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Selection of a halophytic plant for assessing the phytotoxicity of dredged seaport sediment stored on land

J.-P. Bedell, Y. Ferro, C. Bazin, Y. Perrodin

Abstract The filling of dry quarries in coastal areas with sediments dredged in seaports represents a potentially interesting method of recycling of these materials. However, this recycling requires the prior carrying out of an Environmental Risk Assessment of the scenario concerned. For this, the question arose as to the type of plants capable of developing on the surface of such a deposit and the method to implement for assessing the possible phytotoxicity of dredged sediments. To answer this question, we chose to work with halophytic plants to be free from the salt-related effect and to assess only the effect related to the toxic compounds present. Based on the objectives set, these works led to the use of common plants of the French coast, with direct seeding, and with pollution-sensitive plants. Three species of angiosperms, *Armeria maritima*, *Anthemis maritima* and *Plantago coronopus*, were finally tested. As a result of this work, *Armeria maritima* was retained as the most suitable plant for testing the possible phytotoxic effect of dredged marine sediments stored on land. The results obtained with this plant are as follows: germination of 40 % of the seeds

in 31 days, produced biomass of 493 mg FW in 6 months and a capacity to bioaccumulate metal pollutants in roots with 350 and 720 mg/kg DW for Zn and Cu, respectively.

Keywords *Armeria maritima* · *Anthemis maritima* · Halophyte species · Germination · *Plantago coronopus* · Seaport sediments

Introduction

Seaports are confronted with the necessity of regularly dredging harbour bottoms to maintain navigation and thus with the fate of these dredged sediments. The latter can contain various pollutants (heavy metals, organic pollutants, etc.) which, when they cannot be discharged into the sea, are directed to disposal channels on land [deposit in Final Waste Storage Centres, Incineration Centres, etc.] or recycling channels [recycling as materials, agricultural reuse, etc.]. Given the considerable quantity of potentially polluted maritime sediments generated by dredging, these procedures are nonetheless limited economically and environmentally and in terms of the volumes to be managed. This is how the Project SEDIGEST [GESTion Durable des SEDiments de dragage des ports marins–sustainable management of sediments dredged in seaports] addressed the channel “Filling of land cavities of the coastline”, which combines concern for the resolution of nuisance of anthropogenic origin, in this case, the creation of land cavities such as quarries (loss of

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space, landscape deterioration, loss of biodiversity, etc.) with the need for the reuse of dredged seaport sediments. This channel deals neither with unpolluted sediments that can be discharged into the sea nor with the most polluted sediments that are recognized as dangerous waste. It concerns materials that are usually pretreated (granulometric sorting, draining, biodegradation, etc.) and requires carrying out an Environmental Risk Assessment (ERA) of the scenario concerned beforehand. This ERA must be performed to check the absence of risks for ecosystems surrounding the cavity (watercourses, wetlands, terrestrial ecosystems, etc.), as well as the absence of risks to groundwater quality during its implementation.

In the light of the demands imposed by the need to preserve ecosystems and of the target ecosystems identified during this risk assessment, it appeared necessary in particular to have tests to assess the toxicity of seaport sediments to plants growing on the surface of such deposits. Indeed, previous works on dredged canal sediments have shown that these phytotoxicity tests could be included in an assessment methodology for risks related to their deposit (Perrodin et al. 2006; Bedell et al. 2003). A similar phytotoxicity test has also been performed recently on seaport sediment (Bedell et al. 2013). The “matrix” approach used in this methodology implies the performance of bioassays, with exposure of organisms to the complex matrix studied (in this case, the sediment). In practice, this involves exposing each target organism to a range of dilutions of the complex matrix, containing a mixture of different potentially toxic substances. Even if this matrix approach does not enable determining the substance(s) responsible for the effect observed (germination or not, growth or not, etc.), it has the advantage of incorporating different parameters, such as the composition of the material tested, notably in pollutants, but also its physicochemical properties (pH, water or nutrient availability, granulometry, density, etc.).

Other studies have investigated the use of dredged marine sediments for the construction of vegetated and landscaped dikes with the dredged sediments of the Scheldt river near the port of Antwerp (Luyssaert et al. 2001; Pensaert et al. 2005; Piesschaert et al. 2005). The spontaneous vegetation that developed on these deposits is dominated by a few salt-tolerant species such as *Atriplicetum littoralis*, *Matricaria maritima* or *Atriplex prostrata* (Piesschaert et al. 2005). In Mertens

et al.’s (2004) study, the tests of plantation of five species of trees on a mound of sediments taken from the Escaut estuary showed that the white poplar (*Populus alba*) can accumulate high concentrations of Cd and Zn whereas other species are more prone to phytostabilisation despite the high levels of Na and high conductivity of the matrices.

Phytotoxicity tests on marine sediments with glycophyte¹ species have shown their limitations with this type of material. For example, the works of Malcolm et al. (2003) related to measurements of germination and formation of plantlets for a halophyte² and a glycophyte in a nutrient medium in the presence of artificial seawater with “low” (160 mS m⁻¹) or “high salinity” (1,900 mS m⁻¹). In this study, the germinations of both a halophyte and a glycophyte were salt-tolerant. Other works have shown that halophytes, despite their specific nature, germinate better in low-salinity conditions (Ungar 1978; Baskin and Baskin 1998) and that they germinate in saline habitats during periods of low salinity, for example during high precipitation or during low evaporation (Ungar 1987; Shumway and Bertness 1992).

Within the framework of the assessment of risks related to the filling of gravel pits with marine sediments, we chose to work with halophytic plants to be free from the salt-related effect and assess only the potential effect of the pollutants present. This article therefore presents the work of selecting a halophyte species suitable for the ERA of a project to deposit seaport sediments on land involving the revegetation of the deposit surface.

It was conducted in three successive stages: (1) first, using a literature review, we sought plants likely to meet our selection criteria; (2) then, we tested these plants (three in total) to check the integrity of the seed lots bought before using them for assessing the phytotoxicity of a seaport sediment and (3) lastly, on this basis, we made proposals for the use of one of them in the overall risk assessment methodology established during the SEDIGEST project.

¹ Glycophytes are not plants naturally encountered on salty substrates although they can tolerate a certain quantity of salt.

² A halophyte is a plant adapted to salty media or, by extension, to media with high osmotic pressure.

Materials and methods

Selection criteria and physicochemical characteristics of the sediment tested

In order to provide a suitable material for testing, the selected halophytic plants, the sediment had to meet several criteria: (1) it had to be representative of sediments that can no longer be discharged into the sea, taking account of the regulations applicable in France since 14 June 2000 (Alzieu and Quiniou 2001; Gourmelon et al. 2003); (2) it had to be sufficiently contaminated to obtain marked results in tests with the selected halophytic plants (“worst case”) and (3) it could not have undergone any treatments incompatible with plant life, for example, a treatment based on hydraulic binders which generate a very alkaline pH, hindering the revegetation of deposits.

Based on these criteria, a sediment dredged from the port of Toulon in the south of France was selected. The main characteristics of this sediment, notably its contaminant levels, are shown in Table 1, where the two contamination levels (N1 and N2) of the French Regulation of 14 June 2000 “relating to the reference levels to be taken into account for their management” are also provided. The conditions of use of these thresholds are as follows: (1) below level N1, the possible impact is considered negligible, the levels being considered to be comparable to the environmental “background noise”; (2) between levels N1 and N2, a further investigation may be necessary according to the project considered and (3) above level N2, a further investigation is necessary since notable indices point to a potential negative impact of the operation. A specific study must therefore be conducted on the sensitivity of the environment to the substances concerned, with notably an assessment of the foreseeable impact on the environment. Dredged sediments exceeding threshold N2 therefore cannot be discharged into the sea and must be treated before storage on land. Sediments between thresholds N1 and N2 can be, according to the project and local context, in the same situation. In both cases, these sediments then take on the status of waste and their potential impact during their management on land must be assessed. This assessment concerns in particular the ecotoxicological effects of sediments and/or of their emissions. Table 1 shows that the sediment has, as sought, a neutral pH and a high level of contamination, as well as high chloride content. Comparing the

measured concentrations with that of the Regulation of 14 June 2000, the selected sediment presents values above thresholds N1 and N2 for Cu, Pb, most of the PCBs and PAHs but also and above all for TBTs (Table 1). The physicochemical characteristics of sediments were obtained by an approved external factory laboratory that uses several protocols and standards, such as NF EN ISO 6468 for PCB determination, NF EN ISO 11885 for trace element determination, ISO/WD/7981 for HAP and ISO 17353 for organotin compounds.

Selection criteria of halophytic plants to be tested

Literature

We set the following selection criteria for the selection of plants to be tested: (1) species known on the French coast, (2) plant species presenting a germination rate sufficient to reveal a possible inhibiting effect, (3) plant species whose growth lasts at least several weeks and (4) plant species that has already been the subject of works relating to the revegetation of sediment deposits.

Three species of angiosperms *Armeria maritima*, *Anthemis maritima* and *Plantago coronopus* drew our attention. We selected these three species for several reasons: the first is that these three species are halophytes and are present on French coasts. Hence, the revegetation of sediment massifs in coastal areas with these plants would not raise any concerns in terms of ecology since these species are naturally present on the territory. The second reason is the existence of a significant literature for these three species, although comparatively less rich than for other species (cf. *Arabidopsis thaliana*, “model” biological species). A more precise crossed search between these species and the sediment management thematic area also provides a literature that remains relatively sparse regarding these species and seaport sediments. *Armeria maritima* is mainly present in articles that describe studies of estuary sites or coastal areas (for example Rodwell and Pigott 2000), whereas, for example, *Anthemis maritima* and *P. coronopus* are mainly present in articles that describe a paleoenvironmental or even archaeological approach to ancient sediments (Marinova and Atanassova 2006; El-Din Fahmy 1997). However, we note that these literature searches led to the identification of several works conducted with *Armeria maritima* on sites contaminated by heavy metals (Schwartz et al.

Table 1 The main physicochemical characteristics of the sediment

		Sediment tested	Threshold	
			N1	N2
Dry content	(%)	25.0		
pH		7.2		
Conductivity	µS/cm	4,810		
Chlorides	(mg/L)	352		
Loss on ignition	%	7		
Organic matter	%	4.96		
TOC	%	4.02		
Ca	g/kg DW	177		
Fe		32.17		
P		0.35		
Inorganic contaminants (mg/kg DW)				
As		85	25	50
Cd		<LD	1.2	2.4
Cr		58	90	180
Cu		1,053	45	90
Hg		70	0.4	0.8
Ni		31	37	74
Pb		1,188	100	200
Sn		109	276	552
Zn		2,343		
PCB (µg/kg DW)				
PCB 28		<4	25	50
PCB 52		100	25	50
PCB 101		260	50	100
PCB 118		210	25	50
PCB 138		520	50	100
PCB 153		450	50	100
PCB 180		210	25	50
PAH (µg/kg DW)				
Fluoranthene		1,296.7	400	5,000
Benzo b fluoranthene		2,389.1	300	3,000
Benzo k fluoranthene		884.9	200	2,000
Benzo a pyrene		2,189.4	200	2,000
Benzo ghi pyrene		2,851.9	200	2,000
Indeno 1.2.3 pyrene		2,538.6	200	1,000
∑ 6 PAH		12,150.6	1,500	15,000
Total fuels	(mg/kg DW)	3,839		
Organotin compounds (µg Sn/kg DW)				
Tributyltin		2,011	100	400
Dibutyltin		835		
Monobutyltin		1,984		

<LD below detection limit, DW dry weight)

2001; Farago et al. 1980) but not with *Anthemis maritima*. Lastly, we note that our literature search brought out *Armeria maritima* among these three species but not on the basis of toxicity, ecotoxicity or phytotoxicity assessment approaches.

Selected halophytic plants

The application of the selection criteria outlined above therefore resulted in the selection of the three species of angiosperms described below and ordered from a supplier of salt-tolerant plants (Phytosem, Gap, France) which has some experience in the revegetation of coastline soils.

Armeria maritima Also known as “thrift”, “sea thrift” or “sea pink”, this plant belongs to the order Caryophyllales and to the family Plumbaginaceae. It is a plant that likes the sun and rocky ground found along the French coast (Channel, Atlantic). *Armeria maritima* communities are characteristic of an excessively drained soil, often very shallow at all the levels of rocky cliffs, occurring more often in cavities that accumulate thin mixtures of mineral and organic matter or around rocky outcrops. The soils are usually uncultivated: even where there is no inlier high on a cliff, vegetation is usually out of reach of the sea waves. The packaging received consisted of 640 seeds/g.

Anthemis maritima Commonly known as “maritime camomile”, this plant belongs to the family Asteraceae (also called Compositae). It is a perennial plant with thick, glabrous foliage. It likes the sun and sandy soils of heathlands or around the Mediterranean. We also note its presence on Brittany coasts. This habitat is found on the coastal cliffs of seabird (mainly Gulls) breeding sites, under a Mediterranean or thermo-Atlantic climate. The substrate, on limestone or siliceous bedrock, is more or less gravelly and enriched with organic matter (nitrate and phosphate inputs) in relation with the activity of seabirds. The packaging received consisted of 1,816 seeds/g.

Plantago coronopus Also called “Buck’s-Horn Plantain”, this species belongs to the family Plantaginaceae. It is a herbaceous plant, biannual or perennial, hermaphrodite, pubescent or hairy. It grows on dry sandy spots in the west and the south of France. The packaging received consisted of 7,700 seeds/g.

Tests and experiments

Checking the integrity of the lot of selected seeds

These three species were germinated to estimate the integrity of the seed lot received. To do this, we used three Petri dishes containing cotton soaked in distilled water. Fifty milligrams of seeds of each species was spread evenly in each of the dishes; then, the nine samples were placed in a thermostated culture room at 20.5 °C in the dark. About 385 *Plantago* seeds, 69 *Anthemis* seeds and 32 *Armeria* seeds were placed on Petri dishes for germination.

Tests of germination and growth of the three halophyte plants on the sediment

The bottom of an experimental pot was covered with a meshed plastic film. Then the bottom (up to one fifth of the pot height) was covered with wet Fontainebleau sand. On top of this layer of sand, either the sediment sample or the International Organization for Standardization (ISO) reference soil was placed. Five test lots were prepared for each plant species and for each matrix.

The reference soil or ISO substrate (OECD 2010) was a mixture of 10 % peat, 70 % silica [industrial quartz sand, more than 50 % particles must be between 50 and 200 µm and all the particles must be less than 2 mm], 20 % clay (less than 30 % Kaolin) and ≤1 % CaCO₃.

For each plant species and for each matrix, five replicates (or pots) were implemented. An identical number of seeds of each species was deposited onto the surface of the pot and slightly covered by the sediment or by the ISO soil. Cultures were performed at 20.5 °C in a thermostated culture room and with a 16 h photoperiod. Every 3 days, soft spray watering

was performed on each pot with deionised water. The germination criterion was the opening of the seed by the emergence of the 3 mm stem. A general observation of the state of plants was performed, and symptoms such as wilting, dehydration or discolouration of the foliage were noted. The tests in pots were monitored in this way at least once a week with the measurement of the length of aerial parts (from the ground to the maximal height of plantlets).

The growth of plants was monitored during periods ranging between 61 days and 6 months according to the pots used (size), the monitored parameter (biomass) and the tested species.

Assessment of the biomass production of the three halophytic plants

We recovered the whole plants by removing the mass of ISO soil or sediment from each of the pots and by softly shaking so as to remove as many particles from the roots as possible. The plants were then carefully washed while immersed in deionised water, after which the plants were spread on a sheet of paper and the aerial part and the roots were separated with scissors for so they could be measured. To establish the fresh weights, each part of the plant identified was weighed separately.

Measurement of the metal contents in the three halophytic plants

The metal contents (Zn, Cu, Cd and Cr) in the aerial part (stem and foliar part) and roots of the plants were determined as follows. After washing three times with tap water and rinsing three times with deionised water, the plants were dried for 48 h at 70 °C, after which they were crushed in an agate mortar. A sample of 0.5 g of dry matter was then dissolved using a mixture of 8 mL HNO₃ and 2 mL H₂O₂, heated in a CEM-type microwave oven (Mars X). After filtration through ash-free filter paper (Prolabo) (average filtration) and adjustment in a 25-mL phial with ultrapure water, the metals were analysed by inductive coupled plasma (ICP) and flame atomic absorption (Zeeman effect) (Zhao et al. 1994). Certified Reference Material (CRM 281 from BCR) and control samples as Surface Water Level 2 (Spectrapure standards) were used to check the complete recycling of the dissolution and/or the calibration of the metal studied.

Data analysis

All the statistical tests were performed with Statistica©Version 10. A value of 10 % for type I errors was chosen. Post hoc comparisons between samples were made using Fisher's test to determine which values significantly differed.

Results

Integrity of the seed lots

The results of the germination tests on soaked cotton are shown in Fig. 1.

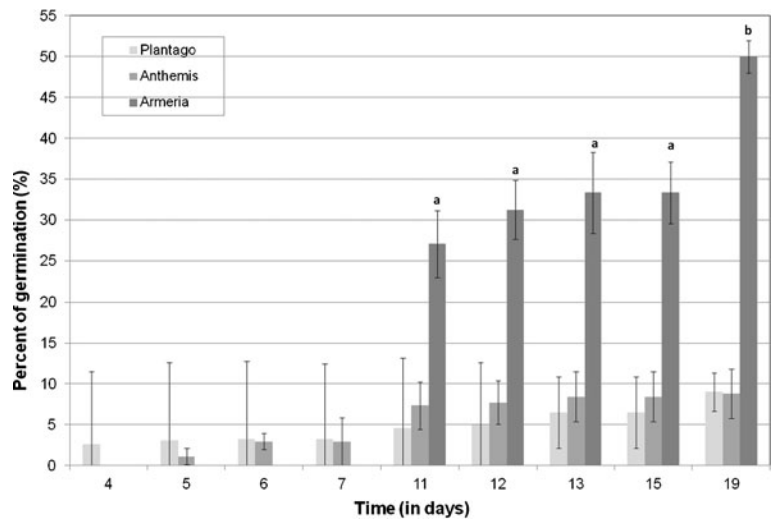
The species *P. coronopus* was the first to germinate on the cotton (after 4 days), but we also noted a considerable variability in this seed lot (Fig. 1). *Armeria maritima* germinated the latest (after 11 days), but this seed lot finally also presented the best germination rate (50 %) with a reduced variability. *Anthemis maritima* presented an earlier germination than *Armeria maritima* but with a much lower yield, of around only 10 %, even after 19 days (Fig. 1). Based on these tests, we concluded that the lot of *Armeria maritima* seeds presented the best homogeneity and that these seeds could be used for assessing a potential toxic effect on their germination. However, it should be noted that we found no or few recommendations to be taken into account for the germination of these three halophyte species on soaked cotton, except for those existing in general for seed germination (darkness, temperature) and that we applied. This could explain the low yields or the large variability observed on some species during these tests.

Phytotoxicity tests on the sediment

Germination tests

The tests in pots on the test sediment yielded very low germination rates for *Anthemis maritima* and *P. coronopus*, with 5.9 and less than 1 % germination, respectively. As for *Armeria maritima*, 38 % on average of the seeds deposited onto its surface germinated after 31 days (Fig. 2).

Fig. 1 Germination monitoring of the different seeds on cotton soaked during 19 days. [Means ($n=3$) \pm SD: histograms with different letters differed significantly (Fisher's test at $p<0.01$)]

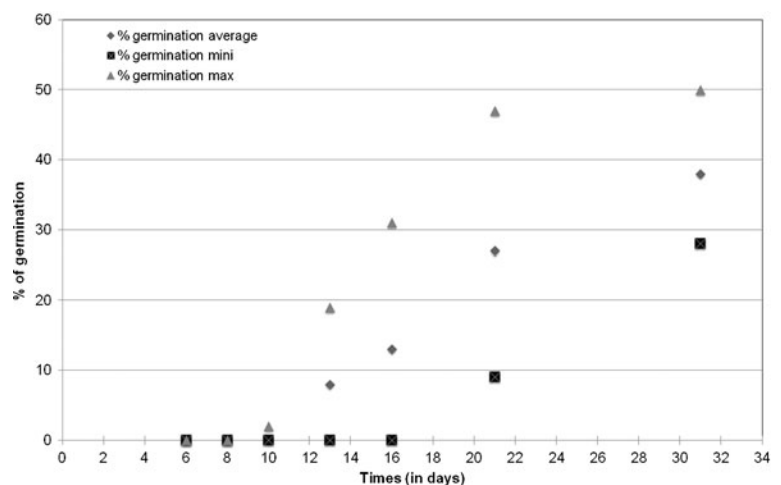


Growth tests

The growth monitoring measurements were performed only on *Armeria maritima*, given the low germination yields obtained with the other species. The results of monitoring *Armeria maritima* growth on the sediment tested are shown in Table 2.

On the tested sediment, about 40 % of the seeds germinated after 31 days, with the first plantlets appearing only after 13 days of culture (Fig. 2). The variability observed throughout the monitoring indicated non-homogeneous germination as already observed on the Petri dish tests with cotton (Fig. 1). However, the yields obtained remained of the same order of magnitude in both cases.

Fig. 2 Percentage of *Armeria maritima* germination on the tested sediment during 31 days ($n=5$)



Although the monitoring was continued for a longer period, we observed that a larger number of plantlets developed on the ISO soil (after 2 months) than on the test sediment (after 6 months) (Table 2).

The ISO "soil" (OECD 2010) is only a culture substrate, a homogeneous mixture of peat, silica, clay and CaCO_3 , and therefore does not provide optimal conditions (nutrient contents for example) for the long-term development of plants. Despite this, besides the number of plantlets, the growth parameters, i.e. the length of the aerial parts and the biomass produced, showed that *Armeria maritima* developed much better on ISO soil than on the test sediment (Table 2). Indeed, the plantlets obtained on the test sediment were fewer and presented very significant lengths for a lower bio-

Table 2 Growth of *Armeria maritima* on ISO substrate and on the tested sediment (n=5)

	Number of seedlings (mean±SD)	Length of aerial part (mean±SD)	Length of root part (mean±SD)	Biomass of aerial part	Biomass of root part
Growth on	(/20 at the start)	(cm)	(cm)	(mg FW/plant) for 61 days old	(mg FW/plant) for 61 days old
ISO substrate (during 61 days)	17.66±1.52	3.82±1.04	4.81±2.17	40.27	9.8
Sediment tested (during 6 months)	11.80±4.12	4.02±1.17	8.10±3.50	25.44 ^a	5.45 ^a

FW fresh weight

^aIn order to compare the values obtained at 61 days old (ISO substrate) with values obtained for 6 months (on sediment tested), we divided by 3 these biomass. Such calculated values are listed in the table for biomass comparison of aerial part and root part of *Armeria maritima*

mass. This may be the sign of an atypical development, probably the expression of some toxicity or of either nutritional or osmotic stress. Moreover, some symptoms of physiological dysfunction (such as chlorosis, the expression of the loss of the chlorophyllous pigment responsible for photosynthesis) were observed on few plantlets developing on the test sediment (results not shown). However, *Armeria maritima* was able to germinate and develop on the test sediment despite its contamination and its initial salinity.

Biomass production

The fresh biomass produced after 6 months culture on the test sediment yielded the results shown in Table 3, with very considerable variations for the species *P. coronopus* and *Anthemis maritima*.

Armeria maritima gave a yield in terms of biomass that was sufficient to reveal an effect and to allow further measurements (bioaccumulation, enzyme activities, etc.). Obviously, this biomass production should be related to the low yield of germination and hence of plantlets likely to develop on the test sediment.

Table 3 Biomass produced by the three species growth in 6 months on the sediment tested. (n=3)

	Total fresh biomass (mg FW total)	
	Mean	Standard deviation
<i>Armeria maritima</i>	492.7	162.5
<i>Anthemis maritima</i>	216.3	156.3
<i>Plantago coronopus</i>	51.7	52.3

Bioaccumulation

Cu concentrations and, to a lesser extent, Zn concentrations were localized in the root system, with the exception of *P. coronopus* which showed an identical distribution between the roots and the aerial parts (Fig. 3). In terms of concentration, we found more Zn and Cu in the roots of *Armeria maritima*. We also noted a preferential accumulation of Cu in the roots of *Anthemis maritima*, which was significant in relation to Zn and to the aerial parts of this plant (Fig. 3). As for Cd and Cr, their localization was mainly in the roots, whatever the species tested (Fig. 3). The three species showed different behaviours with regard to Zn and Cu, but in terms of bioconcentration, *Armeria maritima* accumulated these two elements the most in its roots. This was also the case for Cr and Cd, for which *Armeria maritima* showed the highest concentration values.

Discussion

The tests performed on halophytic plants in order to neglect the effect of NaCl led to the selection of common plants of the French coast with direct seeding.

Finally, three species of angiosperms *Armeria maritima*, *Anthemis maritima* and *Plantago coronopus* were tested. Among these, *Armeria maritima* presented the most homogeneous seed lot and this species was retained for testing a potential effect on seed germination. Moreover, this species showed a sufficient germination and growth rate on the sediment tested. The following discussion therefore focuses on *Armeria*

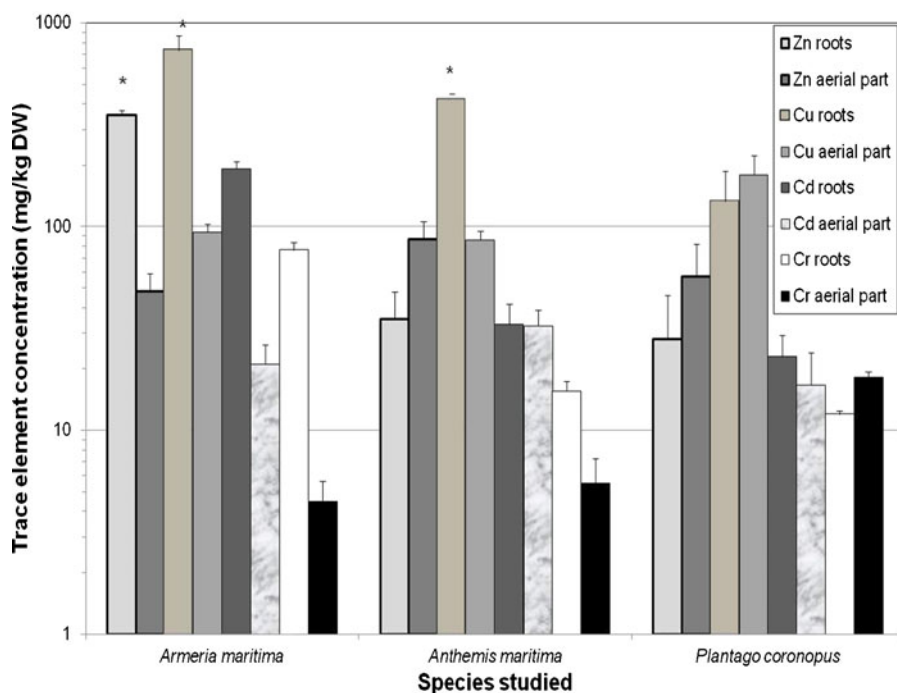


Fig. 3 Zn, Cu, Cd and Cr contents in the roots and the aerial part of the three species tested after 6 months of growth on tested sediment. [Means ($n=3$) \pm SD: histograms with asterisks differed significantly (Fisher's test at $p<0.01$); DW dry weight]

maritima and on its germination and growth potential on a test sediment and, more broadly, on salty sediments.

An important characteristic of halophytic plant seeds which distinguishes them from glycophytes is their capacity to remain dormant in the presence of very high salinity and to germinate later, when salinity is reduced (Ungar 1978). Piesschaert et al. (2005) emphasized that the “zero” management of deposit sites of dredged seaport sediments is recommended if the water and salt levels of the sediment allow the implementation of spontaneous halophyte revegetation. Otherwise, they recommended the implementation of different management options, such as revegetating with mixtures of herbaceous plants on the dikes of the port of Antwerp (Belgium). Using different mixtures of grasses on these dredged seaport sediments, they showed that such plants could develop with an abundance of 40 to 60 % (Piesschaert et al. 2005). In the light of our low germination yields close to these values; the question was raised about performing a hormonal pretreatment of halophyte seeds before using them. However, Hanslin and Eggen (2005) showed that most of the tested species germinated well in pure water without the addition of hormonal treatment

(gibberellin, GA 4+7), except for some species, such as *Lupinus perennis* or *Beta vulgaris*, which showed very little germination, with or without treatment with gibberellin GA4+7. Gibberellic acids and other growth regulators could therefore increase salt-tolerance during germination to reach levels similar to that of halophytic plants (Ungar 1978). The response obtained varies, however, according to the species (Khan and Ungar 2001; Gulzar and Khan 2002; Khan et al. 2002, 2004). These gibberellin treatments are known to eliminate some effects of salinity on germination, and the amplitude of this effect is greater in halophytes than in glycophytes (Ungar 1978). Under saline soil, the germination rate of cress seeds can be reduced; however, compost application retrieves this effect (Lakhdar et al. 2009). Hence, germination in high-salinity conditions for some of the most tolerant species can be overestimated. This is why we preferred not to treat *Armeria maritima* seeds beforehand, so as not to overestimate the germination and growth potential of this plant on seaport sediments.

In the works conducted on Cd accumulation by Redondo-Gomez et al. (2010), the germination of a halophytic Cd-hyperaccumulator *Arthrocnemum macrostachyum* was subjected to an alternating diurnal

regime of light and temperature chosen to mimic the autumn conditions in the Odiel marshes and thus enable the germination of the seeds. This approach enabled the authors to obtain a sufficient germination and growth rate for their analysis. Other experiments with *Armeria maritima* have been conducted after making the seeds germinate in Petri dishes on paper filters soaked in distilled water during 7 days before potting them for tests of tolerance of this species to Zn, Cd and Pb (Olko et al., 2008). This could be a working hypothesis to improve the germination yield of this species.

Germination is based on the emergence and growth of the radicle. The seeds differ regarding their optimum temperature, their capacity to remove the part of the fruit surrounding the seed, their capacity to respond to soaking and in the disappearance or reduction of their germination in the presence of salts (Mayer and Poljakoff-Mayber 1963). However, plantlets of halophytic plants have the capacity to survive and grow in the presence of salts that are toxic for glycophytic plants (Malcolm 1986). Osmond et al. (1980) established that the transition from germination to the establishment of the plantlet is the most critical phase for plant growth. Regarding the growth of *Armeria maritima* on the test sediment, the orders of magnitude obtained in terms of biomass are comparable with other studies owing to the specificity of our growth medium matrices (ISO soil and test sediment). Using seeds of *Armeria maritima* from Scottish salt marshes, Loughman et al. (1983) obtained growth for 60 days of about 90 mg FW/seedling, but within the framework of growth tests with a standard solution and its different nutrients. Köhl (1997) obtained biomasses from 52.5 to 54.1 mg DW/seedling for two salt-marsh ecotypes of *Armeria maritima* after 3 months culture on artificial soil (sand + ion exchanging synthetic resin with nutrients). The biomass values obtained in our experiments are below the abovementioned values, but they come from cultures without any nutrient input and under relatively comparable experimental conditions. Furthermore, Köhl (1997) showed that enrichment in Zn of his artificial soils significantly decreased the biomass produced by these two tested marine ecotypes of *Armeria maritima*. Our test sediment presented high values in Zn but also in other contaminants that can have a limiting effect on growth and on the biomass produced. Moreover, observations of chlorosis and depigmentation on certain *Armeria maritima* plantlets during their growth on the test sediment are indications of

stress (nutrient or osmotic) or toxicity for the plant (results not shown). Although there was no observation of chlorosis in the halophytic plant *Arthrocnemum macrostachyum*, a decrease in chlorophyll and carotenoid contents was observed in experiments with increasing Cd concentrations (Redondo-Gomez et al. 2010).

Armeria maritima showed a good capacity to develop but also to accumulate certain metallic elements of the sediment tested. Previous studies have already shown its capacity to develop on soils with substantial heavy metal contamination (Schwartz et al. 2001; Becker and Brändel 2007; Olko et al. 2008; Anic et al. 2010) and that it was an indicator of Cu mineralization (Neumann et al. 1995; Farago et al. 1980). *Armeria maritima*, under Zn stress on artificial soil, was shown to be a species in which metalliferous and non-metalliferous populations are equally metal resistant in long-term tests (Köhl 1997). Köhl (1997) also suggested that genes related to metal resistance are active independently of the metal supply in the heavy metal populations. More recently, *Armeria maritima* ssp. *halleri* growing on soils enriched with zinc and lead showed a localization of Zn in cell walls, plasmodesmata and vesicles in the leaf glands (Heumann 2002). Previously, copper excretion by leaf glands has been demonstrated in a metal-tolerant *Armeria* species (Neumann et al. 1995). The complex leaf gland functioning in *Armeria maritima* highlights new mechanisms that occur in all salt-plant and metal-tolerant species of Plumbaginaceae.

The limitations of our approach are linked to the low yields achieved (germination and biomass). This point could bring into question our hypothesis of “zero management” direct seeding. Here, we addressed the aspect of bioaccumulation, but it would also be interesting to address that of pollutant stabilization (for example, with the measurement of simple chemical extraction potential) or the state of health (fitness) of the plant, for example, by measuring different enzyme activities as physiological indicators. Similarly, account should be taken of the agronomic quality of the sediment by N/K/P measurements and C/N ratio.

The growth of *Armeria maritima*, although with low yields, was possible on the test sediment. The germination of its seeds, the biomass it produced and its capacity to bioaccumulate pollutants make it an operational tool for revealing the possible toxic effect of a sediment on this plant. This plant is therefore a good

candidate for ERA and, more precisely, for assessing the phytotoxicity of marine sediments.

Stehouwer and Macneal (2003) recorded an increase of germination and fescue seedling establishment after the first leaching event as a response to the salt decrease following compost amendment. Based on this observation, it is possible to conceive an assessment procedure that includes measuring the residual toxic effects of a sediment following an increasingly longer leaching phase with water, by column percolation or by forced leaching in a rotary shaker. This approach using a column-leaching test has been used previously on similar seaport sediment but only to assess the effect of phytotoxicity on germination (Bedell et al. 2013). Thus, on the basis of the rainfall of the site studied and of the residual toxicity results obtained, this would enable in determining the duration required to revegetate the deposit, notably by halophytic plants such as *Armeria maritima*.

Conclusion

Of the three halophytic species tested, *Armeria maritima* presented the most homogeneous seed lot. This species was therefore retained for expressing a possible toxic inhibitory effect on the germination of halophytic plants. Besides it being present in the existing literature, the seed lot of this species was suitable for performing germination and growth tests. Moreover, the germination rate and the growth and the yields obtained were sufficient on the test sediment to reveal a possible phytotoxic effect. Finally, its bioaccumulation capacities make it a good candidate for the ERA of a seaport sediment deposit within the framework of a quarry filling scenario and a suitable bioindicator for characterizing the potential toxicity of dredged seaport sediments. These different results also enabled us to select this species as sufficiently tolerant for immediate seeding, but further information on its long-term behaviour and its development with other species are still required.

The restoration of saline habitats in general and the revegetation of marine sediments in particular pose major challenges, since very few plants can establish under high-salinity conditions and thus enable phytorestation. In order to improve the revegetation potential of these deposits, solutions could include mixing the superficial sediment layer of the deposit with

local soil or covering the sediments with a layer of arable soil. However, these approaches are not completely satisfactory and considerably increase the costs of restoration, thereby emphasising the interest of using halophytic plants.

The use of phytorestation on dredged saline sediment is relatively new. Therefore, for the purpose of promoting the restoration and the revegetation of maritime sediment deposits, it is necessary to continue works in the domain and in particular to improve knowledge of the halophyte species that can be established on these deposits.

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