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Alma Heckenroth [a, b], Jacques Rabier [a], Thierry Dutoit [a], Franck Torre [a], Pascale Prudent [c], Isabelle Laffont-Schwob [a, *]

[a] *Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France*
[b] *ORTEC OGD — Ortec Générale de Dépollution, Parc de Pichaury, 550 rue Pierre Berthier, CS 80348, 13799 Aix en Provence Cedex 3, France*
[c] *Aix Marseille Univ, CNRS, LCE, Marseille, France*

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A B S T R A C T

The aim of this study was to develop an effective and non-destructive method for the selection of native Mediterranean plants with phytoremediation potential based on their spontaneous recovery capacities. The study site consisted in a mixed contaminated soils (As, Cu, Pb, Sb, Zn) in the vicinity of a former lead smelting factory abandoned since 1925 in the Calanques National Park (Marseille, southeastern France).

We developed an integrated characterization approach that takes into account topsoil metal(loid)s (MM) contamination, plant community composition and structure and mesologic parameters without using destructive methods. From a statistical selection of significant environmental descriptors, plant communities were described and interpreted as the result of spontaneous recovery under multiple stresses and local conditions (both natural and anthropogenic). We collected phytoecological and MM topsoil data using field monitoring and geographic information system (GIS) on a pollution hotspot where natural plant communities occur.

The results of the multivariate analysis performed between species and descriptors indicated that a century of MM pollution pressure produced a significant correlation with plant community dynamics in terms of composition, diversity and structure, leading to the co-occurrence of different plant succession stages. Thus, these successions seemed linked to the variability of anthropogenic disturbance regimes within the study site.

We recorded high topsoil contamination heterogeneity at the scale both of the plot and of the whole study area that suggested a heterogeneous MM distribution pattern dependent on the source of contaminants and site environmental variability. We identified 4 spontaneous plant communities co-occurring through a MM contamination gradient that could be used later from degraded to reference communities to define ecological restoration target combined to phytoremediation applications with respect to local conditions. Our results suggested that some of the native plant species such as *Coronilla juncea* and *Globularia alypum* might be tolerant to high mixed MM soil concentrations and they could thus be used for phytostabilization purposes in polluted Mediterranean areas in regard to their life-traits.

Our non-destructive methodology led both to the selection of tolerant native plant species and communities and identification of highly polluted priority intervention areas through the study site where phytostabilization should be implemented. Furthermore, by analyzing succession dynamics linked to contamination patterns throughout the area and spontaneous recovery of native tolerant vegetation, our methodology opens up broad perspectives and research fields for ecological restoration for Mediterranean protected and contaminated areas based on ecosystem trajectories and new approaches for the integrative management of polluted soils.

1. Introduction

Metals and metalloids (MM) have been spread worldwide by human activities, contaminating industrial, urban and natural soils in the vicinity of metallurgical industry areas (Alkorta et al., 2004; Khan et al., 2008; Wuana and Okieimen, 2011). Such soils with high levels of MM increase the physico-chemical pressure on the environment that often leads to sparse vegetation and extensive surface areas of bare soil. Wind and water erosion is consequently more extensive on these soils that can be transformed into sources of pollution for surrounding environments (Affholder et al., 2014; Testiati et al., 2013; Wong, 2003), increasing the risks of pollution exposure. As an alternative to conventional soil reclamation techniques such as excavation or containment, phytotechnologies and more specifically phytostabilization applied to soil diffuse pollution are sustainable and environmental-friendly techniques (Ali et al., 2013) that can be used as ecological engineering methods to restore polluted ecosystems (Li, 2006; Marchiol et al., 2013; Suchkova et al., 2014). For natural contaminated soils in particular, located within protected areas where conventional techniques could not be applied without extensive or total destruction of ecosystems, phytotechnologies using local plant species represent an integrative, ecological and sustainable solution for pollution control. For this purpose, it is important to investigate the vegetation that grows spontaneously at these sites in order to select native plant species adapted to local conditions, that can be used simultaneously for phytoremediation and ecological rehabilitation purposes (to decrease soil erosion, increase biodiversity and/or functionality and/or naturalness). According to Baker et al. (2010) the tertiary metal and metalloid contaminated sites, which correspond to naturally non-metal-enriched environments that have been contaminated by industrial emissions, host tertiary plant communities able to tolerate MM toxicity selected from the local non-contaminated habitats. These plant species can also be described as pseudo-metallophytes according to their capacity to establish and grow in both non-contaminated and MM contaminated environments (Favas et al., 2014; Tordoff et al., 2000). Furthermore, in the Mediterranean semi-arid context, local plant species are able to tolerate strong water stress. These adaptations are particularly required for the ecological rehabilitation of MM polluted areas (Frérot et al., 2006; Mendez and Maier, 2008; Moreno-Jiménez et al., 2011). Moreover, within the context of protected or sensitive habitats, it is important to avoid disturbances linked to the introduction of exotic or invasive plant species in a natural environment such as those routinely used in phytoremediation projects such as *Miscanthus sp.* (Mench et al., 2009; Nsanganwimana et al., 2014). Screening the local spontaneous plant communities is thus necessary in order to understand the interactions between species, environment and disturbances through the expression of different ecological functionalities (Disante et al., 2010; Párraga-Aguado et al., 2013). These parameters are then determinant for the success of restoration projects for polluted areas.

On the Mediterranean coast of Marseille, south-eastern France, the former lead smelting factory of Escalette, which ceased activity in 1925, is a brownfield to date still left unreclaimed. Its activities strongly contributed to the mixed contamination of the surrounding area (Affholder et al., 2014; Testiati et al., 2013), which is located within the first French peri-urban National Park, founded in 2012. The particularity of this site is that within the same brownfield are included urban and natural areas combining major anthropogenic disturbances (urban and industrial pollution, trampling, erosion) and biodiversity-richness, and consequently it is the focus of major conservation issues (Affre et al., 2015). At this site, recent studies have shown the capacity of certain native plants to grow spontaneously on highly contaminated soils and their phytostabilization potential (Affholder et al., 2013, 2014; Laffont-Schwob et al., 2011; Testiati et al., 2013). These observations correspond indeed to one century of spontaneous recovery of the tolerant native plant communities after high industrial disturbances (Laffont-Schwob et al., 2016). Previous results have also highlighted MM pollution hotspots in the vicinity of the brownfield (Rabier et al., 2014), including some occurring in particular in natural areas as the result of slag and atmospheric deposits or ashes from former industrial structures such as the horizontal chimney (Affholder et al., 2013; Testiati et al., 2013). Accordingly, there is a need for more extensive screening of local plants in relation with the diversity of spontaneous plant communities, adapted to local heterogeneous environmental conditions, which may be able to persist on the land or be resilient for future phytoremediation applications.

In this study, we focused on the area which was under the influence of the smelter horizontal chimney, where both high mixed contamination and spontaneous native vegetation occur, in order to perform a precise and georeferenced characterization of this particular ecosystem. Our aim was to assess changes in vegetation structure and/or composition caused by contaminants and other mesologic parameters to highlight the most MM-tolerant native plant species throughout heterogeneous environment. We developed a protocol without using destructive methods, in conformity with the regulations regarding this protected area that consisted in X-ray fluorescence field equipment for soil MM analysis, coupled with phytoecological monitoring relative to plant distribution and cover and discriminant environmental parameters. We thus developed an integrative methodology not based on plant MM uptake but on plant distribution and assemblages, habitat characterization and life-traits. The aim was to characterize plant communities in a context of MM heavy contamination in order to identify tolerant native plants that can be used to optimize the phytostabilization potential of the vegetation (improvement of soil quality by C and N soil input, creation of habitats for microorganisms, erosion control, MM chelation) and to favor restoration of contaminated protected areas while limiting future pollution dispersion.

2. Materials and methods

2.1. Site description

The Escalette former lead smelting factory area (WGS84 coordinates: N = 43.22578°, E = 5.35059°), located on the south coast of Marseille, south-east France (Fig. 1), is a territory combining housing, brownfield, slag heaps and natural Mediterranean landscapes. The factory, which was in operation from 1851 to 1925, has to date not been reclaimed. Since 1925, MM pollution of the site and its surroundings has been directly and indirectly caused by the slag deposits in the vicinity and by dispersion of contaminants from the polluted buildings, chimney ashes and dumps by weathering and bioalteration processes (Testiati et al., 2013). The factory structure is characterized by a horizontal chimney, built along a ridge over approximately 300 m, culminating at an altitude of 140 m (Fig. 1). The horizontal smelter chimney area is one of the most contaminated sites in the area, with high As, Cu, Pb, Sb and Zn mixed-pollution (Affholder et al., 2014) due to ash deposits along the chimney flue and their dispersion into the vegetation and soils located nearby. However, from a phytomorphological point of view, this area seems very similar to the Calanques natural landscapes, characterized by a mosaic of spontaneous plant communities that constitute calcareous xero-thermophilous shrublands (Mediterranean matorral), grasslands and stands of Aleppo pine (interactive Escalette map.kml).

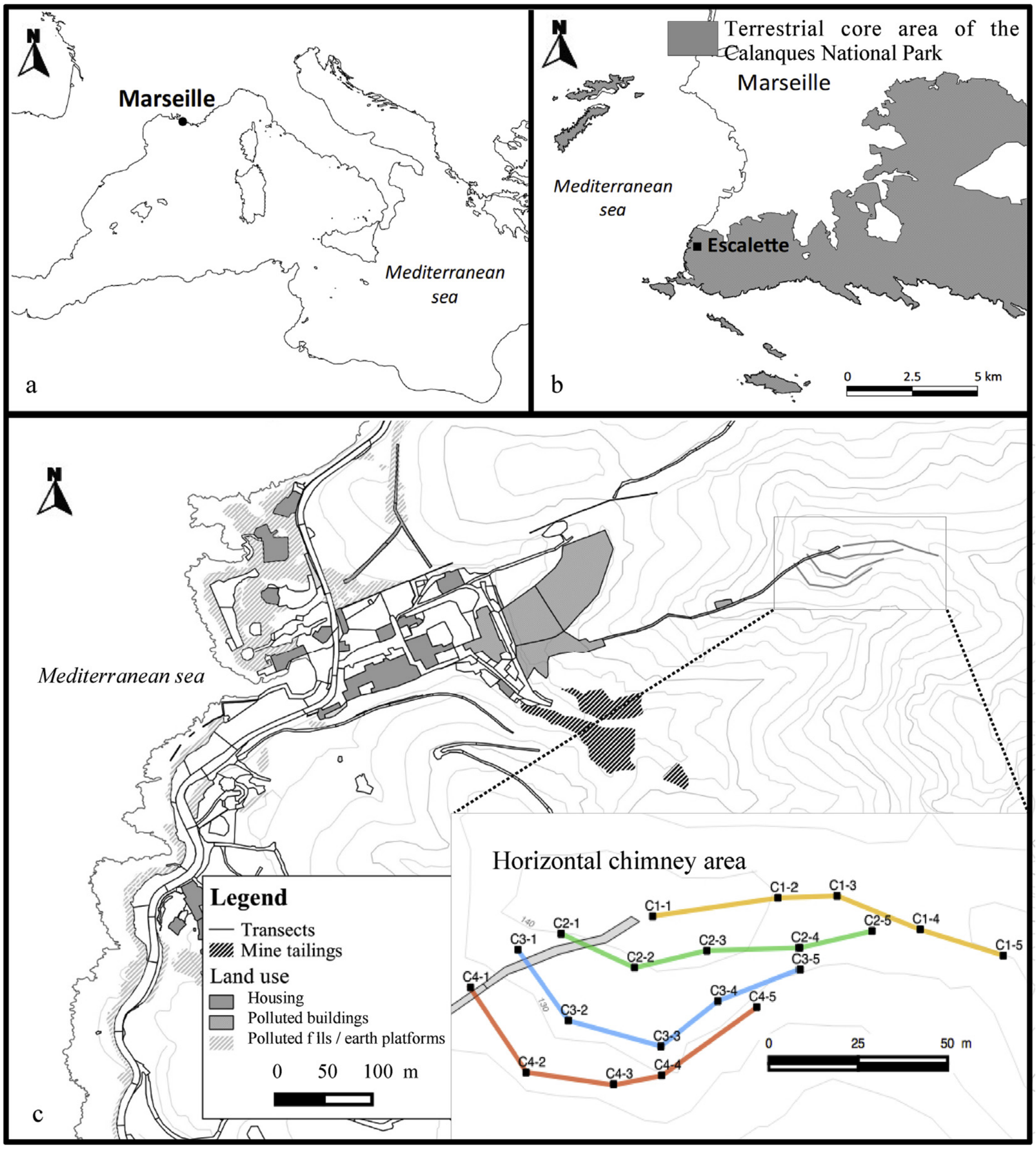

Fig. 1. Location of Escalette study site in the urban area of Marseille in the Mediterranean basin (a), within the terrestrial area of the Calanques National Park (b). Location of the experimental plots at the study site (c): transect 1 is represented by a yellow line, transect 2 by a green line, transect 3 by a blue line and transect 4 by a red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The bioclimate of this area is thermo-Mediterranean (Quézel and Médail, 2003), on the basis of thermal criteria. According to Aillaud and Crouzet (1988) this location should be considered in an intermediary position between Mediterranean semi-arid and arid area on the basis of pluviometry data recorded in the urban area of Marseille (north of the study site) and at Cap Croisette (south of the study site). Prevailing winds are north-north-easterly (Mistral) and easterly, and contribute to increase water stress, especially the Mistral which blows about 135 days a year. Substrate is dominated by karstic limestone and calcareous bare rocks are frequent. Soils are usually thin (less than 50 cm thick), oligotrophic, originating from calcareous parent material (calcosoil with surface colluvia), and ranging from leptosols to cambisols.

2.2. Sampling design

Soil and plant sampling conducted in previous studies in the vicinity of the former factory highlighted the horizontal chimney area (Fig. 1) as a pollution hotspot (Affholder et al., 2014; Guillamot et al., 2014; Testiati et al., 2013). This area was also colonized by spontaneous vegetation and was thus selected in this study for phytoecological and soil MM monitoring. We considered that the major sources of pollution in this zone, identified as the pipe and the outlet of the horizontal chimney, could generate a pollution gradient through the area. We therefore used the chimney pipe and outlet as reference points for the monitoring transect design and used 4 transects of 100 m length from the chimney ruins, located on contour lines, in order to take into account the effect of topography on contaminant dispersion by runoff (Fig. 1). Preliminary floristic inventories were conducted in spring 2012 (results not shown) to define the minimum surface area by using successively larger size sampling areas (Cain, 1938). Testiati and collaborators (2013) showed that the soil Pb contamination varied between 12.8 g/kg and 130 g/kg on a 50 m transect located on both sides of the chimney. Thus, a compromise was decided on in order to take into account the minimum sampling area, estimated at around 32 m^2, and the expected heterogeneity of MM topsoil contamination (Marchiol et al., 2013; Testiati et al., 2013) in order to achieve the optimum sampling plot design according to an area of steep terrain: a rectangular surface area of 32 m^2, with an 8 m length in the line of slope and 4 m width, considering that we could thus reduce the contamination heterogeneity related to the drop in altitude, compared to a square plot. We located 5 plots per transect and an identification code was assigned to every plot as follows: C1-2, with 1 coding for transect number 1, and 2 for plot number 2 (Fig. 1). It should be noted that it was impossible to select, at local scale (Escalette) and even at the scale of the whole massif of the Calanques, a control site free of any contamination (Affholder, 2013) where our experimental design could have been replicated. The selection of a distant control site in another of the peri-urban hills of Marseille could have generated some bias because of significant changes in the environmental parameters (slope, exposure, bedrock, soil, local climate, etc.), land use history (former traditional land uses) which may have changed the composition and spatial and temporal dynamics of the present vegetation in comparison with our study site (Knoerr, 1959).

2.3. Phytoecological sampling

Phytoecological inventories were conducted over the whole surface area of the plots using the 'strata' protocol from the BASECO model for floristic and mesologic data (Gachet et al., 2005). Plant species were determined using the Fournier (1961), Pavon (2014) and Rameau et al. (2008) flora. Compared to the model, the record sheet was adapted to the field characteristics by adding new classes of strata. The covering percentage of rock, litter and each vegetation stratum then each plant species were recorded using 6 coefficients coding for a semi-quantitative visual estimate of cover (0: cover = 0%, 1: cover < 10%, 2: cover = 10%–25%, 3: cover = 25%–50%, 4: cover = 50%–75%, 5: cover = > 75%) (Table 1). Because of the observation of several qualitative classes for stone cover (rocks, blocks, stones) and vegetation vertical structure (herbaceous, low shrubs, shrubs, high shrubs, trees), a synthetic index was calculated to take into account both cover and class diversity of these two variables (Table 1) for statistical analysis:Index $= n\sum_{i=1}^{n} Ci$ where n is the number of represented classes within a sampling plot and C the coefficient score for each class i (Table 1). Soil depth was measured at the center (n = 1) and at each corner of the plot (n = 4) after removal of surface stones and litter and using a steel rod and a hammer until we reached the rock. The value conserved for further analyses corresponds to the mean depth of the plot (n = 5). Species richness corresponded to the number of plant species per plot and Shannon Index (Table 1) was calculated following the formula $H' = -\sum[p_i \times \log(p_i)]$ where $p_i = n_i/N$ and n_i = abundance of species i and N = total abundance of all species using the function "diversity" of R 3.0.2 vegan package (Borcard et al., 2011; Oksanen et al., 2014). We also calculated Pielou's evenness index J (Pielou, 1969) from Shannon-Weaver H' and species richness S: $J = H'/\log(S)$, as suggested by Oksanen and O'Hara in vegan package (function diversity, R documentation, Oksanen et al., 2014). These indices were used as descriptive (Table 1) but not as explanatory variables for multivariate analysis as they were calculated from species data.

2.4. Soil analysis

We performed soil MM analyses in the field using a portable X-Ray Fluorescence (pXRF) analyzer (Niton® XLt 792W, Thermo Scientific) with the probe placed directly on the soil surface (Table 1). Use of this non-destructive field apparatus is of particular interest in the context of a protected natural park where samples of organic and inorganic materials are strictly controlled. Moreover, pXRF analysers are recognized as a way of measuring soil contaminants as they offer rapid and simultaneous multi-elemental analysis in solid conditions with minimum sample treatments and with suitable limits of detection (Horta et al., 2015; Martin-Peinado et al., 2010; Paulette et al., 2015; Weindorf et al., 2013). In order to take into account the wide heterogeneity of MM surface soil contamination (e.g.: Sb soil concentrations ranging from 1280 to 9030 mg/kg in a transect of 50 m on either side of the damaged chimney (Testiati et al., 2013)), we measured MM at 24 points per plot, each 1 m-distant each and 0.5 m from the plot edge. At each point, stones and litter were removed to perform analysis on bare topsoil samples (5 cm max deep) and to reduce heterogeneity under field conditions. The pXRF analyses were performed during 120 s per

Table 1
Descriptive statistics of environmental and pollution variables extracted from field sampling and GIS data (GIS: Geographic Information System, pXRF: portable X-Ray Fluorescence).

Environmental variables	Units	n	Minimum	Maximum	Mean	Median	SD	Data sources
Species richness (S)	(–)	24	7	25	19	19	4	Sampling
Shannon Diversity Index (H')	(–)	24	1.78	3.18	2.83	2.88	0,30	Sampling
Pielou Evenness Index (J)	(–)	24	0.92	0.99	0.98	0.98	0.01	Sampling
Soil depth	cm	24	0	41	14	12	11	Sampling
Light intensity index	(–)	24	2.5	4.0	3.5	3.5	1	Sampling
Stones cover index	(–)	24	8	24	19	21	5	Sampling
Litter cover	coef.	24	2	5	3.0	3	1	Sampling
Vegetation cover	coef.	24	3	5	3.4	3	0.7	Sampling
Vertical structure index	(–)	24	18	60	30	26	13	Sampling
Altitude	m	24	122	140	133	132	6	GIS
Slope	%	24	0	42	23	24	11	GIS
Chimney nearest distance	m	24	2	102	35	33	26	GIS
Chimney outlet distance	m	24	5	103	44	42	22	GIS
Chimney mean distance	m	24	4	103	39	38	23	GIS
Chimney-plot height difference	m	24	−3	15	5	6	5	GIS
Metals and metalloids in soils								
Arsenic	mg/kg	467	86	72,473	6069	8998	2876	pXRF
Copper	mg/kg	467	90	3089	616	346	523	pXRF
Iron	mg/kg	467	3120	166,704	26,330	12,512	24,628	pXRF
Lead	mg/kg	467	461	272,877	27,480	34,824	15,138	pXRF
Antimony	mg/kg	467	0	18,988	1557	2437	670	pXRF
Tin	mg/kg	467	0	4446	451	605	253	pXRF
Zinc	mg/kg	467	233	142,723	11,262	16,469	5554	pXRF

point, which corresponded to a maximum measurement time for this equipment and reduced the detection limits as far as possible (Kalnicky and Singhvi, 2001).

2.5. Geographic information system data

After field monitoring, additional variables were added to the plot data using Quantum GIS versions 1.8.0 Lisboa and 2.2 Valmiera completed by GRASS open source software. We used GIS to create a local Digital Elevation Model (DEM) in order to extract altitude and slope data at the center of the sampling plots (Table 1). We calculated precise distances between plot centers and different presumed sources of MM pollution or anthropogenic disturbances. These parameters, such as nearest distance to the horizontal chimney or distance from the chimney outlet (Table 1), were used to estimate the plot spatial location through the expected contamination gradients represented by topsoil MM concentrations.

2.6. Statistical analysis

For each MM XRF sampling point, equipment relative error was calculated to obtain a mean value of the relative error for each element based on all the measurements. We only kept MM concentrations reported in Table 1 for which the equipment mean relative error was $\leq$20% (results not shown), as this value corresponds to the precision required to consider quantitative results for field environmental applications (Kalnicky and Singhvi, 2001). Thereafter, means, medians, standard deviations of selected MM soil concentrations per plot ($n = 24$, except when missing data) were used for further statistical treatments. We calculated the MM dispersion ranges of each experimental plot using JMP (JMP Pro 10) to characterize the contamination heterogeneity within the study area.

We performed linear regression and multivariate analyses using R 3.0.2. software (R Core Team, 2014) along with stats, vegan and ade4 packages (Dray et al., 2007; Oksanen et al., 2014). As a first step, explanatory variables were treated separately: we computed environmental data (Table 1) in a canonical constrained ordination for forward selection of the relevant explanatory variables using the ordistep function (Blanchet et al., 2008) (Table 2). All the MM previously selected were retained for the second step since identified as relevant industrial contamination descriptors (Table 2). We also calculated Spearman correlation coefficients *r* from the environmental variables to estimate variables redundancy and help with their selection (results not shown). A principal component analysis (PCA) was performed on these variables to obtain a graphical representation of variable correlations.

In order to avoid the rare plant species to have too much influence on the canonical analysis, we used a Hellinger transformation on the species dataset (Borcard et al., 2011; Legendre and Gallagher, 2001). Then, we used a redundancy analysis (RDA) on all selected variables (both environmental and MM data) compiled within the same table to describe relationships between plant species composition and environmental and pollution descriptors observed at the scale of the sampling plots. We used permutation tests to test the significance of RDA results and canonical axes ($\alpha = 0.001$) (Legendre et al., 2011). K-means partitioning (Hartigan and Wong, 1979) was used on RDA species scores to delineate clusters corresponding to groups of species on the first two principal directions. The most relevant number of groups was determined following a between-group variance criterium as proposed by Brewer et al. (2007).

We calculated contamination factors (CF) from topsoil MM concentrations measured in this study and the results obtained by Affholder et al. (2013, 2014) for the local background (CF = mean MM topsoil concentration on a plot/mean MM topsoil concentration of the local background). Then, these CF were used to estimate a multi-contamination level corresponding to a pollution load index $\left(PLI = \sqrt[5]{CF_{As} \times CF_{Cu} \times CF_{Pb} \times CF_{Sb} \times CF_{Zn}}\right)$ (Rashed, 2010).

3. Results

3.1. Metal and metalloid distribution

For each element, the results showed a wide heterogeneity of the topsoil MM concentrations, both within each plot and between plots (Fig. 2). This variability was greater on plots where the highest MM mean concentrations occurred. We recorded a significant decrease of As, Cu, Pb, Sb, Sn and Zn concentrations with increasing distance from the chimney (p-value < 0.01, Table 3). Nevertheless, as there was no significant linear regression between contamination and position of the plots along the transect (results not shown), we considered plots as independent replicates for further interpretation.

When looking closely to spatial representation of As and Pb classes of mean concentrations by plot, values appeared greater when closer to the chimney (Fig. 3). Because of the high variability within the plots, these differences were only significant when looking at plots C1-1, C2-1 and C2-2, which are more contaminated in As than C1-2 to C1-5, C2-4 to C2-5, C3-5, C4-2 to C4-3 ($p < 0.01$, supplementary material 1). On the basis of Pb concentrations, C1-1, C2-1 and C2-2 were more contaminated than C1-2 to C1-5, C2-4 to C2-5, C3-5 and C4-3 ($p < 0.01$, supplementary material 1). These tendencies are quite similar for the other industrial MM (supplementary material 1). Considering As, Cu, Pb, Sb and Sn (Fig. 2), the hotspot of pollution was identified as plot C2-2 which is the closest plot to chimney outlet to be located downstream (Figs. 1 and 3) and showed significantly higher values for these elements compared to all the other plots ($p < 0.01$, supplementary material 1). For Fe as for Zn, C2-2 mean concentrations were not significantly different than those recorded in C1-1 and C3-3 which were respectively located upstream and downstream of C2-2 (Fig. 3, supplementary material 1). On the upmost transect (C1), the most contaminated plot C1-1 considering all recorded MM corresponds to the creeping

Table 2
List of the variables successively retained by forward selection (999 permutations) based on Akaike Information Criterion (Akaike, 1973). ***, **,* respectively coding for p-value $\leq$ 0.001, 0.01 and 0.05). AIC: Akaike Information Criterion. F: F-test value.

Explanatory variables for RDA	AIC	F	p-value	Abbreviation	Decision
Chimney distance	−18.42	2.79	0.001***	Chimney_D	selected
Chimney distance + Vertical structure	−19.04	2.37	0.001***	Veg_struct	selected
Chimney distance + Vertical structure + Stone cover	−19.32	1.93	0.004**	Stones_cov	selected
Chimney distance + Vertical structure + Stone cover + Altitude	−19.44	1.67	0.018*	Alt	selected
Chimney distance + Vertical structure + Stone cover + Altitude + Arsenic	*−19.37*	*1.42*	*0.086*	*As*	*excluded*

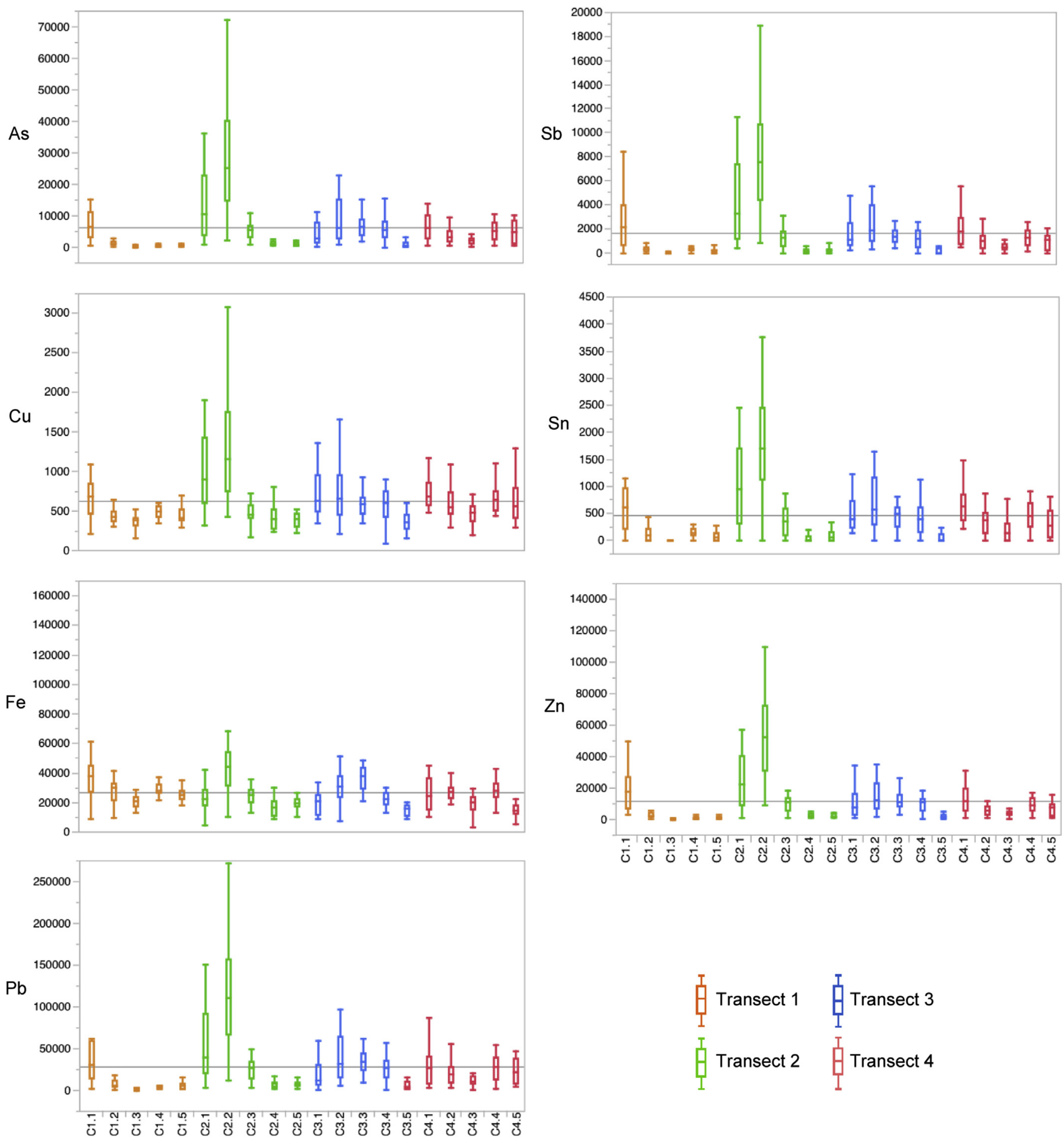

Fig. 2. Boxplots of soil MM distribution ranges within the experimental plots. Each transect is represented by a different color (C1: orange, C2: green, C3: blue, C4: red). MM concentrations are given in mg/kg. Mean, 1st and 3rd quartiles were used to build the boxes. The vertical line within the box represents the median sample value. The ends of the box represent the 3rd and 1st quartile, respectively. The whiskers are determined by the upper and lower data point values (not including outliers). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chimney outlet ($p < 0.01$, supplementary material 1). Then, the MM concentrations were significantly lower on transect C1 from the second plot. The same pattern were observed on C2 for the plots located below the ridge, corresponding to C2-3 to C2-5 that showed significantly lower concentrations, except for Fe, compared to C2-1 and C2-2 ($p < 0.01$, supplementary material 1). C3-5 was significantly less contaminated in Pb and Sb than the other plots on transect C3. Considering the location of the plots from upstream to downstream below the chimney outlet (Fig. 3), there were no significant difference of As and Pb contamination between C2-3, C3-1 to C3-4, C4-1 to C4-2 and C4-3 to C4-4 ($p < 0.01$, supplementary material 1).

Table 3
Coefficients of the linear regressions performed between MM and distance from the chimney (Chimney_D data). Only significant results are presented.

MM	Multiple R-squared	Adjusted R-squared	*p-value*
As	0.39	0.36	*0.003*
Cu	0.40	0.37	*0.003*
Pb	0.39	0.36	*0.003*
Sb	0.41	0.38	*0.002*
Sn	0.44	0.41	*0.001*
Zn	0.37	0.33	*0.004*

3.2. *Environmental variables*

The two first axes of the PCA of environmental variables were considered. PCA1 and PCA2 explained respectively 46% and 14% of the total variance. On the first axis of the PCA correlation circle (Fig. 4), the industrial MM markers As, Cu, Pb, Sb, Sn and Zn (positive part of axis 1) were highly correlated between them and negatively correlated with increasing distances from the chimney (negative part of axis 1). The graphic representation also highlighted the positive correlation existing between Cu and As, Pb, Sb, Sn and Zn ($0.87 \leq r \leq 0.96$, p-value < 0.001). On the positive part of axis 2, the vertical structure of vegetation seemed to be correlated with slope, litter and vegetation cover, as indicators of the closing of the vegetation by scrubs and trees. When looking at Spearman coefficients, it appeared that this correlation was only significant for vertical structure and litter ($r = 0.54$, p-value < 0.05).

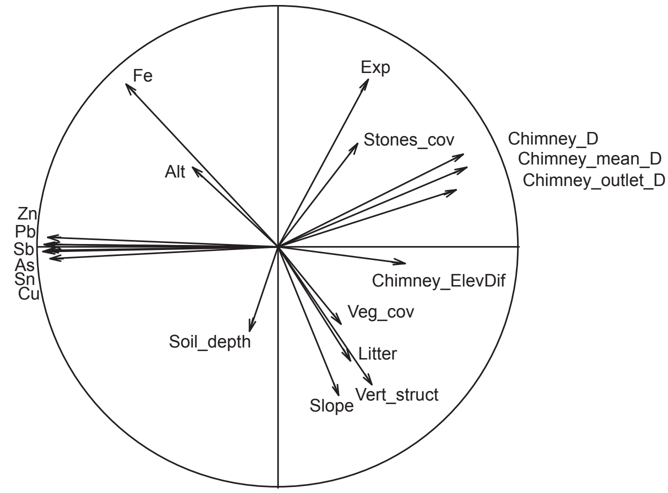

Fig. 4. Correlation circle of the PCA performed on environmental variables (Cumulative projected inertia = 60% on the two first axes). Alt: Altitude, Chimney_D: chimney-plot nearest distance, Chimney_mean_D: chimney-plot mean distance, Chimney_outlet_D: chimney outlet-plot nearest distance, Chimney_ElevDif: chimney-plot height difference, Exp: Light Intensity Index, Litter: Litter cover, Stones_cov: stone cover, Veg_cov: plant cover, Vert_struc: Vertical plant structure.

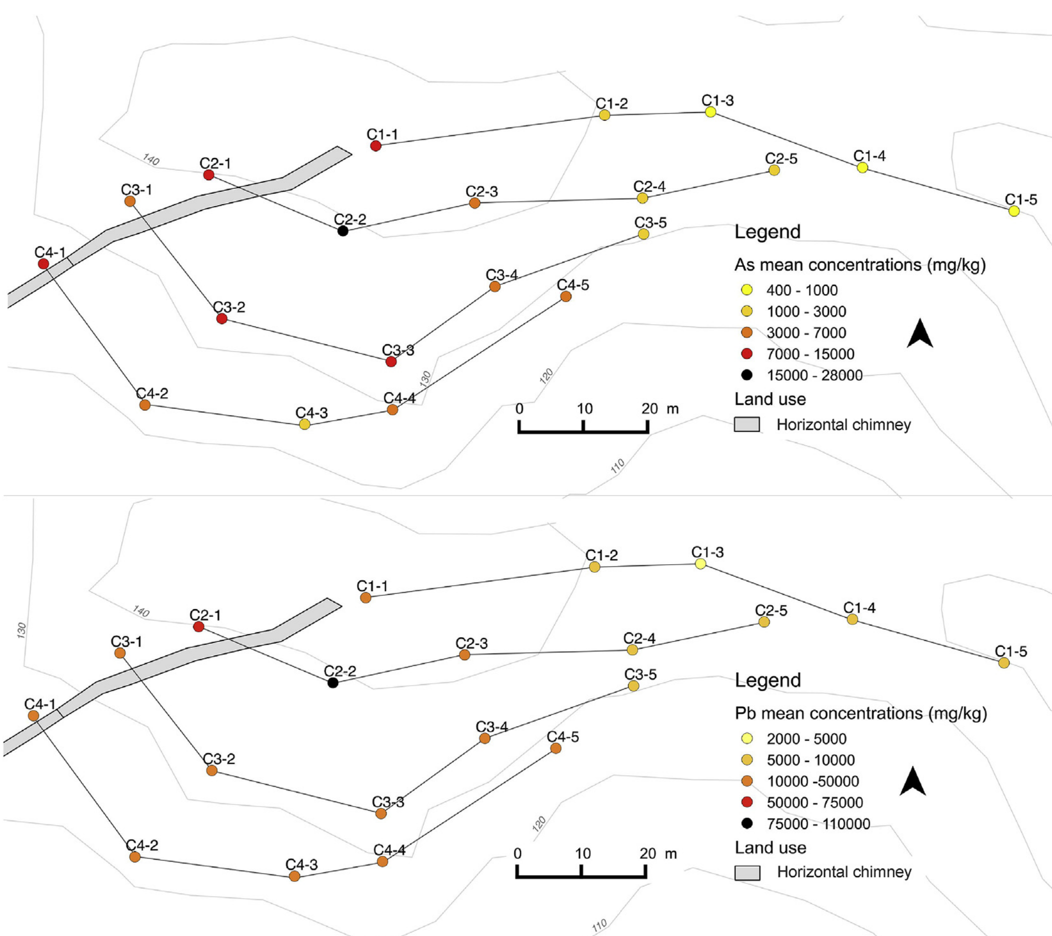

Fig. 3. Spatial distribution of lead (Pb) and arsenic (As) mean soil concentrations (n = 24).

3.3. *Vegetation composition and structure in relation with environmental variables and MM contamination*

The RDA analysis showed a statistically significant relation between selected descriptors (Table 2) and vegetation community (p-value of the model < 0.001) explaining 31% of the total variance on the two first canonical axes (with RDA1 and RDA2 explaining respectively 17% and 14% of the total inertia; p-value of the axis $= 0.004$ and 0.003 respectively) (Fig. 5). *K*-means analysis highlighted four groups of species based on RDA species scores. Clusters 1, 2, 3 and 4 contained respectively 23, 7, 14 and 4 plant species (Fig. 5). The negative part of RDA axes 1 and 2 seemed to be related to the closure degree of the vegetation highlighted by the vegetation structure variable. The closest habitats are characterized by species corresponding mostly to group 4 of the cluster analysis composed by the tree species *Pinus halepensis* and shrub species as *Rhamnus alaternus*, *Asparagus acutifolius* and *Smilax aspera* (Fig. 5 and supplementary material 2). In contrast, the positive part of axis 1 was represented by native shrubs and herbaceous species that belonged to groups 1 and 2 of the cluster analysis and characterized by higher stone cover percentage and more open habitats. Both first and second axes showed a gradient of pollution as highest MM concentrations were located on the negative part of axis 1 and the positive part of axis 2 and negatively correlated with greater distance from the chimney ($-0.91 \leq r \leq -0.81$; p-value < 0.001), which appeared clearly as the principal source of MM (Fig. 5). Thus, species scores along these axes could be used to classify them according to their tolerance to high levels of pollution. When taking into account the species mean scores on both axis 1 and axis 2 (results not shown), it appeared then that the native shrub species *Coronilla juncea*, *Coris monspelliensis* and *Globularia alypum* were the most positively correlated with MM concentrations. Similarly, *Avenula bromoides, Bromus madritensis*, *Linaria supina*, *Lobularia maritima*, *Lysaria arvensis*, *Reichardia picroides*, *Piptatherum caerulescens* and *Plantago lagopus* were the herbaceous species the most positively correlated with MM concentrations. The tree species *Pinus halepensis* was also among the most MM correlated species. In contrast, the species with the least representative scores were typical Matorral shrubland species such as *Allium acutiflorum*, *Cistus albidus*, *Fumana leavipes*, *Quercus coccifera*, *Rosmarinus officinalis* and *Thymus vulgaris*. These species belonged to group 2 of the cluster analysis, which seemed positively correlated with increasing distance from the pollution source.

4. Discussion

4.1. *Spatial heterogeneity of the soil contamination*

Topsoil of the site was highly contaminated by MM, as some metallic species mean values were as high as 1 to 10 percent of the soil mass (Fig. 2). In previous studies (Affholder et al., 2013, 2014) the local background of soil contamination in the Calanques massif was estimated at 5, 7.5, 43, 3 and 66 mg/kg for As, Cu, Pb, Sb and Zn, respectively (no data for Sn, Table 4). Results for the studied area clearly evidenced high contamination of soils, with mean values (Table 5) up to 1200, 80, between 500 and 600 and up to 170 times the respective local background concentration for As, Cu, Pb, Sb, and Zn, respectively. In relation to threshold values under French legislation for contaminated soils (Table 4), mean values of the study area soils were up to 209 times higher for As, up to 6 times higher for Cu, up to 275 times higher for Pb and up to 55 times higher for Zn (Table 4). In the absence of values for France, the Belgian “intervention values” for industrial soils were considered (Table 4). They are defined as the soil concentrations in the case of which an intervention will be systematically carried out. The study area soil mean concentrations were up to 20, 1.2, 20 and 9 times higher for As, Cu, Pb and Zn, respectively (Table 1). Furthermore, the PLI showed important values on the study area (Table 5). In comparison, the highest PLI obtained by Affholder et al. (2014) was 120 at the site considered then as the site contamination hotspot. These concentration levels were characteristic of the smelter activity pollution mostly caused by particular and direct ash deposits in the vicinity of the factory (Marchiol et al., 2013; Regvar et al., 2010). They highlighted the extent of the soil diffuse contamination through the natural areas in the vicinity of the Escalette former smelter (Tables 4 and 5) and the need to manage the pollution in conformity with polluted site and soil legislation.

The highest contamination levels were recorded at plots directly influenced by flying ash and contaminated materials coming from the chimney ruined pipe, according to Testiati et al. (2013). Although the results confirmed that the horizontal chimney and especially the outlet downstream area were the principal source of MM throughout the study area, leading to a metal gradient correlated with pipe distance ($p < 0.01$, Table 3), the soil contamination was very heterogeneous both at study site (5000 m^2) and plot scales (32 m^2) (Table 1 and Fig. 2). This spatial heterogeneity of the MM soil content could be linked to the variability of the topography and substrates, such as rocky outcrops providing protection against contamination in some cases or increased MM pollution by generating preferential runoff flows in others (Affholder et al., 2014). The transect lower contamination levels from plot C1-2 to C1-5 compared to C1-1 were certainly linked to the topographic situation of these plots, located along the ridge line and protected from the past and present source by a rocky outcrop corresponding to C1-1 location (Figs. 1 and 3). Furthermore, the last plots of transects C2 (from C2-3) and C3 (C3-5) showed lower levels of contamination and could thus be considered as less directly influenced by ash deposits from the chimney as they were also located below the rocky outcrop.

On the other hand, the results highlighted the plot located just down of the chimney exit (C2-2) as the most contaminated in As, Cu, Pb, Sb and Zn. Similarly, the plots located downstream from the chimney outlet showed similar high MM levels that highlighted a contamination pattern which could be linked to preferential runoff promoting MM transfers, especially for As (Vangronsveld et al., 2009). Thus, in the context of steep slope (plots mean slope $=$ 24%, Table 1) and highly polluted environment, this study could suggest an interesting approach to selecting priority areas to restore in order to reduce the runoff by the use of local native plants (De Baets et al., 2007; Durán-Zuazo et al., 2004). The distribution pattern observed was less visible for Fe, meaning that the distribution of this element could be linked both to local background, for which concentrations were estimated around 10 g kg^{-1} DM (Affholder et al., 2013), and anthropogenic sources such as industrial use of pyrites (FeS_2) and chalcopyrites ($CuFeS_2$) (Laffont-Schwob et al., 2016). In this way, it could provide additional results to use spatial and topographic analyses such as LIDAR data (Dassot et al., 2011) and GIS in order to better understand contamination patterns throughout the area by 3D representations of the MM distribution heterogeneity.

4.2. *Ecosystem recovery and metal tolerance*

Despite the heavy MM soil contamination, there was an important recovery of the local ecosystem in the form of a spontaneous colonization of the polluted study area by local native plants in a secondary plant succession series (Fig. 5, supplementary material 2). The multivariate analysis emphasized the vegetation heterogeneity at the scale of study area as well as at the smaller scale of the plot (several tens of square meters): the cluster analysis

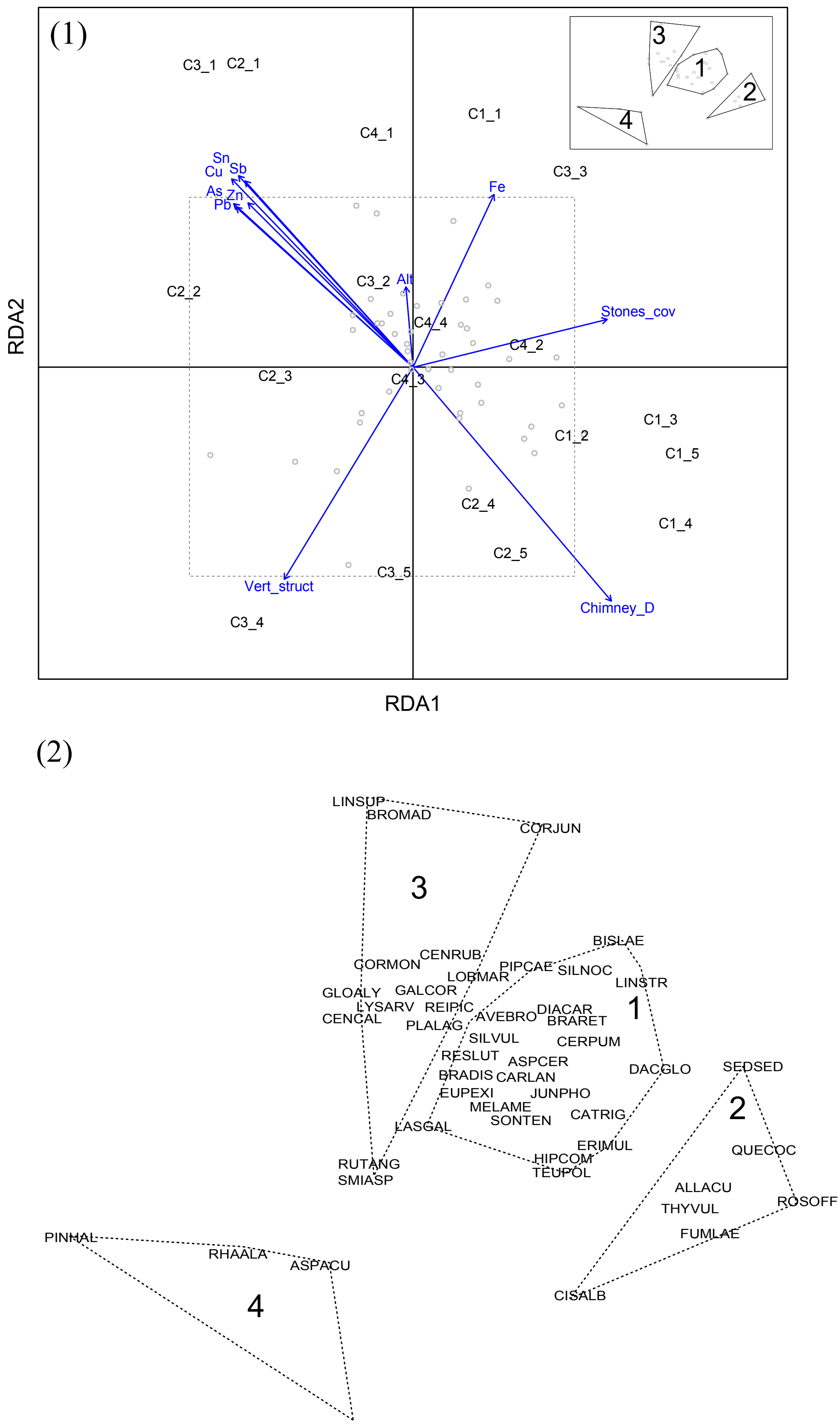

Fig. 5. (1) Triplot representation of the RDA performed on data (sites identification codes are in black, species are represented by grey points and descriptors by blue arrays). Cumulative projected inertia = 31% on the two first axes. R-adjusted square = 28%. (2) K-means clustering representation of species based on the RDA species scores on the first two axes. The choice of four groups of species appeared to express an optimized between-group variance (see procedure in Brewer et al., 2007). For species abbreviations, see the supplementary material 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
MM concentrations in soils of the study site local background (Affholder et al., 2013, 2014), reference values in French (Darmendrail et al., 2000) and Belgian soils and threshold MM soil concentrations under French and Belgian legislation (Arrêté ministeriel 08/01/1998 in Journal Officiel 26, 1998 of the French Government; Afnor NF-U-44-041, 1985; Décret relatif à la gestion des sols 08/12/2008 of Wallon Parliament). All concentration values are in mg/kg of soil dry mass (DM).

Metals and metalloids in soils (mg/kg DM)	As	Cu	Pb	Sb	Zn
Local background MM soil concentrations (n = 5)					
	5	7.5	43	3	66
MM soil concentrations reference values in France					
n	–	787	790	–	804
Total concentrations in French soils (min-max)	–	<2 to 107	2 to 3088	–	<5 to 3820
Median	–	13	34	–	80
Mean	–	15	65	–	149
Threshold values authorized in soils	29	100	100	–	300
MM soil concentrations reference values in Belgium					
Reference values in natural soils/industrial soils	12/12	14/14	25/25	–	67
Threshold values in natural soils/industrial soils	30/50	40/120	120/385	–	120/320
Intervention values in natural soils/industrial soils	220/300	80/500	170/1360	–	215/1300

Table 5
Contamination factors (CF) and pollution load index (PLI) based on As, Cu, Pb, Sb and Zn topsoil mean concentrations at the plot and local background. For min and max values of CF and PLI, the plot where these values were recorded is specified in subscript.

	CF_{As}	CF_{Cu}	CF_{Pb}	CF_{Sb}	CF_{Zn}	PLI ($n_{ETMM} = 5$)
Min	$81_{C1\text{-}3}$	$50_{C3\text{-}5}$	$56_{C1\text{-}3}$	$13_{C1\text{-}3}$	$12_{C1\text{-}3}$	$33_{C1\text{-}3}$
Moy ± SD	1228 ± 1285	82 ± 30	636 ± 575	497 ± 569	169 ± 186	340 ± 313
Max	$5647_{C2\text{-}2}$	$174_{C2\text{-}2}$	$2576_{C2\text{-}2}$	$2397_{C2\text{-}2}$	$828_{C2\text{-}2}$	$1381_{C2\text{-}2}$

performed on RDA species scores revealed 4 plant clusters characterized by plant species of different biological type (Raunkier, 1934) and strategies (Grime, 1977), that showed the variability through each community. If these results made interpretation of the vegetation occurrence through environmental gradients complex and could be an explanation for the low explanatory percentage of the redundancy analysis first axes, the four clusters could be linked to several plant communities corresponding to successional stages that developed through the study area environmental heterogeneity (Fig. 5).

Cluster 3 contained a majority of MM tolerant species based on mean species scores on axes 1 (negative part) and 2 (positive part). This group was composed of a majority of small stress-tolerant species which could correspond to a highly constrained habitat with specialized and pioneer species such as the leguminous *Coronilla juncea*. As some ubiquitous and ruderal species were also represented within this group, such as *Centranthus sp.*, *Lobularia maritima*, *Lysaria arvensis* and *Reichardia picroides*, it could be considered as a habitat highly disturbed by high levels of pollution and soil artificialization caused by the proximity of the horizontal chimney, as shown by analysis of the soil composition performed by Testiati et al. (2013). *Globularia alypum*, an early-successional stage native shrub (Maestre and Cortina, 2004), was also part of group 3 as one of the most MM tolerant shrub species. This cluster was composed both of open environment species (positive part of axis 2) and forest environment species (negative part of axis 2), depending on their position on RDA2.

Group 4 was representative of a young spontaneous forest community characterized by *Pinus halepensis*, *Rhamnus alaternus*, *Asparagus acutifolius* and *Pistacia lentiscus* from the most MM tolerant species to the least (Fig. 5). The species richness of this group was low, suggesting strong competition for light between species that could be linked to the dominant presence of *Pinus halepensis* (Maestre and Cortina, 2004).

Group 1 was composed of both herbaceous and shrub species which could represent transitional habitats between highly tolerant communities (group 3) and typical Matorral shrubland species that seemed less tolerant of MM pollution on the basis of their RDA scores (group 2). Plants of this group were mostly representative of open, heterogeneous and disturbed environments as shown by the co-presence of species with different life strategies (Gachet et al., 2005; Grime, 1977). This habitat, that contained few shrubs (*Erica multiflora*, *Juniperus phoenicea*), could thus represent a mid-successional stage towards a ruderal scrubland characterized by stress-tolerant and chamaephyte-type species such as those mainly found within group 2. As groups 3, 1 and 2 were composed both of pioneer and middle-successional species, they could correspond to a transitional stage of young scrub habitats, with group 2 corresponding to a more advanced successional stage and less disturbed habitats. We cannot exclude that the habitats close to the chimney could also have been more disturbed by human presence and disturbances as the chimney was regularly cleaned out during smelter operation. Nowadays, the chimney is still a frequented area as confirmed by the presence of numerous hunting trails, hides and cartridges throughout this area. Cluster 2 like cluster 4 could also correspond to more homogeneous and specialized habitats, as they were represented by only a few species (respectively 7 and 4). In this context of heavy MM contamination, these transitional stages could provide some reference trajectories for contaminated ecosystem restoration (Clewell and Aronson, 2013) from the highly disturbed communities of groups 3 and 1, to target communities represented by group 2.

The visual estimation of plant cover ranged between 25% and >75% on sampling plots, reflecting the plant community recovery since about one century (Laffont-Schwob et al., 2016). The lack of bare soils and the absence of a significant correlation between plant cover and contamination level highlighted the presence of native non-metalliferous plant tolerance mechanisms (Ernst, 2006) that enabled the natural implementation of re-colonization processes independently of the MM soil contamination. It is also expected, that resilience capacities of native plant communities could have been conditioned by the time gap needed for soil spontaneous recovery (Schaeffer et al., in press), which corresponds to the improvement of their quality and functioning, after the end of disturbances linked to toxicity caused by acid deposits (Laffont-Schwob et al., 2016). That highlights the need for further investigation to assess the local plant-soil-microorganism interactions occurring within the mycorhizosphere in order to provide ecologically effective solutions to manage the MM soil contamination (Khan, 2005) throughout the Calanques national park, especially in a Mediterranean context (Barea et al., 2011).

4.3. Plant strategies, life-traits and MM tolerance

Previous studies in Mediterranean metal-enriched areas have already shown the interest of using spontaneous native plants for phytoremediation and restoration purposes (Boukhris et al., 2015; Mendez and Maier, 2008; Párraga-Aguado et al., 2014), as they developed metal-tolerance and are already adapted to local conditions (Baker et al., 2010; Marchiol et al., 2013). At our study site, plant communities were mostly composed of perennial native Mediterranean plants corresponding to different biological types (Raunkier, 1934) and life strategy (Grime, 1977) on the basis of the BASECO database (Gachet et al., 2005). By using life-traits and excluding the annual species (therophytes), a selection grid can be obtain and lead to selection of native plants with phytostabilization potential (Table 6).

In our study, some native plant species, such as *Coronilla juncea* and *Globularia alypum* for shrubs, and *Biscutella leavigata*, *Lobularia maritima*, *Piptatherum caerulescens*, *Silene vulgaris* for perennial grass and forbs, showed significant positive correlations with the MM contamination level that suggested their higher tolerance to pollution compared to the other plants of the community (e.g. *Cistus albidus*, *Rosmarinus officinalis*, *Thymus vulgaris*). *C. juncea* is a leguminous shrub that has been already used in Mediterranean ecological restoration projects, and has proved of interest for soil recovery both in non-contaminated (Padilla et al., 2009) and metals-contaminated areas (Alguacil et al., 2011; Carrasco et al., 2011). Another study carried out on Mediterranean metal-contaminated soils assessed the effects of organic amendments on the phytostabilization capacity of the native plants *C. juncea* and *Oloptum miliaceum* (syn. *Piptatherum miliaceum*) (Kholer et al., 2014). Previous studies in the Escalette factory area were conducted on the phytoremediation capacity of *G. alypum* and concluded that this native shrub is a good candidate for MM phytostabilization with regard to bioaccumulation (BCF) and translocation factors (TF) (Testiati et al., 2013). Testiati et al. (2013) showed a restriction of TF when root concentrations were higher and that BCF were ≤ 1 and TF < 1 for As, Cu, Pb and Zn. *Biscutella leavigata* and *Silene vulgaris* were identified as heavy metal tolerant species in the literature (Ernst, 2006), with metallophyte populations found in mining areas (Bringezu et al., 1999), and the former is more specifically known as a metal hyperaccumulator (Anderson et al., 1999; Wenzel and Jockwer, 1999; Wierzbicka and Pielichowska, 2004). The genus *Linaria* was also reported in the literature to be metal tolerant with some species growing on serpentine Cr and Ni rich soils (Freitas et al., 2004). More generally, native plants identified in Table 6 mostly belong to Caryophyllaceae, Brassicaceae, Poaceae and Fabaceae, families which are well known in the literature for their MM tolerance (Kabata-Pendias and Pendias, 2001).

In order to avoid too much competition between species, we should be careful to not introduce species with invasiveness potential, that have a greater ability to grow on disturbed soils and could become a threat for less competitive native species. Among the selected tolerant native species, some of them, especially those with a reduced MM translocation, such as the fast-growing herbaceous and small shrubs, could be used as nurse species or ecosystem engineers (Jones et al., 1994) in order to promote the later establishment of a more diverse plant community (Markham et al., 2011; Párraga-Aguado et al., 2014). These engineer species should thus be used to improve soil quality by nutrient and/or organic matter input occurring within the rhizosphere where soil-plant-microorganisms interactions take place (Barea et al., 2011;

Table 6
Species life-traits and native plant tolerance to MM at study site C. Abbreviations: Family: AST = Asteraceae, BRA = Brassicaceae, CAR = Caryophyllaceae, FAB = Fabaceae, LAM = Lamiaceae, PLA = Plantaginaceae, POA = Poaceae, PRI = Primulaceae. Biological types (Raunkier, 1934): Ch = chamephyte; G = geophyte; H = hemicryptophyte; NPh = nanophanerophyte; Th = therophyte. Strategies (Grime, 1977): C = competitor; R = ruderal; S = stress-tolerant. Seed dispersal type: Anem = anemochore; Baro = barochore; Zoo = zoochore. MM tolerance: based on "species" scores from RDA analysis, +++ = 1st quartile; ++ = 2nd quartile; + = 3rd quartile; - = 4th quartile. Data collected from Baseco database (Gachet et al., 2005) and Fournier (1961), Pavon (2014), Rameau et al. (2008) flora.

Plant species	Family	Biological type	Strategy	Habitat	Seed dispersal type/flowering	Substrat	Study area C	
							As, Cu, Pb, Sb, Sn, Zn tolerance	K-means group
Biscutella laevigata	BRA	H	S	Rocky environments, shrublands etc.	Anem/May to August	Alkaline to lightly acid	++	1
Lobularia maritima	BRA	Ch (H ou Th)	RS	Dry environments	Anem/March to September	Neutral to alkaline	+++	3
Cistus albidus	CAR	Ch (NPh)	CSR	Shrublands	Anem-Zoo/May to June	Variable	–	2
Dianthus caryophyllus	CAR	H	S	Dry grasslands, rocky environments	Anem/June to September	?	+	1
Silene vulgaris	CAR	H (Ch)	R	Diverse environments (dry to humid)	Anem/June to August	Neutral	++	1
Coronilla juncea	FAB	NPh	SC	Thermophile shrublands	Zoo/April to June	Neutral to alkaline	+++	3
Rosmarinus officinalis	LAM	NPh	SC	Shrublands	Baro-Zoo/January, April, July, October	Alkaline to lightly acid	–	2
Teucrium polium	LAM	Ch	SC	Shrublands, dry grasslands, etc.	Baro/May to August	Alkaline	–	1
Globularia alypum	PLA	Ch (NPh)	SC	Shrublands, pine-forests	Anem-Zoo/April to June	Alkaline to neutral	+++	3
Avenula bromoides	POA	H	SR	Rocky grasslands and shrublands	Anem-Zoo/May to July	Alkaline to neutral	++	1
Brachypodium retusum	POA	G (H)	CS	Dry grasslands, shrublands, pine-forests	Anem-Zoo/May to July	Alkaline to acid	+	1
Piptatherum caerulescens	POA	H	CS	Rocky shrublands of coastal massifs	Anem/April to June	Alkaline to neutral	++	1
Coris monspeliensis	PRI	Ch (H)	SR	Rocky environments, shrublands, etc.	Anem-baro/April to July	Alkaline to neutral	+++	3

Cortina et al., 2011; Krumins et al., 2015; Ottenhof et al., 2007; Wenzel et al., 1999; Wong, 2003). Some others species could provide a MM filter by reducing the leaching and runoff and thus their availability for less tolerant species. This approach would later promote the establishment of less tolerant but phytostabilization-competent shrubs such as *Rosmarinus officinalis* (Affholder et al., 2013, 2014; Testiati et al., 2013), after soil quality improvement.

5. Conclusion

In natural protected areas, it is important to target certain specific plant species for further analysis without causing an additional environmental impact during the first steps of a research project that focuses on MM tolerant plant species selection. The non-destructive and cost-effective field methodology we have developed, combined with life-traits data, represents a good compromise that allowed more extensive screening of the spontaneous vegetation with phytostabilization potential and was less restrictive than usual initial studies on contaminated areas that were based on a few species MM plant-uptake characterizations (Conesa et al., 2007; Marchiol et al., 2013). The agreement between the results of multivariate analysis and the data found in the literature validates these methods concerning the identification of native plants with phytoremediation potential; and communities characterization and life-traits allow to refine the plants selection. The results provides original data about some Mediterranean native plant abilities to grow in a heavy MM mixed contamination, with CF and PLI reaching record level in natural areas surrounding Escalette factory. In the context of the ecological restoration of MM contaminated areas, the characterization of plant communities throughout variable local conditions provides critical informations in order to identify an ecosystem reference trajectory for achieving the restoration objective. Thus, to ensure the effective management of soil contamination, it is important to combine two approaches, based on native plant metal tolerance and life-traits on one hand, and the analysis of successional stages and plant assemblages on the other hand. Our results have shown the capacity of native plants to establish on a MM polluted area, and highlight that some particular species were more tolerant to heavy MM contamination, but involving different mechanims (e.g. phytoaccumulation, phytostabilization). In order to achieve a targeted selection of native plants, further analyses are needed based on plant life-traits and plant-soil-microorganism interactions linked to their metal-tolerance. These experiments are already in progress concerning two identified tolerant native shrubs i.e. *C. juncea* and *G. alypum*. Further field trials will give an assessment of this plant selection methodology in regard to restoration objectives. This highlights the importance of both community and species ecology characterization in order to achieve an effective selection of the most suitable native plants to use with regard to the restoration objective and MM management strategy. In the complex study site context, only projects that take into account both ecological restoration aspects (biodiversity, functional diversity, reference ecosystem and trajectory) and pollution management objectives (reduction of contaminant transfers) should be considered in order to achieve the effective rehabilitation of the protected and polluted areas.

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