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Ahlem Ellili [1,2], Jacques Rabier[1], Pascale Prudent[3], Marie-Dominique Salducci[1], Alma Heckenroth[1], Mokhtar Lachaâl[2], Isabelle Laffont-Schwob[1*]

[1] Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France. (ahlem.ellili@gmail.com, jacques.rabier@imbe.fr, marie-dominique.salducci@imbe.fr, alma.heckenroth@imbe.fr, isabelle.schwob@univ-amu.fr)

[2] Faculty of Sciences of Tunis, Physiology and Biochemistry of Plant Response to Abiotic Stresses Unit, University of Tunis El Manar, 1060, Tunis El Manar, Campus Universitaire Farhat Hached B.P. N° 94 – Rommana 1068, Tunisia (ahlem.ellili@gmail.com, mokhtar.lachaal@fst.rnu.tn)

[3] Aix Marseille Univ, CNRS, LCE, Marseille, France. (pascale.prudent@univ-amu.fr)

* Corresponding author

Isabelle Laffont-Schwob

Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, 3 place Victor Hugo, case 4, 13331 Marseille cedex 3, France

Phone:+33 (0)4 13 55 12 30, Email: isabelle.schwob@univ-amu.fr

Abstract

In polluted protected areas, using phytoremediation raises the question of the choice of the plant species to select. As an example, *Atriplex halimus* has been identified as a proliferative plant species that needs to be eradicated in the Calanques National Park (PNCal). Since it has been proven that the spontaneous populations of this plant species could phytostabilize shore waste deposits generated by past industrial activities within the PNCal territory, its status seems controversial, presenting a dilemma between biodiversity management of a protected area and ecological solutions for pollution management. To address this issue, we assessed the ability of *A. halimus* to grow on different soils from this territory, in order to estimate the potential invasiveness of this plant in this territory. Petri dish germinations and pot-growth experiments showed 50% germination of seeds collected on local individuals from the most polluted PNCal soil and 20% growth reduction of seedlings. Soil analysis showed that limitation of growth was caused by high pH value and sparsely available micronutrients as well as metal and metalloid contamination. Our results suggested that local populations of *A. halimus* may stabilize the highly metal and metalloid polluted salt-affected soils of the PNCal, with low seed germination potential lowering the eventuality of a propagation over the PNCal territory. As a consequence of this study, the administration of the PNCal decided not to remove *A. halimus* populations along the polluted coastline until another solution to prevent pollution dispersal had been found. This laboratory approach may be extended to other similar situations where plant species may be evaluated not only in term of phytoremediation potential but also in term of biodiversity preservation.

Keywords: *Atriplex halimus*, ecological management, metals and metalloids, phytostabilization, potential invasiveness.

1. Introduction

Problem statement

Most of the papers dealing with *in-situ* soil phytoremediation examine persistence of selected agronomic plant species in polluted sites and mainly use efficiency of the pollution management as the major criteria of success (Vangrosveld et al., 2009; Kidd et al., 2015). Apart from this mainstream approach, the use in phytoremediation of native plants to avoid introduction of non-native and potentially invasive species that may result in decreasing regional plant diversity has been discussed (Mendez and Maier, 2008) and pilot assays using local plant assemblages for phytostabilization in polluted protected areas have been incremented (Heckenroth et al., 2016). However in certain cases, the status of invasiveness or even more of indigeneity is still controversial for some plant species and new criteria of evaluation of the compliance of these plants for phytoremediation of protected polluted areas have to be investigated. Moreover, although the great majority of invasive species are introduced, occasionally native plant species may become invasive, spreading rapidly into previously unoccupied habitats according to Simberloff (2011), these new habitats may correspond to recently polluted habitats. In France, many national parks host polluted soils in their territory (Desrousseaux and Ugo, 2016) and worldwide this situation occurs regularly (Mazurek et al., 2017; Armendáriz-Villegas et al. 2015). Amongst invasive plant species, some of them are really good candidates for phytoremediation such as *Miscanthus* X *giganteus* or alimurgic species (Bandiera et al., 2016). However, in protected areas, phytoremediation approach favour phytostabilization i.e. use of plant cover to reduce pollutant mobility in soils rather than phytoextraction i.e. use of the ability of some plants for metal translocation in their aerial parts involving the removal of the aerial parts. Thus, determining the benefit of ecological services vs the proliferation risk of these controversial plant species in polluted protected areas is important to assess and also better understanding the local parameters and specially soil characteristics that enable or not their proliferation and their ability for metal phytostabilization.

Atriplex halimus: example of a controversial species in a protected area

To illustrate this situation, the plant species *Atriplex halimus* (Amaranthaceae), a xerohalophyte that mainly grows in salt-affected nitrophilous and/or degraded soils with a high tolerance to drought (Walker et al., 2014), may be an expressive case study in the Mediterranean area. This plant species has a high tolerance to metal and metalloid elements (MM) and numerous papers dealing with its potential use for phytoremediation have recently been published, showing the growing interest in this plant species (Caçador and Duarte, 2015; El-Bakatoushi et al., 2015; Manousaki and Kalogerakis, 2009; Márquez-García et al., 2013; Pardo et al., 2014; Pérez-Esteban et al., 2013; see reviews by Lutts and Lefèvre, 2015; Walker et al., 2014). *Atriplex halimus* has been widely cultivated as forage or wind barrier in much of the world (Otal et al., 2010; Walker et al., 2014), although it originated in the Mediterranean Basin (Ortíz-Dorda et al., 2005; Walker et al., 2014). In South East France (Mediterranean coast), spontaneous populations of *A. halimus* are found in polluted coastal soils of a protected area i.e. the Calanques National Park (PNCal). Though part of the populations present on the site may be native of Ibero-Provençal origin, according to Ortíz-Dorda et al. (2005), the Mediterranean National Botanical Conservatory (MNBC), a French authority for plant conservation, considers that many ornamental individuals of this species planted as hedges near housing might have escaped from neighbouring gardens and originated most of the populations currently occurring along the coast of the PNCal on the basis of phytosociological criteria. Therefore the MNBC suggested the eradication of *A. halimus* from the territory of the PNCal in the same way as the notorious invasive species *Carpobrotus edulis* in Mediterranean (Affre et al., 2010). However, the composition of the soil near the coastal road in the PNCal was definitely altered by slag deposits from past industrial activities from the 19th to the mid-20th century (Laffont-Schwob et al., 2011; Daumalin and Laffont-Schwob, 2016), and it has been demonstrated that stands of spontaneous *A. halimus* phytostabilize these polluted soils, preventing soil erosion, diffuse pollution and contamination of the food web (Rabier et al., 2014).

This turns to a cornelian dilemma for the PNCal between plant conservation including invasive plant eradication and maintaining pollution management to prevent pollutant transfer to terrestrial and sea biocoenosis. Therefore, a set of effective parameters has been examined to provide decisional criteria for the PNCal.

Methodological approach

A genetic approach for the study of the PNCal *A. halimus* populations would be too expensive, time-consuming and probably not effective for determining the autochthonous status or not of these populations. In addition to showing high genetic variability (Abbad et al. 2004; Ortíz-Dorda et al. 2005), local populations may have been subject to microevolution processes due to the driving force of high MM pollution, as has been recently proven for another pseudo-metallophyte (Słomka et al., 2011).

An ecological approach on life traits may be more accurate to evaluate the potential of invasiveness of this species on a specific territory. *A. halimus* mainly spreads via seeds that may be transported by animals, air or water and, to a lesser extent, can propagate vegetatively (Walker et al., 2014). A potential spread of this species at the expense of other native plant species, especially rare ones, needs to be taken into account. Therefore the invasiveness potential may be evaluated as the ability of this species to germinate and grow in soil conditions differing from those in which its currently develop. While the tolerance of *A. halimus* to MM in coastal areas has been demonstrated previously on older *in situ* individuals, no information is available on its capacity to establish on the more or less polluted soils of this territory. More scientific information on the ecological preferendum of *A. halimus* is thus necessary. As a first step, the ability of local *A. halimus* populations to germinate and grow on various soils from the PNCal territory needs to be assessed. Therefore, an ex-situ experiment was conducted in laboratory on the germination, seedling growth and MM accumulation capacities and on chemical and ecological traits of *A. halimus* in various PNCal soils. The results are discussed in a global perspective to propose a methodological approach for the selection or keep up of non-native or potentially invasive plant species in polluted protected areas.

2. Materials and methods

2.1. Study area and soil sampling

The current PNCal territory was the site of metallurgical and chemical industrial activities from the mid-19th century until the beginning of the 20th century. Silver–galena ore was treated by pyrometallurgical processes during this period (Daumalin and Raveux, 2016). The former Escalette factory (in operation from 1851 to 1924) was the one that had the most intensive activity in the area. The factory, located on the lower slopes of a hill, was characterized by a horizontal smelter chimney as the condensing system. The ruins of this chimney are still present today. In well-constructed flues, the deposit could yield from 2 % to 3 %, while loss dispersed in the smoke could amount to around 10 % of the lead produced in the ore, depending on its quality (Percy, 1870). Slag was deposited on the old factory site, but was also scattered along the coast in several main deposits and as roadfill. Six sites were selected for this study (supp. data 1), located in the peri-urban area of Marseille, south-east France, i.e. Calanque de Saména (SA, 43°13.749' N; 5°20.960' E) and Calanque des Trous (TR, 43°13.233' N; 5°20.766' E), corresponding to soils from moderately polluted seashore sites where *A. halimus* grows spontaneously; Escalette Chimney (E.C, 43°13.584' N; 5°21.32'

E) and Escalette Slagheap (E.S, 43°13.454' N; 5°21.126' E), corresponding to soils from the former Escalette smelter heavily polluted by two different steps of the smelting process; Cap Croisette (CC, 43°12.812' N; 5°20.899' E), a site exposed to very low pollution and seaspray; Sormiou (SO, 43°12.806’ N; 5° 24.964’ E), a site exposed to very low pollution and no seaspray; and in order to have a reference condition, was chosen as a control substrate a loamy horticultural soil (CN, mixture of peat moss, sphagnum peat moss, wood fiber and plant cultivation support composted with NPK 8-2-7, Botanic®). The areas were chosen on the basis of previously published data on metal and metalloid contamination in the PNCal area (Laffont-schwob et al., 2016) and according to similar physico-chemical characteristics of the soils (except level of MM contamination). All soils are pooled samples of five sub-samples collected on the top soil: for SA, TR and CC, soils are from the root zone of *Atriplex* populations as previously described (Rabier et al., 2014); for E.C., E.S and SO, soils samples were collected under mattoral plant cover (Testiati et al., 2013; Affholder et al., 2013).

On each of the six selected sites, soil samples were collected from the top 15 cm after removal of the litter. In order to obtain representative samples of each site, a composite soil sample was achieved by mixing 5 subsamples of equal weight. These 5 subsamples were collected 2 m apart in a cross pattern at each site, and were sieved on site to 2 mm mesh. Each composite soil sample was stored in a plastic bag until returned to the laboratory.

An *ex-situ* assay by pot experimentation in the laboratory was conducted with these composite soils, to assess the germination and growth capacities of *A. halimus* from seeds collected on spontaneous individuals in the area on these MM and salt-affected soils.

2.2. Soil physico-chemical analyses

In the laboratory, soil composite aliquots were air-dried at room temperature and then ground to pass through a 0.2 mm titanium sieve (RETSCH zm 1000 with tungsten blades) before analyses. Physico-chemical parameters of soil, i.e. salinity, pH, conductivity, texture, total organic carbon and total Kjeldahl nitrogen content, and trace and major element concentrations, were determined on 3 analytical replicates of each soil composite sample per site.

Measurements of pH (ISO 10390, 2005) and salinity (calculated from conductivity) were determined by potentiometry in a 1:5 soil:water suspension using a Multi 3420 SET B-WTW pH meter and ECmeter (Baize, 2000).

Total organic carbon (TOC) quantifications were carried out with a Jena Analytic TOC-N/C2100S, coupled with HT1300 solid module (ISO 10694, 1995). Total Kjeldahl nitrogen content was measured with Büchi 323 digester and distillation units (ISO 11261, 1995).

For MM analyses, soil samples were mineralized in a microwave mineralizer (Milestone Start D) using *aqua regia* (1/3 HNO_3 + 2/3 HCl). The solutions obtained for soil mineralization were filtered with a 0.45 µm mesh and the metal levels were determined by ICP-AES (JobinYvon Horiba, Spectra 2000) for As, Cu, Fe, Mn, Pb, Sb and Zn (Lotmani et al., 2011); flame AES (Thermo Scientific ICE 3000) was used for Na and K measurements. Cd was not taken into account since results showed concentration levels far below 1ppm. Quality assurance controls and accuracy were checked using standard soil reference materials (CRM 049-050, from RTC-USA) with accuracies within 100 ± 10 %. Results are presented in supp. data 2.

The following analyzes were performed by the Laboratoire Developpement Méditerranée at Alès (Gard, France). The soil texture was measured on five non-decarbonated fractions (NF X 31-107, 2003), the exchangeable P (P_2O_5) was determined according to the Joret Hébert method (ISO 11263, 1995), the cation exchange capacity (CEC) was measured according to the Metson method (ISO 23470, 2007), extraction of the exchangeable cations was carried out with ammonium acetate (ISO 23470, 2007).

2.3 Seed collection

A. halimus seeds were collected in September 2013 from 5 individuals of the spontaneous population at Escalette (highly polluted site, supp. data 1), then pooled and stored in paper bags at room temperature until use. No information is available on this population regarding its potential ornamental origin, but it was confirmed that the chromosome number of this populations was diploid (2n = 2x = 18) (Snow, 1963), corresponding to the phenotype of the subsp. *halimus* originating from France and Spain, according to Le Houérou, 1992; McArthur and Sanderson, 1984; Talamali et al., 2004; Walker et al., 2005. This population is thus characteristic of the particular conditions of *A. halimus* populations in the area.

2.4. Seed germination experiment

Seeds were removed from their bracts by dragging them over sandpaper. The seeds were surface-sterilised just before use by immersion in 5 % (v/v) sodium hypochlorite for 10 min and rinsed three times in sterile water, a treatment that does not affect germination parameters or seedling characteristics (Muñoz-Rodríguez et al., 2012).

The seeds were germinated under controlled conditions with 12/12 h of day/night at 24/20 °C, respectively, constant humidity 60 %, that have been proven to be appropriate conditions to enhance the percentage of germination in *A. halimus* (Muñoz-Rodríguez et al., 2012). The Petri dishes were put in a plant growth chamber (SANYO MLR-351H). The light was provided by fluorescent lamps that produce an average photosynthetic photon flux density of 300 µmol/m^2/s. Germination tests were carried out in Petri dishes filled with 40 ml of every soil type (corresponding to 20 g of composite soil (sieved to 2 mm) from each site and 5 g of control substrate (CN), due to the different substrate densities). These different soils were watered with distilled water to near field capacity. Ten replicates of 30 seeds were used for each soil type. Germination was monitored every 2-days by counting the number of germinated seeds per Petri dish (Keiffer and Ungar, 1997). Seeds were considered to have germinated after radicle emergence.

2.5. Growth conditions and plant morphology

The six composite soils (sieved to 2 mm) and the control (CN) were distributed in individual 120 ml-paper pots (100 ml of dried soil per pot from the six PNCal sites or CN) in order to get 10 pots for each soil type. These pots were placed in a plant growth chamber under the same previously described conditions and were sprayed with distilled water until soil water-holding capacity (WHC) was reached. WHC had been previously determined for each soil type.

Seedlings from the Petri dishes were transplanted into the pots, being careful to keep the seedlings from one type of soil in the Petri dish in the same soil in the pot (7 types of soils i.e. 6 sites and CN, 5 plants per pots, 10 replicates per type of soil). Seedlings were grown for 63 days and they were watered with distilled water every two days to restore the initial WHC.

Plant height (shoots) and number of leaves were measured every 7 days.

At the end of the experiment, the plants were carefully removed and then separated into roots, stems and leaves. Roots were thoroughly washed with tap water to clean off soil particles and were rinsed with deionized water (three successive rinses of 30 s each), then gently blotted between paper towels. Morphological parameters (root length, shoot length, and leaf number) were measured on each plant.

Leaf surface areas were determined using a scanner and ImageJ software (URL: http://rsbweb.nih.gov/ij/).

Shoot and root fresh weights (FW) were determined immediately. The pooled root samples per pot were oven-dried at 40 °C for 48 h to determine the root dry weight (DW). Dry weight determination was done identically from pooled shoot samples per pot.

Hydration rates (HR) were calculated on shoots and roots of plants grown on the different soils, as described elsewhere (Rabier et al. 2007)

The root and shoot dry material was ground separately for metal analysis. Finally, MM concentrations were analyzed in root and shoot parts.

2.6. Non-destructive plant physiological index measurements

At three different stages (35, 55 and 63 days), plant physiological indices were estimated optically using a Multiplex® 3 non-destructive measurement equipment (FORCE-A, Orsay, France; Agati et al., 2011). This portable fluorimetric device uses fluorescence technology with multiple excitations to measure constitutive and induced epidermal phenols, flavonols, anthocyanins, chlorophylls and a chlorophyll-to-flavonoid ratio referred to as nitrogen balance index (NBI). Different combinations of the blue-green, red and far-red fluorescence signals at the various excitation bands could be used as indices of the different compounds (Agati et al., 2011; Cerovic et al., 2008). Though we used the term 'anthocyanin indices' because the apparatus was developed for relative measurement of anthocyanin, the red pigments of *A. halimus* are betalains, as for other members of the Amaranthaceae with a similar absorbance spectrum (Lavene, 1995; LoPresti, 2015). For each type of soil, the average of five measurements was made per pot and was repeated for the 10 pot replicates. Before starting the measurement by Multiplex, we covered the soil of each pot with pieces of blackboard wall stickers to avoid fluorescence of organic matter from soil.

2.7. Plant elemental analysis

Before analysis, dried shoot and root plant samples from the 10 replicates were pooled separately. They were then ground to pass a 0.2 mm mesh titanium sieve and three aliquots were analyzed by sample. About 0.5 g of dry matter was digested with the microwave digestion system Milestone start D with a HNO_3, H_2O_2 and ultra-pure H_2O mixture (volume proportion ratio 2:1:1). Extracts were analyzed for pseudo-total metals and metalloids (MM) i.e. Cu, Fe, Mn, Pb, and Zn content, using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, JY 2000 Jobin Yvon Horiba) and by graphite furnace AAS (Thermo Scientific ICE 3000) for As and Sb, while flame AES (Thermo Scientific ICE 3000) was used for Na and K measurements. Three analytical replicates were performed for each type of plant sample. Standard plant reference materials (DC 73349) from China National Analysis Centre for Iron and Steel (NSC) were analyzed as part of the quality control protocol (accuracies within 100 ± 10 %).

To evaluate the phytoremediation ability of the plants, bioaccumulation factors (BCF) and translocation factors (TF) in each soil were computed according to the following formula (Yoon et al., 2006):

$BCF = C_{root}/C_{soil}$ (1)

$TF = C_{shoot}/C_{root}$ (2)

Where C_{soil}, C_{root}, C_{shoot} are average concentrations of trace elements in soils, roots and shoots, respectively. These factors indicate the importance of pollutant transfer from soils to roots (BCF) and from roots to shoots (TF).

A quantitative approach of the multi-element contamination can be made based on the pollution load indices (PLI) calculated for each soil following Rashed (2010):

$$PLI = \sqrt[5]{CF_{As} \times CF_{Cu} \times CF_{Pb} \times CF_{Sb} \times CF_{Zn}} \quad (3)$$

with $CF_{MM} = [MM]_{considered\ soil}/[MM]_{SO}$

CF being the soil contamination factor and Sormiou (SO) being considered as the reference area for this study since MM soil concentrations at this site are very close to the local background of contamination in the PNCal area (Affholder et al., 2014). A PLI value strictly above 1 indicates that the soil may be considered as polluted.

2.8. Root symbiosis observations

Before the final harvest, an individual from each pot was removed for root symbiosis analysis (10 replicates per soil type). The roots of these plants were rinsed first under tap then deionized water, pooled in three replicates for each soil type and stored in alcohol (60 %, v/v) at room temperature until proceeding. To limit the use of highly toxic products, we used the method described by Vierheilig et al. (1998) for staining fungal structures before observation. First roots were soaked for 3 min in 10 % KOH bath at 80 °C then rinsed and stained with Pelikan ® blue ink, in a 5 % acetic acid solution at 90 °C for 3 min. The roots were mounted on slides. For each sample, five slides were prepared, each containing 10 root fragments of 1 cm length. Therefore the results are based on the observation of 50 fragments of roots per replicate. The arbuscular mycorrhizal (AM) structures were sorted into mycelium (M), vesicles (V) and arbuscular (A) structures, using optical microscopy at 100 and 400 magnifications.

The structures of dark septate endophytes (DSE) i.e. septate melanized mycelia and microsclerotia were observed. The percentages of each symbiotic structure (A, M, V and DSE) observed in the samples were independently estimated for each soil type, using the formula of Zhang et al. (2010):

Percentage of fungal structures observed = 100 * number of fragments where the structure was observed / number of observed fragments. Information about root symbioses may indicate the ability of the plant to interact with edaphic micro-organisms, playing a role in plant pollutant tolerance but also giving an indication of the potential occurrence of symbionts in polluted soils (biological characteristic of soils).

2.9. Statistical analysis

Statistical analyses and control carts were performed for all data using JMP 11 statistical software (SAS Institute, Cary, North Carolina, USA). *A. halimus* germination percentages, MM concentrations in plant parts and soils, plant stress indices and fungal root colonization percentages were compared using non-parametric Wilcoxon rank sum test (Kruskal-Wallis test) and Wilcoxon each pair test.

3. Results

3.1. Soil characterisation

Except for the control substrate (CN) with a pH value ca. 6, all soils of the PNCal area were alkaline including the technosols (E.C and E.S), being characteristic of local limestone soils (Fig. 1). SO had the lowest conductivity and SA the highest, but lower than 2000 µS/cm. Therefore, according to natural soil saline classification and conductivity data, all the PNCal soils, as well as CN, may be considered as non-saline.

TOC values in all PNCal soils were very low except for SO, with an average value of 110 mg/g. The TOC values were 14 (CC) to 3.5-fold lower (SO) than CN. No NTK differences were observed for SA and SO compared to CN, although other NTK values were up to 7-fold lower (E.C, E.S) than CN. The fertility of these soils could be thus considered as low.

The texture of soils from the coastal sites (SA, TR and CC) was sandy loam as well as for CN, but with less coarse sand for the latter (Fig. 1). The granulometry of SO soil corresponded to clay loam while the technosols (E.C, E.S) were sandy clay loam with coarser sand for E.S.

According to agronomic criteria (Fig. 1), soils from the coastal sites contained highest concentrations of exchangeable Na but also of the major fertility elements (P, Ca, Mg, K), with less exchangeable P for TR. The technosols (E.C, E.S) and the soil from SO had a low content of exchangeable Na but also of exchangeable P, Ca, Mg, K. As expected, CN presented good fertility indices.

The E.S and E.C technosols had the highest Pb and Zn contents whose varied from 1 to 3 %, and a very high content of As in E.C (about 0.3 %), but also high values of Fe, i.e. 1 to 5 % as for all PNCal soils (Fig. 2 and supp. data 2).

We used the PLI to compare the relative load of the mixed pollution of the various soils. PLI for E.C and E.S soils were at least 20-fold higher than all the other soils i.e. $PLI_{E.C} = 216$ and $PLI_{E.S} = 203$ whereas $PLI_{SA} = 6$, PLI_{TR} & $PLI_{SO} = 4$, $PLI_{CC} = 1.4$ since $PLI_{CN} = 1.3$. This revealed a potentially high ecotoxicological risk associated with both technosols i.e. ingestion or inhalation of soil particles may expose biocoenosis to non negligible effects of trace elements.

Following these analyses, the loamy horticultural soil (CN) could be considered as control soil and the soil of CC and SO were the least PNCal polluted soils, being also the least seaspray-affected of the four seashore soils.

3.2. Germination test

The percentage of germination after 14 days was 80 % in the CN (Fig. 3), while significantly lower for all the other soils tested (it varied from 20 to 60 %). However, the germination of this plant species was mostly affected by the combination of alkalinity and salinity (TR, Fig. 1). The germination percentage at 4 days was negatively correlated both with soil pH and conductivity (supp. data 3). The results also showed a weak negative correlation after 14 days with Zn (r_s = - 0.45, p-value < 0.0001) and Cu (r_s = - 0.45, p-value < 0.0001). Very weak negative correlations were observed for As (r_s = - 0.30, p-value < 0.0001) and Pb (r_s = - 0.28, p-value < 0.0001). The negative correlation between germination and pH increased with time, while the correlation with exchangeable Na and conductivity decreased. The negative correlation between germination and soil Zn concentration increased with time. These results can be interpreted as a rapid osmotic effect for germination inhibition by conductivity, with possibly a partially transitory priming effect for Na (Capron et al., 2000) and a progressive inhibition by MM, mainly Zn.

These results demonstrated the ability of *A. halimus* to germinate up to 50 % even in the case of soils containing up to 2 500 mg/kg of As and up to 30 000 mg/kg of Pb near the horizontal chimney (E.C), and the limitation of germination potential by pH alone or combined with salts for all the PNCal soils.

3.3. Growth traits

Overall, the growth traits expressed as percentages compared to control can be classified by increasing order of effects, as follows: survival (given by the number of individuals) < shoot and root lengths < shoot and root fresh weights < leaf surface area (Fig. 4). In other words, amongst growth traits, leaf surface, length and plant weight were the most significantly inhibited with 60-90 % reduction compared to control. After germination, individual loss continued during plantlet growth up to 10-20 % for TR whereas no significant losses were observed for the four other PNCal soils. Reductions of all parameters except survival for E.C and SO soils were significantly greater than those for SA soil. The growth traits were not significantly inhibited for SA and only concerning leaf surface and shoot fresh weight for CC. The growth traits not significantly inhibited were survival, shoot and root length for E.S and, shoot length and root fresh weight for TR.

3.4. Plant chemical traits

After 63 days, the highest indices of flavonol, betacyanin (refered as antocyanin index for Multiplex® index) and phenol were obtained for plants grown in SO soil, whereas phenol and betacyanin indices were the lowest in CN soil. Chlorophyll indices were the most stable, with significantly lower values in E.S, E.C and CC soils than in other soils.

Monitoring of plant chemical traits with non-destructive index measurements (Fig. 5) enabled observation of a time-dependent response. As a first step, betacyanin indices decreased in basal leaves and stems in CN and SA and increased in SO soils, immediately followed by an increase in chlorophyll index in all soils except SO, then in phenol indices in all soils and finally a decrease in flavonol indices. The latter was concomitant with the appearance of necrosis in the basal leaves in E.C and E.S. Moreover, there were no specific visual symptoms of toxicity and/or necrosis on the other leaves and heterogeneity of betacyanin pigmentation developed in the same way from rib to limb on CN soil as on the PNCal soils. The general trend of the curves (Fig. 5) showed that this physiological succession was faster for the control. Thus part of the response of chemical traits may be due to the physiological stage of the seedlings.

3.5 Metal and metalloid transfer to plants

Concerning non-essential elements, the highest accumulations of Pb and Sb in roots were measured in E.C and E.S soils and for As in roots, only in E.C soil (Fig. 2). For CC and SO soils, Sb and As concentrations in roots were similar to those of the control whereas Pb concentrations were still above those of CN. In some of the PNCal soils, Pb, As and Sb concentrations in roots were not those of normal tissue concentrations but those of possibly harmful concentrations (Markert, 1994; Prasad et al., 2006). The major pollutants in root tissues were As and Pb in E.C soil, with 3 439 mg/kg and 24 444 mg/kg respectively, in accordance with the composition of the slag deposit from the smelter industrial activity. Metals and metalloids (As, Pb, Sb, Zn, Cu) present in soils were weakly transferred to the roots (BCF < 1) (supp. data 4), except in the case of E.C for As, Pb and Zn, of CN for Cu, of SA for Cu and Sb, of CC for As, Cu and Zn, and of SO for Cu. Even in the soil of E.C, there was a selective transfer of each of these elements between the soil and the root parts, as Pb was better transferred to root than Sb and Zn (Fig. 2). The transfer coefficients from soil to aerial parts were always < 1 for As, Cu, Fe and Pb (supp. data 4). TF values were more than 1 in the case of K and Na for all conditions. TF values were only > 1 for Zn in the case of CN, CC and SO. Mn was present in soils at moderate to high concentrations, with higher concentrations in E.S, SO and SA soils. However, its transfer to shoots was particularly low in E.C, E.S and SA soils, although Mn TF value was high for the CN.

The elements that are regulators of osmotic pressure, e.g. Na and K (Fig. 2), were preferentially accumulated in shoots in all the soils tested. However, the concentration ratios between Na and K in shoots were strictly above 1 for TR and SA, and below 0.5 in all the other PNCal soils, while this ratio was equal to 0.51 for CN (supp. data 5).

3.6. Root fungal colonization

Two types of fungal structure were observed in *A. halimus* roots i.e. arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). Both kinds of symbiotic microorganisms were present in all root systems for each of the PNCal soils. A discriminant analysis was applied on the percentages of each fungal structure (Fig. 6). Along the first dimension, there was a separation between CN and CC, with a higher occurrence of AM colonization, and E.C and E.S with more DSE. In the second dimension, more arbuscules were observed in roots from CC and SO soils. With this analysis, three main groups may be identified: (i) CN, (ii) CC, TR, SA and SO, (iii) E.C and E.S. A strong correlation was detected between DSE colonisation percentages and As, Pb and Zn concentrations in soils, with Spearman coefficients of 0.70, 0.63 and 0.56, respectively (p-value $\leq$ 0.01). DSE colonisation percentage was also negatively correlated with available P in soil (Spearman coefficient of -0.55 at p-value $\leq$ 0.01).

4. Discussion

4.1 Do PNCal soils have good agronomic properties?

The metal and metalloid contamination of PNCal soils has previously been partially studied and the diffuse pollution of these soils has been discussed in separate publications (Affholder et al., 2013; Heckenroth et al., 2016; Rabier et al., 2014; Testiati et al., 2013). However, in this study, soil characterization was completed by fertility parameters for the seashore sites. Soils were alkaline with pH ranging from 7.5 to 8.5, typical of calcareous areas, but the highest exchangeable Ca and K values found for the soils from less polluted areas of the seashore were associated with the presence of scrap materials such as lime mortar for Ca, and for K an origin that could be linked to bricks and tiles with aminosilicate of potassium as the active substance (Testiati et al., 2013). The soils from the seashore were mainly sandy clay soils, while the granulometry of the technosols from E.C and E.S had no relation with the mineralogy of natural soils. There was more fine dust in E.C without coarse sand and coarser sand and less fine dust in E.S soil. Technosol from E.C seemed to be very compact when dry, like real clay soil.

Concerning soil pollution, the strong correlation between As and Pb, particularly for E.C soil, was probably related to their deposition as lead arsenate, while the correlation between As and Sb was explained by their belonging to the same group in the periodic table in relation to the same behaviour during the ore treatment and dust deposit in soils (Testiati et al., 2013). Sb concentrations were low in soils except for E.S and E.C, where As, Pb, Zn and Cu, whose toxicity is more well-known, were also present at high concentrations.

Zn concentration was locally important (Testiati et al., 2013), which could be explained by differences in ore origin during the smelter activity and use of pyrite in the former sodium carbonate factories at the vicinity of the smelter factory (Daumalin and Raveux, 2016).

The results of Pb and TOC for SO were a little above those of natural soils, but SO was not exempt from previous anthropogenic activities that took place at the beginning of the 19th century (Fressoz, 2013; Daumalin and Raveux, 2016).

In summary, the PNCal soils have low fertility with high pH, high Fe content and low nutrient and TOC contents. The anthropogenic addition of materials maintained or increased pH and Fe concentration, but lowered TOC. Fertility was slightly increased for seashore sites in relation with domestic anthropogenic nitrogen deposition and a covering layer of excavated loam (Rabier et al., 2014). The positive common denominator of these soils is that high pH, Fe and Mn oxides and low TOC lowered MM mobility and limited their transfer into plants and into marine ecosystems (Bert, 2012; Dang et al., 2014; Lenoble et al., 2015; Lin et al., 2008; Martínez-Sánchez et al., 2011). The higher fertility level compared to the other PNCal soils of the coastal area where spontaneous *A. halimus* populations are exclusively localized for the moment may be a driving parameter for *A. halimus* development.

4.2. Is A. halimus able to germinate in all the PNCal soils?

The results showed that pH, Na and Zn were involved in *A. halimus* germination inhibition. The data of pH and soil composition were in accordance with those of studies of germination inhibition whose differentiated the effect of alkalinity from salinity (Chen et al., 2010; Ma et al., 2015). Sodium soil concentration may explain the delay of germination shown by the characteristic S-shaped aspect of germination for seashore soils which has been observed for some other *Atriplex* species and other Chenopodiaceae (Capron et al., 2000; Katembe et al., 1998). The effect of multiple metals and metalloids is difficult to appreciate because they occur simultaneously in the field and their presence is correlated with other factors such as salinity and alkalinity. The correlation between Zn and germination rate was weak, which is in accordance with other studies under controlled conditions showing no effect of separated Cu, Pb or Zn treatments for A. *halimus* sub. *schweinfurthii* (Lotmani et al., 2011) and separated Cu or Zn treatments for *A. halimus* sub. *halimus* (Márquez-García et al., 2013).

Finally, our results indicated that the potential for widespread dissemination of *A. halimus* by seed germination is very limited in the PNCal soils. Previous test of germination with an agronomic plant species i.e. *Raphanus sativus* showed a percentage of germination of 83±6, 72±9 and 90±3 on E.C, SO and CN soils respectively (unpublished data), confirming the low capacity of *A. halimus* to develop on other soils than the type on which it spontaneously develops compared to an agronomic species.

4.3. A. halimus growth inhibition in PNCal soils

Reduced additional individual losses were shown during plant growth monitoring for TR. However, a growth inhibition of as much as 60-80 % for the less polluted soils was observed. This demonstrated a negative impact on growth of these mainly oligotrophic soils, independently of their pollution level. This is in accordance with the studies by other authors on *A. halimus* (Martínez-Fernández and Walker, 2012), which have shown that nutrient supply rather than heavy metals (Pb, Zn) limits growth of *A. halimus*. Moreover, *A. halimus* grows well in nutrient-rich solution (Lutts et al., 2004) and is described as halo-nitrophilous (Muñoz-Rodríguez, 2012; Walker et al., 2014). Coastal sites of the PNCal are popular and often crowded during summer, generating illicit waste deposits. Moreover, permanent indigenous human presence at the site of Marseilleveyre can be dated back at least to 600 AC (Bouffier and Garcia, 2014). This could coincide with the introduction of *A. halimus* species among the halonitrophilous taxa of the Chenopodiaceae, though older evidence of the presence of *Atriplex* species has been found in pollen from Calanques sediment (Romey et al., 2015 and 2014). However, it is not possible to distinguish between the species by their palynogical character (Abel-Schaad et al., 2014; Fernández-Illescas et al., 2010). Though a potential spread of *A. halimus* at the expense of native leguminous plant species does exist in theory, especially considering *Astragalus tragacantha*, a protected native plant species on the seashore and, *Coronilla juncea,* another native plant species, at the chimney site, it is limited by the nitrogen contribution of these species which is probably negligible in comparison with the anthropogenic nitrogen sources. These results are in accordance with our previous results as *A. halimus* is spontaneously present on disturbed soils from the seashore and inland in presence of waste from building, but not on the more polluted part of the former smelter nor in undisturbed mattoral. Moreover, it has been previously demonstrated that *A. tragacantha* shows no difference of growth in a soil analogous to TR compared to a loamy reference soil after two years of culture (Laffont-Schwob et al., 2011) although *A. halimus* had a significantly reduced growth in TR compared to CN.

Consequently, for the PNCal, if it seeks to manage *A. halimus* dispersion due to its potential invasiveness, the priority action would be to reduce dog droppings and organic matter deposits resulting from human practices that favour preferential habitats for *A. halimus* rather than to eradicate *A. halimus* individuals themselves.

4.4. Root symbiosis: the secret weapon to success for A. halimus?

Symbionts were ubiquitous in all root systems, whatever the PNCal soil tested, indicating their occurrence in soils, in contrast to what it is observed on more recently disturbed soils with reduced fungal inoculum (Brundrett, 2009). As previously confirmed from aerial photography archives, the soils from these sites, heavily contaminated and disturbed during the industrial period, have been less disturbed since 1960 for the seashore sites and ca. 1950 for E.C as the roof of the smelter horizontal chimney had clearly been destroyed (Rabier et al., 2014; IGN 1943; IGN 1950). It was probably destroyed by bombing during the battle of Marseille in August 1944 (Duchêne and Contrucci, 1998). It appears that a sufficient period of time has passed for a spontaneous symbiont spore bank to be formed in these polluted soils. Moreover, antimony microbial tolerance of soils from the same site has been shown by respiration measurements, demonstrating an adaptation of the PNCal soil microbial populations (Guillamot et al., 2014). Even if the genus *Atriplex* was previously quoted as nonmycorrhizal (Brundrett, 2009), recent papers showed the occurrence of root symbioses in many species belonging to this genus (Sonjak et al., 2009). The occurrence of symbionts in the roots of *A. halimus* has already been proven *in natura* in the Calanques National Park (Laffont-Schwob et al., 2011; Rabier et al., 2014) suggesting an old occurrence of this plant species in the area. As previously discussed, though these sites are peri-urban, which explains the spontaneous presence of nitrophilous species such as *A. halimus*, the perturbation of the entisol was sufficiently old to allow the development of a spontaneous fungal spore bank. The positive correlation between MM and DSE is in accordance with several authors (Ban et al., 2012; Xu et al., 2015; Zhang et al., 2008), and a previous work on *Rosmarinus officinalis* at E.C (Affholder et al., 2014). However contrasting results can be found in the literature and are related to the plant's strategy to increase its tolerance to MM (see review by Göhre and Paszkowski, 2006). Recently, there has been a surge in research focused on how DSE could restrict the uptake of metals by their host plants and especially the interactions of metal ions with melanins (Felix et al., 1978; Fogarty and Tobin, 1996; Mandyam and Jumpponen, 2005; Mugerwa et al., 2013; Stainsack et al., 2003). However, the ability of fungal symbionts to limit metal transfer from soil to *A. halimus* may not be the only factor involved. It has recently been proven that *A. halimus,* with its own biosynthesis of enzymes and antioxydants, may bind, sequestrate and reduce the harmful effect of trace metals (El-Bakatoushi et al., 2015). Consequently the occurrence of this plant species in coastal polluted soils may not increase the potential transfer of MM to the food chain and may favour phytostabilization.

4.5. Decision-making criteria for the PNCal to maintain or eradicate A. halimus

In response to the question of whether *A. halimus* is able to grow on more or less polluted or salt-affected soils from the PNCal - in other terms, in soils different from those in which it spontaneously grows, - the answer is yes. However, a high loss of 50 % at the germination stage was observed. The results also showed 80% growth reduction for the surviving plants in the two technosols (E.C and E.S.). Moreover, the controlled conditions used mimicked the field conditions except for xericity, providing more favourable conditions than the *in natura* ones. The nitrophilous character of *A. halimus* also limits its potential for the colonization of new territory. Thus, the oligotrophic and less polluted soils of CC and SO showed a significant reduction of growth, while that of SA exhibited better growth results and higher chlorophyll indices associated with more widely available micronutrients, despite its significant pollution.

Given the low translocation factors, our results confirm that the individuals of *A. halimus* growing naturally on polluted soils along the coast do not present a risk of transfer of pollutants to aerial parts and the food chain and are tolerant to MM. It would appear that the germination and growth of this species require disturbed soil sites with increased macronutrient availability and associated symbionts. On the basis of these results, the PNCal is considering maintaining *A. halimus* stands in the polluted soils of the coastal area until a better ecological solution for pollution containment is found.

5. Conclusion

From this case study, we wanted to reconsider the simplistic vision of pollution treatment vs biological conservation in polluted protected areas. In various cases, plants spontaneously growing in polluted areas are adapted to pollution and may prevent pollutant transfer. The potential invasiveness of these pollutant-tolerant plants in such field may be easily experienced by analysing biotic and abiotic factors favouring their germination and growth in ex-situ assays and give decisional tools for the protected area managers.

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Figures

Fig. 1: Physico-chemical characteristics of the PNCal soils and control. Parameters: EC: electrical conductivity, TOC: total organic carbon, NTK: total Kjeldahl nitrogen, Exch. P_2O_5: exchangeable P mg/kg P_2O_5, Exch. Na, K, Mg, Ca: exchangeable Na, K, Mg, Ca (mg/g), CEC: cationic exchange capacity (Metson cmol+/kg). Sites: CN: control; SA: Calanque de Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou.

Fig. 2: Average metal and metalloid concentrations (logarithmic scale, mg/kg of dry weight, DW) in soils, shoots and roots of *A. halimus* (n=3, $p \leq 0.05$). Sites: CN: control; SA: Calanque de Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou. Different letters above mean values (n=3, $p \leq 0.05$) mean significant difference (Wilcoxon test).

Fig. 3: Effect of PNCal soil contamination and/or salinity on germination of *A. halimus* during the 14 days of the experiment. Different letters above mean values (n = 10, $p \leq 0.05$) indicate significant difference (Wilcoxon test).

Fig. 4: Growth traits and survival (as percentage of control) for *A. halimus* in the PNCal soils, n= 10, $p \leq 0.05$). Sites: CN: control; SA: Calanque de Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou. Different letters above mean values (± standard error (SE)) indicate significant difference for the same parameter (Wilcoxon test).

Fig. 5: Monitoring of chlorophyll, flavonol, anthocyanin and leaf epidermal phenol indices in *A. halimus* leaves depending on PNCal soils types (n=10, p ≤0.05). Different letters following curves indicate significant difference for the same parameter (Wilcoxon test).

Fig. 6: Canonical plot of results of discriminant analysis with the percentages of each fungal structure (A: arbuscule, M: mycelium, V: vesicle and DSE: dark septate endophytes), and the 7 soil types as parameters (n=3 and $F_{(24,39)}$=7.67, P<0.0001; Wilk's Lamda = 0.002; Canonical1=59.9%, Canonical2=28.3%). For each category, inner and outer circles represent the 95% confidence intervals for the means and the 50% prediction intervals, respectively. The seven soil types explain 59.9% and 28.3% of the observed variance in fungal occurrence on axis 1 and 2, respectively.

Supplementary data:

Supp. data 1: Map of soil sampling locations (SA: Calanque de Saména; TR: Calanque des Trous; CC: Cap Croisette; E.C: Escalette Chimney; E.S: Escalette Slagheap; SO: Sormiou) and seed sampling location (E) in the Calanques national park (PNCal).

Supp. data 2: Average metal and metalloid concentrations (mg/kg of dry weight) in soil samples from each soil type. Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, p≤0.05, Wilcoxon test).

Supp. data 3: Evolution of Spearman correlations between germination percentages and soil parameters up to 15 days after imbibition (n= 10, p ≤0.05). ρX,Y: Spearman correlation, X=G: germination percentage, Y=pH, EC, TOC, (Na): Na concentration, (Zn) Zn concentration. The Line of Fit element shows a linear regression with confidence intervals in shady colors.

Supp. data 4: Bioaccumulation factor (BCF) and translocation factor (TF) values for each metal and metalloid in *A. halimus* growing on the different soil types. Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, $p \leq 0.05$, Wilcoxon test).

Supp. data 5: Na/K ratio in different soil types, and in root and shoot of *A. halimus* growing on these different soils. Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, $p \leq 0.05$, Wilcoxon test).

Figure1

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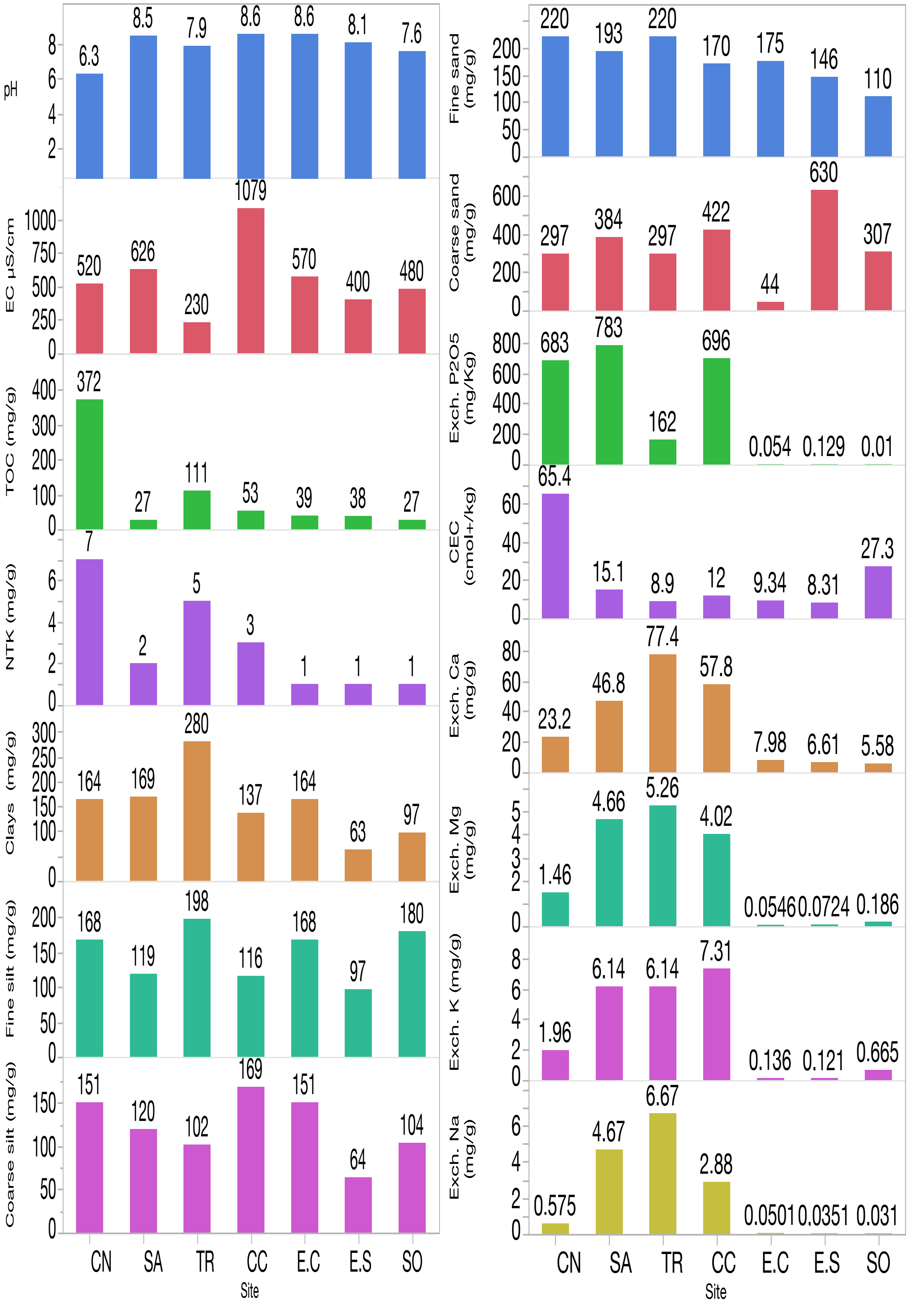

Figure2
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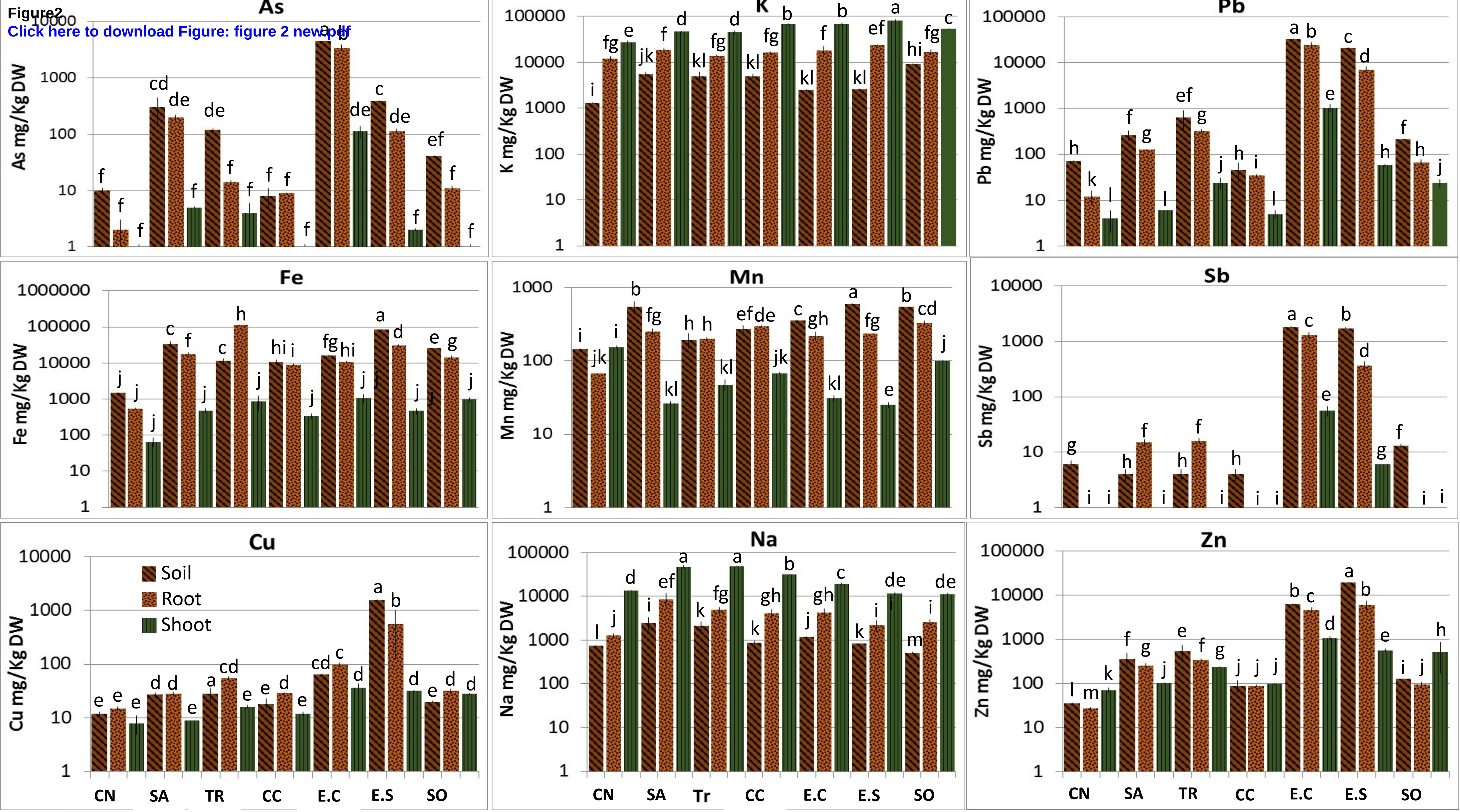
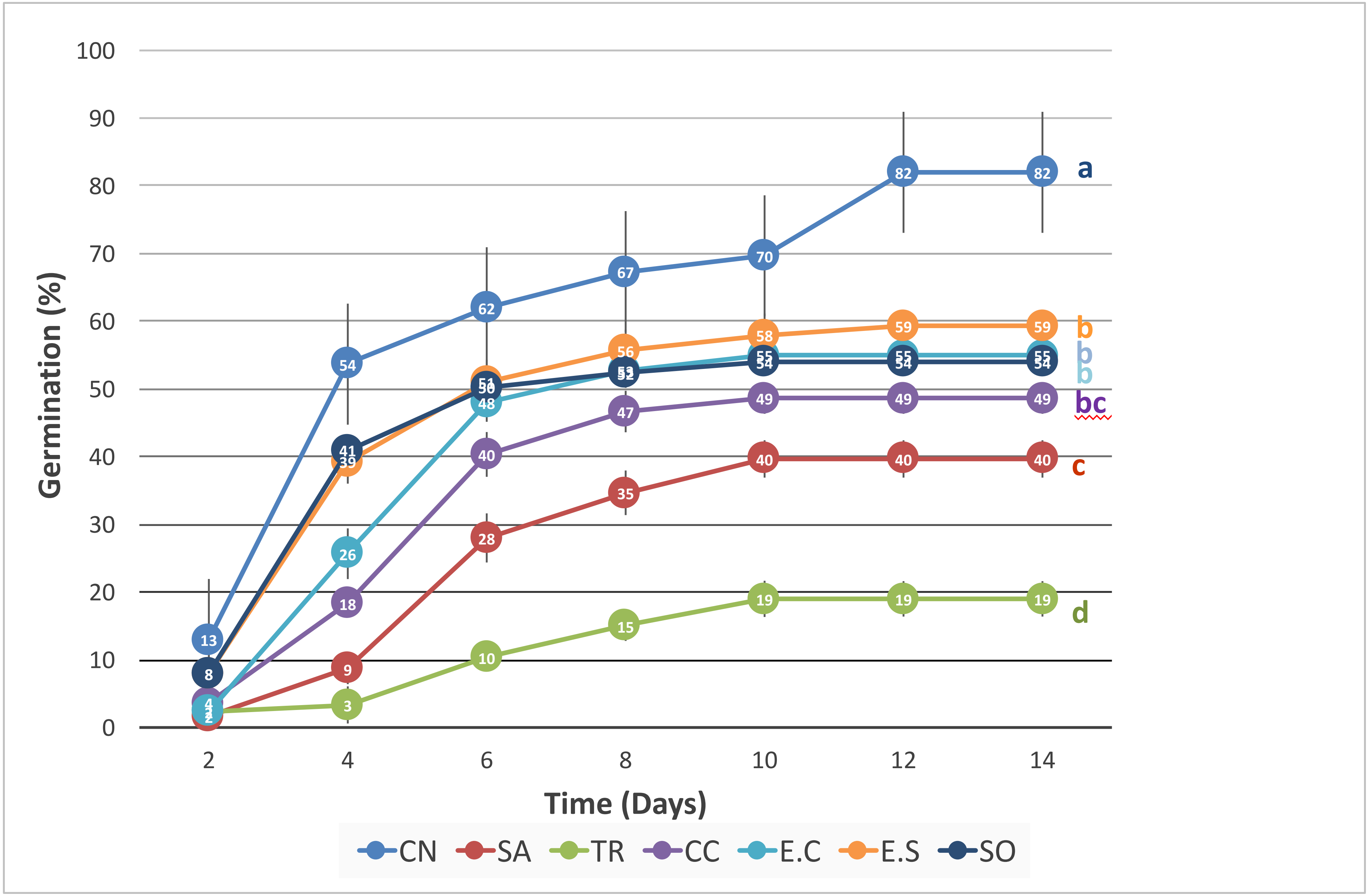


Figure3


Germination (%)
100
90
80
70
60
50
40
30
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0
2
4
6
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12
14
Time (Days)
a
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bc
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TR
CC
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Figure4

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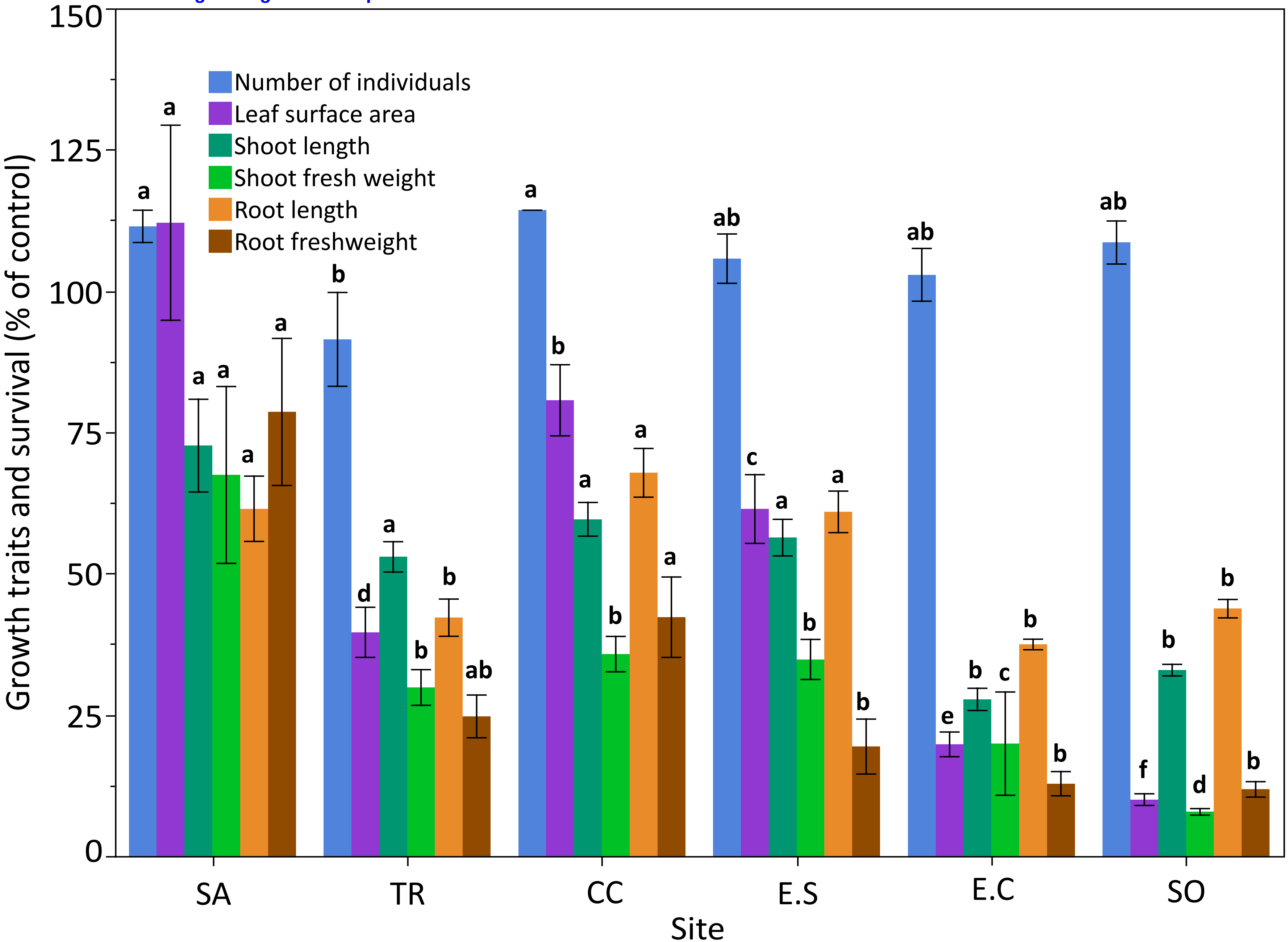

Figure5

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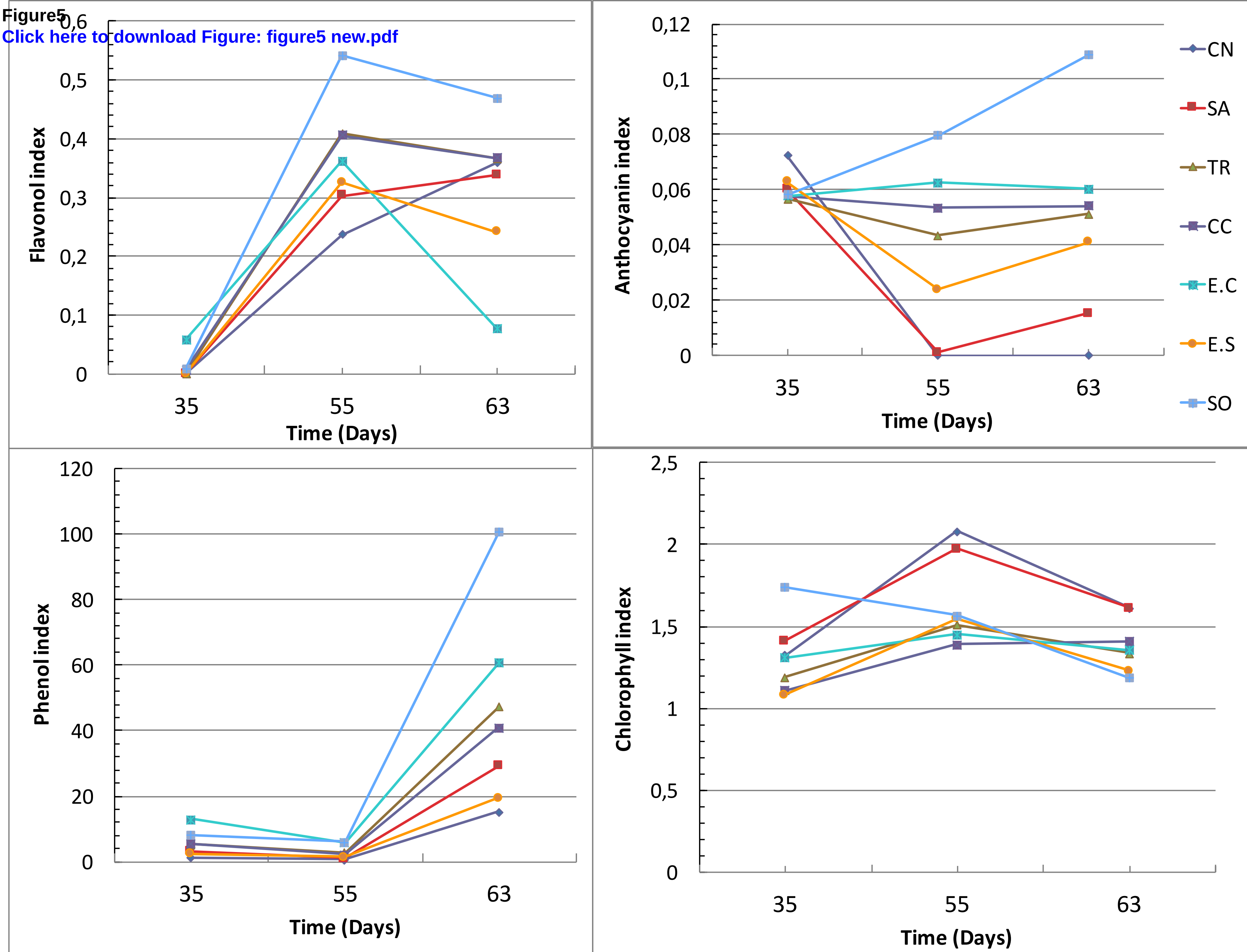

Figure 6

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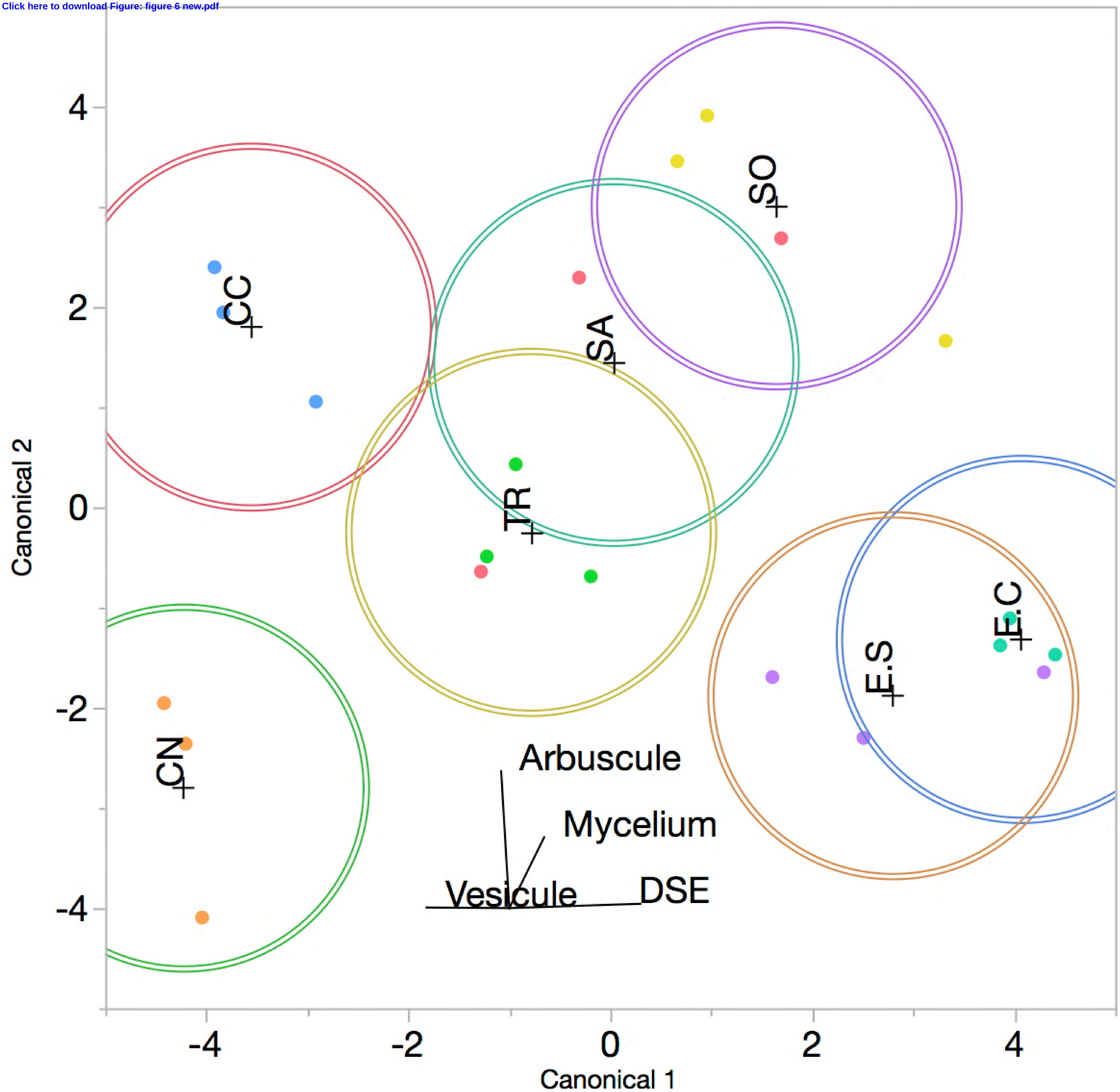
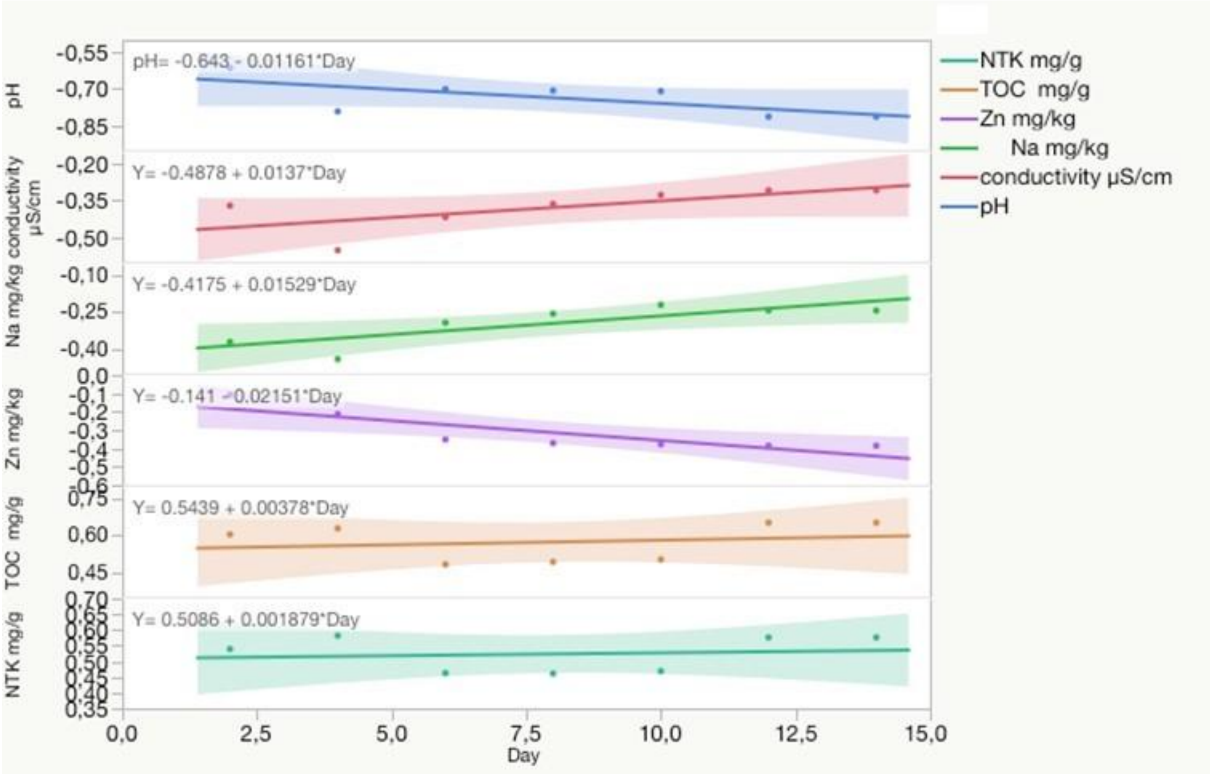


Supplementary data 2: Average metal and metalloid concentrations (mg/kg of dry weight) in soil samples from each soil type.

Soil origin	Element concentrations (mean ±SE, $mg.kg^{-1}$)								
	As	Cu	Fe	K	Mn	Na	Pb	Sb	Zn
Control (CN)	10±1b	12±1c	1491±14d	1303±13d	143±2d	737±11bc	70±3c	6±1c	35±1d
Calanque de Saména (SA)	301±139b	27±3c	33860±6535 b	5440±704b	540±108b	2486±804a	263±63c	4±1c	355±134cd
Calanque des Trous (TR)	13±4c	28±8c	11566±1788c	4918±1328bc	192±44cd	2084±566ab	628±283c	4±1c	528±200c
Cap croisette (CC)	8±3c	18±5c	10600±1654c	4978±588bc	271±35cd	867±108bc	45±21c	4±1c	88±26d
E. Chimney (E.C)	2470± 58a	110±1b	9270±231c	2499±82c	370±6bc	1202±17ab	28800±808a	2520±58a	7670±173b
E. Slagheap (E.S)	120±3bc	2100±58a	47500±1328a	2565±64c	760±17a	846±1bc	16200±462b	1720±46b	15200±289a
Sormiou (SO)	41±1c	20±1c	25993±264b	9174±29a	550±3ab	515±14c	210±3c	13±1c	126±1cd

Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, $p \leq 0.05$, Wilcoxon test).



Supplementary data 3: Evolution of Spearman correlations between germination percentages and soil parameters up to 15 days after imbibition (n= 10, p ≤0.05). ρX,Y: Spearman correlation, X=G: germination percentage, Y=pH, EC, TOC, (Na): Na concentration, (Zn) Zn concentration. The Line of Fit element shows a linear regression with confidence intervals in shady colors.

Supplementary data 4: Bioaccumulation factor (BCF) and translocation factor (TF) values for each metal and metalloid in *A. halimus* growing on the different soil types.

Soil origin	Elements								
	As	**Cu**	**Fe**	**K**	**Mn**	**Na**	**Pb**	**Sb**	**Zn**
					BCF				
Control (CN)	0.25±0.02c	1.22±0.05b	0.37±0.01e	9.11±0.58a	0.48±0.01c	1.74±0.08e	0.17±0.03b	0.16±0.01b	0.78±0.03bc
Calanque de Saména (SA)	0.77±0.12b	1.03±0.06bc	0.53±0.04d	3.52±0.19d	0.47±0.04c	3.75±0.77bcd	0.51±0.05b	4.0±0.4a	0.8±0.1bc
Calanque des Trous (TR)	0.120±0.003c	0.03±0.00e	0.24±0.00f	5.45±0.07c	0.26±0.01d	5.79±0.52a	0.02±0.00b	0.01±0.00b	0.02±0.00e
Cap croisette (CC)	1.2±0.1a	1.72±0.15a	0.85±0.05b	3.31±0.16d	1.09±0.05a	4.81±0.57abc	0.91±0.14b	0.25±0.02b	1.07±0.10b
E. Chimney (E.C)	1.39±0.09a	0.88±0.03c	1.16±0.04a	7.25±0.96b	0.59±0.04b	3.58±0.37cd	13.84±3.43a	0.2±0.08b	1.43±0.23a
E. Slagheap (E.S)	0.95±0.03b	0.39±0.03d	0.65±0.01c	9.33±0.08a	0.310±0.002d	2.56±0.21de	0.44±0.02b	0.21±0.01b	0.40±0.03d
Sormiou (SO)	0.26±0.02c	1.61±0.07a	0.55±0.02d	1.86±0.12e	0.60±0.02b	4.92±0.37abc	0.32±0.03b	0.07±0.00b	0.75±0.05c
					TF				
Control (CN)	0.62±0.05a	0.58±0.09b	0.12±0.02a	2.36±0.18e	2.25±0.05a	10.79±0.57a	0.44±0.1a	1±0a	2.58±0.18ab
Calanque de Saména (SA)	0.03±0.00c	0.32±0.02c	0.03±0.00ef	2.5±0.11de	0.11±0.01d	6.89±1.13bc	0.05±0b	0.06±0.00b	0.41±0.02e

Calanque des Trous (TR)	0.28±0.07b	0.29±0.02c	0.08±0.02bc	3.24±0.17cd	0.24±0.02bc	10.56±0.94a	0.07±0.01b	0.06±0.00b	0.68±0.02d
Cap croisette (CC)	0.17±0.00b	0.42±0.01bc	0.04±0.00de	4.18±0.15ab	0.23±0.00bc	8.41±0.79ab	0.15±0.01b	1±0a	1.10±0.03c
E. Chimney (E.C)	0.03±0.00c	0.38±0.04c	0.1±0.01cd	4.45±0.63a	0.14±0.01cd	4.88±0.49c	0.04±0.01b	0.05±0.01c	0.23±0.02ef
E. Slagheap (E.S)	0.02±0.00c	0.04±0.00d	0±0f	3.46±0.09bc	0.11±0.01d	5.62±0.52bc	0±0b	0.02±0.00d	0.10±0.01f
Sormiou (SO)	0.14±0.01b	0.89±0.04a	0.07±0cd	3.33±0.22c	0.31±0.01b	4.67±0.36c	0.39±0.05a	1±0a	1.91±0.11b

Different letters following means ± standard error (SE) in a column indicate significant difference ($n=3$, $p\leq0.05$, Wilcoxon test).

Supplementary data 5: Na/K ratio in different soil types, and in root and shoot of *A. halimus* growing on these different soils.

Soil origin	Soil Na/K	Root Na/K	Shoot Na/K
Control (CN)	0,5656±0,0008a	0,109±0,004c	0,51±0,03b
Calanque de Saména (SA)	0,42±0,02c	0,42±0,05a	1,03±0,04a
Calanque des Trous (TR)	0,33±0,01d	0,35±0,05a	1,09±0,08a
Cap croisette (CC)	0,1741±0,0003e	0,25±0,02b	0,470±0,003b
E. Chimney (E.C)	0,481±0,003b	0,25±0,01b	0,280±0,004c
E. Slagheap (E.S)	0,329±0,002d	0,091±0,008c	0,139±0,001d
Sormiou (SO)	0,056±0,0004f	0,16±0,02b	0,207±0,003d

Different letters following means ± standard error (SE) in a column indicate significant difference (n=3, $p \leq 0.05$, Wilcoxon test).

support data 1

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