaf area (cm^2 g^{-1}) of *Ranunculus acris* for the soil series A and B and a 50-day growth period (*n*=4, except for B (80 %) *n*=2)

with the progressive addition of soil F. Decrease in soluble P concentration may reflect sorption of phosphates on hydrous Fe oxides and clay as for arsenates and molybdates. Iron and Mn (hydr)oxides can react by sorption and co-precipitation with cations, e.g., Zn, Cd, Cu, and Ni, and anions, e.g., arsenates (As(V)) (Violante et al. 2010), molybdates (Caporale et al. 2011), and phosphates (Yan et al. 2010). Such pH-dependent reactions may simultaneously occur with oxyanions and cations as soil pH was near 7 in the soil series A. Total soil Na and K were similar in the soils F and A, and decreases in soluble Na and K concentrations may mirror the increase in soil CEC. Metal mobilization by dissolved organic compounds is reported for Zn, Cr, and Cu (Alvim Ferraz and Lourenço 2000), Ni (Tipping et al. 1998; Wells et al. 1998),

and As (Kalbitz and Wennrich 1998). Due to gradual increase in soil OM derived from the soil F, dissolved OM might raise in the soil-pore water, but its potential effect was not evidenced here on soluble As, Ni, and Cu concentrations, whereas soluble Cr concentration transiently increased in the soils A (60) and A (80). Conversely, in the riverbank soil series of the Jalle d'Eysines River, displaying gradual increases in OM and clay contents but steady soil pH, total, and soluble soil Cr concentrations were negatively correlated (Marchand et al. 2014).

In the soil series B, total and soluble soil As, Cd, Mg, Mo, Zn, Na, and K concentrations were positively correlated. For Mo, this confirmed previous findings with the Jalle d'Eysines riverbank soils (Marchand et al. 2014). Increased total soil Mo as well as slightly lower OM and clay contents may explain higher soluble Mo in the soils B (80) and F. Higher hydrous Fe oxide and clay contents in the B soil series likely explain low and steady soluble As, Ni, Fe, and P concentrations compared to the soil series A. Slight soluble As increase in soils B (80) and F may be related to lower clay and OM contents. Organic matter is able to react with arsenite and arsenate, limiting As mobility through sorption (Goldberg 2002) and formation of insoluble complexes (Wang and Mulligan 2006). Conversely, dissolved organic matter (DOM) can promote As desorption from Fe/Mn (ox)hydroxides (Bauer and Blodau 2006). Increase in soluble K concentration was mainly due to higher total soil K in the soil B and, may be, to changes in soil texture from silty clay loam to silty loam. Total soil Na and Mg were faded in the soil series B and consequently soluble Na and Mg concentrations decreased. As soil OM and clay contents were high in both the soils B and F, soluble Cu concentration remained steady and lower than in the soil series A. Further investigations on the TE speciation in the solid phases and the soil-pore water, e.g., extended x-ray absorption fine structure (EXAFS), μXANES imaging, and DGT, are needed to better characterize the influence of soil texture and TE-bearing

Table 6 Two-way ANOVA (type II) for analyzing the influence of the soil series A and B (soil type and dilution rate as factors) after a 50-day exposure period on leaf dry weight yield, total leaf area (TLA), and specific leaf area (SLA) (*n*=4)

		Df	Mean sq	F value	p (>F)
Leaf DW yield	Soil type	2	0.7	2.6	0.08
	Dilution rate	4	0.4	0.7	0.61
	Soil dilution rate*	4	0.8	1.5	0.21
	Residuals	37	4.9		
TLA	Soil type	2	11,514	2.1	0.13
	Dilution rate	4	14,491	1.3	0.28
	Soil dilution rate*	4	15,333	1.4	0.25
	Residuals	43	117,848		
SLA	Soil type	2	32,158	4.3	*0.02*
	Dilution rate	4	23,484	1.6	0.07
	Soil dilution rate*	4	4174	0.3	0.8
	Residuals	35	130,925		

The value in italics is statistically significant (*$p<0.05$)

Df degree of freedom, *Mean Sq* mean square value, *p* p-value

phases on TE exposure in the studied soil series (Davison and Zhang 2012; Grafe et al. 2014).

Relationships between the foliar ionome, morphological traits, and indicators of soil TE exposures

The soil fading did not significantly affect the leaf DW yield, the TLA, and the SLA of *R. acris* on the 50-day exposure period for both soil series A and B. Thus, a dilution effect on foliar TE concentrations due to changes in leaf biomass across each soil series was unlikely. Similarly, PSII maximum efficiency of *R. acris* was not impacted by the gradual soil TE contamination. Indeed, photosystem II activity and foliar traits of *R. acris* gave coinciding responses for each soil series studied.

In plant photosynthesis, Zn is involved in the catalytic function of the chloroplastic b-carbonic anhydrase (b-CA) enzyme, which rapidly inter-converts CO_2 and H_2O into HCO_3 ions. Additionally, Zn-finger proteins regulating transcription through site-specific interactions play a role in photosynthesis (Yruela 2013). Under high Zn exposure, a decline in the photosynthetic function may occur (Cambrollé et al. 2012). Foliar Zn concentrations of *R. acris* were in compliance with common concentrations in aboveground plant parts, i.e., 20–100 mg kg^{-1} DW (Blum et al. 2012) and slightly higher than those of this species along the Jalle d'Eysines river (37–55 m kg^{-1}, Marchand et al. 2014) due to a higher Zn exposure (Fig. 5). Phytotoxicity symptoms usually become visible at foliar Zn concentration over 300 mg kg^{-1} DW (Marschner 2011). Stress-enzyme activities however indicate early phytotoxic effect in leaf dwarf bean when 100 mg Zn kg^{-1} DW is exceeded (Mench et al. 2000). This upper critical threshold

value was surpassed in *R. acris* from the soils A (80), B (80), and F. Indeed, 2-week-old beans grown on the soil F showed a slight decrease of the root biomass and increased activities of guaiacol peroxidase, isocitrate dehydrogenase, and malic enzyme in primary leaves, ranking this soil as slightly phytotoxic (JO Janssen, Hasselt University, personal communication). Here, photosystem II activity was not affected, as well as leaf growth parameters, and the foliar ionome did not evidence antagonism with Zn. For both soil series A and B, the foliar Zn concentrations of *R. acris* did not reflect the total Zn concentrations in the soil and the soil-pore water. This confirmed previous findings suggesting *R. acris* as a Zn excluder storing more Zn in its belowground parts (Marchand et al. 2014).

Cadmium is a non-essential element for plants, easily taken up by roots, likely following the Zn and Fe uptake pathways (Hassan and Aarts 2011). It is also transported through membranes by the natural resistance associated macrophage protein (NRAMP) family (Pottier et al. 2014). Phytotoxic effects of excess Cd are dose-dependent (Semane et al. 2010; Lin and Aarts 2012; D'Alessandro et al. 2013). Foliar Cd concentrations of *R. acris*, i.e., 0.1–0.3 mg Cd kg^{-1}, were very low compared to the phytotoxic range (5–700 mg kg^{-1}, Chaney 1989), similar to those for *R. acris* sampled along the Jalle d'Eysines River (0.1–0.3 mg Cd kg^{-1}, Marchand et al. 2014), and in line with common Cd concentrations in aerial plant parts (0.05–0.5 mg kg^{-1}, Blum et al. 2012). Bonanno (2013) reported Cd concentrations in roots, stems and leaves of *Typha domingensis* Pers., *P. australis* (Cav.) Trin. ex Steud., and *Arundo donax* L. below 0.1 mg kg^{-1} DW, suggesting macrophytes act as Cd excluders. Conversely, Marchand et al. (2014) found higher foliar Cd concentrations, e.g., up to
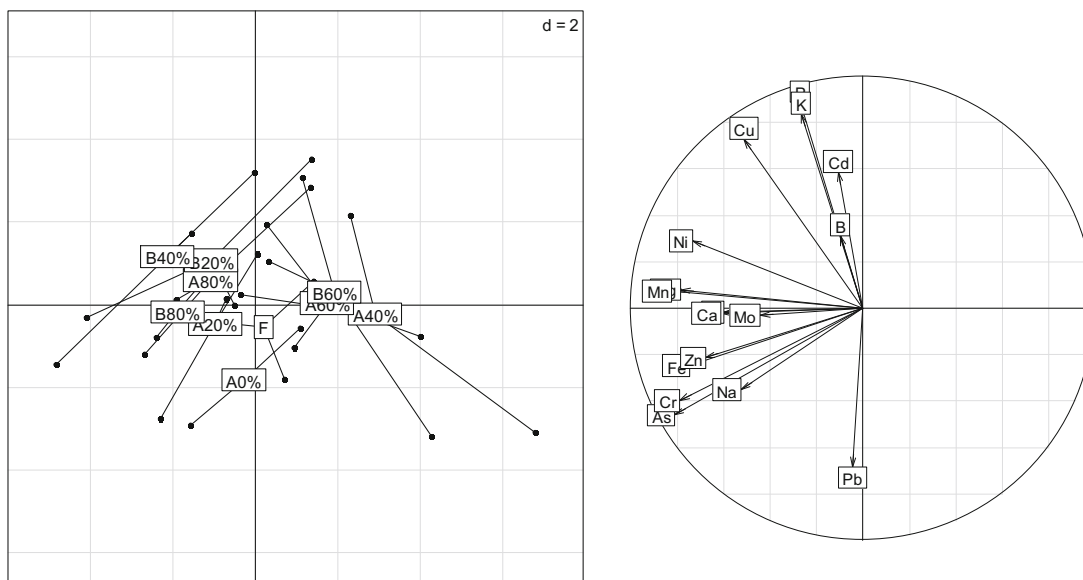

Fig. 5 Principal component analysis (PCA) on foliar Al, As, B, Cd, Ca, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, K, Na, and Zn concentrations of *R. acris* grown on the soil series A and B (PC1, 33.9 %; PC2, 21.2 %)

1.13 mg kg^{-1} in leaves of *Iris pseudacorus* L. The interspecific diversity, but also the development stage of macrophytes, shoot biomass, and sampling time, in line with soil physico-chemical and microbial conditions are key factors for foliar Cd concentrations and may explain such variations (Wu et al. 2013). Our results confirmed the lack of relationship between foliar Cd concentration of *R. acris* and soluble Cd concentration in the soils. This plant species was likely a Cd excluder in both soil series A and B and not a relevant biomonitor for both total and soluble soil Cd.

Soluble Ni concentrations in the soil series A, i.e., 2.9–15.9 μg L^{-1} slightly surpassed the values for the Jalle d'Eysines riverbank soils, i.e., 3.1–5.3 μg L^{-1} (Marchand et al. 2014), but foliar Ni concentrations in this pot experiment were at least 3-fold lower compared to those for *R. acris* leaves collected along the Jalle d'Eysines riverbanks, i.e., 1.2–3.3 mg kg^{-1} (Marchand et al. 2014). No correlation was found between Ni concentrations in *R. acris* leaves, the soil and the soil-pore water, which disagreed with Marchand et al. (2014). Similarly, soluble Cu concentrations in the soil series A, i.e., 11.1–31.2 μg L^{-1} were slightly higher than the values for the Jalle d'Eysines riverbank soils, i.e., 11.1–19.6 μg L^{-1}, but foliar Cu concentrations of *R. acris* grown on the soil series A were 2-fold lower compared to those sampled on the Jalle d'Eysines riverbanks, i.e., 15.8–16.9 mg kg^{-1} (Marchand et al. 2014). The lack of correlation between foliar Cu concentration of *R. acris* and total and soluble soil Cu in both soil series A and B confirmed previous findings (Marchand et al. 2014).

Foliar Na concentrations of *R. acris* were positively correlated with both total and soluble soil Na for the soil series A and B. Similarly, foliar Mo concentrations were positively correlated with both total and soluble soil Mo for the soil series B, but negatively for the more sandy soil series A. A bias resulting from the high variability of foliar concentrations on this soil series prevented to clearly rule on the relationship between the Mo concentration in the soil and the pore water and the leaf Mo content. In line with Marchand et al. (2014) reporting no significant correlation between total and soluble soil Mo and the foliar Mo concentration of *R. acris*, this plant species would not be relevant for biomonitoring Mo without accounting for the soil type.

Even for the soil F with high TE contamination, photosystem II activity and leaf morphological parameters did not indicate deleterious effects on *R. acris* growth. This agreed with previous findings showing no major adverse effects of this soil on the abundance and diversity of the springtail communities, no deleterious effect on the soil bacterial communities and a vegetation cover close to 100 % in plots at the PHYTOSED Scale 1-platform (Bert et al. 2012). Plant assays accounting for stress-enzyme activities may however be relevant to detect early phytotoxic effects if any in the soil series A and B (Kumpiene et al. 2014).

Conclusions

Relationships between total TE concentrations in the soil and soil-pore water, foliar ionome of *R. acris* L., its photosystem II activity and foliar morphological traits were assessed for a sandy (A) and a silty clay loam (B) soil series obtained by fading a TE-contaminated technosol developed on dredged sediments (soil F) by two riverbank soils. The TE concentrations in soil-pore water of both soil series did not reflect the gradual increase in soil TE contamination. For Zn, Cu, and Cd, the partition coefficient Kd between the soil and the soil-pore water was influenced by the soil series texture. Increases in total soil TE did not affect leaf DW yield, total and specific leaf areas, and the photosystem II activity of *R. acris* over a 50-day exposure period. The foliar ionome of *R. acris* was not effective for biomonitoring total and soluble element concentrations in both soil series A and B, except for Na. Such results highlight that total TE concentration in the soil but also (1) the soil texture, (2) its physico-chemical parameters, (3) TE chemical speciation, and (4) the plant species strategy implemented for facing a TE exposure are key factors when selecting a macrophyte for biomonitoring TE exposure in a riverbank soil. Additional parameters such as (5) the development stage of the plant, (6) the intraspecific variability as a driver of TE tolerance and TE transfer to aerial parts in macrophytes, (7) the bacterial and fungal communities in the soil, and the soil-pore water and the plants (e.g., endophytic bacteria) should be considered in further investigations. Such set of drivers may limit the use of rooted macrophytes as relevant biomonitors of TE exposure.

Compliance with ethical standards The manuscript has not been submitted to more than one journal for simultaneous consideration.

The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (please provide transparency on the re-use of material to avoid the hint of text-recycling ("self-plagiarism")).

A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g., "salami-publishing").

No data have been fabricated or manipulated (including images) to support your conclusions

No data, text, or theories by others are presented as if they were the author's own ("plagiarism"). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for

verbatim copying of material, and permissions are secured for material that is copyrighted.

Consent to submit has been received explicitly from all co-authors, as well as from the responsible authorities—tacitly or explicitly—at the institute/organization where the work has been carried out before the work is submitted.

Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

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