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Assessment of the Phytotoxicity of Seaport Sediments in the Framework of a Quarry-Deposit Scenario: Germination Tests of Sediments Aged Artificially by Column Leaching

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Abstract The aim of the Sustainable Management of Sediments Dredged in Seaports (SEDIGEST) project is to assess the risks of treated port sediments for terrestrial ecosystems when deposited in quarries. We simulated the "ageing" of these sediments up to the "moment" when plants can germinate. Sediments were leached by water percolating through a laboratory column. Sediments 1 and 2, taken from the port of Toulon (France), were dried and aired. Sediment 3, taken from the port of Guilvinec (France), was stabilised with lime. Phytotoxicity was evaluated on the three artificially aged sediments using germination and early development tests (48 h to 7 days) by Phytotoxkit F^{TM} bioassays. The three dilutions tested were performed with the reference "ISO substrate" and with *Lolium perenne* sp. (rye grass), *Sinapis alba* (white mustard), and *Lepidium sativum* (watercress). The tests performed with sediments 1 and 2 showed (1) a decrease of their toxicity to the germination of the species selected following leaching and (2) that *L. perenne* was the most sensitive species. The tests performed with sediment 3 showed that it was improper for colonisation even after leaching simulating 16 months of ageing. These germination tests on aged sediments identified the effects of leaching and made it possible to appreciate the capacity of the sediments to allow colonisation by plants.

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Seaport sediments are often highly contaminated by heavy metals (RNO 1988; Andral et al. 2004; Lafabrie et al. 2008; Schintu et al. 2009) and organic compounds, such as organotins, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (Gomez-Gutièrrez et al. 2007; Mille et al. 2007; Cassi et al. 2008). In France, regulations for the management of dredged marine sediments are based on two levels of contamination (N1 and N2) set out in the French Decree of June 14, 2000 "relating to the reference levels to be taken into account for their management" (Alzieu and Quiniou 2001). The conditions for using these thresholds are as follows: (1) lower than level N1 = the potential impact is deemed negligible because the contents are considered to be comparable with environmental background contamination, (2) between level N1 and level N2 = additional investigation may be necessary depending on the project considered, and (3) higher than level N2 = additional investigation is necessary because the significant indices recorded give rise to the assumption that the operation has a potentially negative impact. It is therefore necessary to perform a specific study focused on the sensitivity of the environment to the substances concerned, in particular, with the assessment of the foreseeable impact of the latter. Dredged sediments higher than threshold N2 cannot therefore be discharged into the sea and must be treated before storage on land. Sediments with levels between thresholds N1 and N2 can be considered as being in the same situation as a function of the project and the local context. In both cases, the potential impact of these sediments during the time they are managed on land must be assessed (Peijneneburg et al. 2005). This assessment concerns, in particular, the evaluation of ecotoxicological effects linked to sediments and/or their emissions (Gourmelon et al. 2003). Nonetheless, relevant legislation currently fails to define an ecotoxicological test to assess the impact of sediments on terrestrial environments. The choice of the organisms to be tested is very important when evaluating the ecotoxicity of contaminated sediments.

Studies on seed germination are considered representative of short-term impacts and above all allow evaluating the effects of acute toxicity. These tests have shown a significant decrease of the germination of certain species in soils and sediments contaminated by different metallic trace elements (Adam and Duncan 2002; Chen et al. 2002; Maila and Cloete 2002; Bedell et al. 2003a). Plant germination and growth tests are bioassays often performed in the domain of ecotoxicology to identify acute toxicity. Different standards, both French (AFNOR 1982, 1986) and international (ISO 1995; Organisation for Economic Co-operation and Development [OECD] 2006), are applicable. These bioassays assess in particular the potential inhibition of seed germination by solid matrixes. In addition, different studies have shown the usefulness and efficiency of these phytotoxicity tests for evaluating the toxicity of environmental (soils, sediments) and anthropic (compost, wastewater treatment plant sludge [WWTP], sewage sludge) matrixes (Fuentes et al. 2004; Bedell et al. 2006; Czerniawska-Kusza et al. 2006; Oleszczuk 2008; Oleszczuk et al. 2012). Thus microplate studies using *Lepidium sativum* led to the evaluation of the toxicity of WWTP, composts rich in PAHs and heavy metals (Oleszczuk 2008), sediments of eutrophic rivers (Czerniawska-Kusza and Kusza 2011) and sediments of urban canals containing fuel derivatives, PAHs, and heavy metals (Czerniawska-Kusza et al. 2006). Recently such tools have also been used to evaluate the influence of activated carbon and biochar on the phytotoxicity of air-dried sewage sludges to *L. sativum* (Oleszczuk et al. 2012). Last, in addition to the contaminants present in marine sediments, residual salinity can harm plant life. The ability of a plant to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water and maintain ion homeostasis (see interesting review of Parida and Das 2005). Thus, sediment salinity can harm plants and their development in the same way as can sediment heavy-metal content (Tiquia and Tam 1998; Chen et al. 2002).

Our study was performed in the framework of the Sustainable Management of Sediments Dredged in Seaports (SEDIGEST) project (www.sedigest.org). This project, financed by the French programme ANR PRECODD, is intended to formulate a method for assessing the potential risks of treated seaport sediments to terrestrial ecosystems in a quarry-fill scenario. In the framework of this scenario, our objective was to determine the level from which deposited sediment could be colonised by plants. Consequently, this entailed simulating "the ageing" of these sediments, particularly under the action of rain, until reaching the "moment" at which plants can germinate and colonise the deposit. This is why we simulated leaching by percolating water through sediments placed in columns to obtain sediments "aged" by leaching from 1 to 16 months. The assessment of phytotoxicity was performed on three sediments aged artificially by carrying out a germination test. Our aim in this study was to examine the potential phytotoxicity of aged sediments and verify whether inland plant seeds are suitable for assessing the toxicity of seaport sediments.

Materials and Methods

Sediments

Choice and Characteristics

The sediments studied were chosen first for their representativeness of the treatment solutions considered for their management (SEDIMARD Programme [http://www.ports-developpementdurable.com/ports_plaisance_developpement_durable/2009/res/aqua.pdf2]), and, second, for their level of contamination (the requirement for a response in the framework of this methodological development programme) and their physicochemical characteristics. Thus the sediment used here came from the SEDIMARD programme in which these three treatments were performed after sieving to eliminate the coarse fraction (gravels, etc.). Sediment 1 (Toulon 0–20) came from the French port of Toulon (district 83) and was dredged in April 2008, dried and aired for 5 months (April to September 2008), and then screened at 0–20 mm (end of November 2008). Sediment 2 (Toulon fines) also came from the port of Toulon. It was taken on March 31, 2006, cleaned (beginning of April 2006), and dried and aired for 5 months (April to September 2006). Sediment 3 (Guilvinec) was taken from the French port of Guilvinec (district 29) in January 2007, dried and aired for 5 months, and then stored until April 2008. It was then screened at 0–20 mm, stabilised in lime and hydraulic binder (April to May 2008), and crushed before being screened again at 0–20 mm. The main characteristics of these sediments after these treatments, notably their contaminant contents, are listed in Table 1, which also includes the two levels of contamination (N1 and N2) of the French Decree of June 14, 2000. The physicochemical characteristics of the sediments were obtained previously at the end of SEDIMARD programme by using several protocols or standards described 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.

Table 1 Main physicochemical characteristics of the sediments

Sediment	1 (Toulon 0–2)	2 (Toulon fines)	3 (Guilvinec)	Threshold N1	Threshold N2
Dry content (%)	24.2	25.0	20.9		
pH	7.6	7.2	12.3		
Conductivity (µS/cm)	3,880	4,810	3,180		
Chlorides (mg/L)	635	352	382		
Loss on ignition (%)	11.5	7	8		
Organic matter (%)	18.53	4.96	7.83		
TOC (%)	10.3	4.02	5.3		
Ca (g/kg DW)	119	177	134		
Fe	23.9	32.17	16.18		
P	0.56	0.35	0.24		
Inorganic contaminants (mg/kg DW)					
As	99	85	16	25	50
Cd	<LD	<LD	<LD	1.2	2.4
Cr	48	58	48	90	180
Cu	1,098	1,053	728	45	90
Hg	7	70	<LD	0.4	0.8
Ni	28	31	<LD	37	74
Pb	772	1,188	185	100	200
Sn	128	109	40	276	552
Zn	2,068	2,343	665		
PCB (µg/kg DW)					
PCB 28	<4	<4	<10	25	50
PCB 52	50	100	40	25	50
PCB 101	150	260	60	50	100
PCB 118	130	210	70	25	50
PCB 138	180	520	80	50	100
PCB 153	280	450	5,080	50	100
PCB 180	100	210	50	25	50
HAP (µg/kg DW)					
Fluoranthene	3,161.8	1,296.7	7,270	400	5,000
Benzo b fluoranthene	2,782.5	2,389.1	2,860	300	3,000
Benzo k fluoranthene	1,265.9	8,84.9	1,620	200	2,000
Benzo a pyrene	3,114.5	2,189.4	4,350	200	2,000
Benzo ghi pyrene	3,220.5	2,851.9	2,210	200	2,000
Indeno 1.2.3 pyrene	3,107.9	2,538.6	2,010	200	1,000
Σ6 HAP	41,509.7	12,150.6	20,320	1,500	15,000
Total fuels (mg/kg DW)	5,903	3,839	3,541		
Organostannic compounds (µg Sn/kg DW)					
Tributyltin	3,748	2,011	NM	100	400
Dibutyltin	8,336	835	NM		
Monobutyltin	26,473	1,984	NM		

<LD lower than detection limit, *NM* not measured, *DW* dry weight

These three sediments present high levels of contamination compared with thresholds N1 and N2 (Table 1). Thus, the values of copper (Cu) and lead (Pb) PCB (e.g., PCB118 and PCB 153), and benzo[k]fluoranthene for the three sediments selected were greater than the N1 and N2 thresholds (Table 1). These sediments with high levels of contamination were chosen at the beginning of the study to generate a clear response from the plants in view to validating the methodology. Thus, the level used does not correspond to a level of contaminant pollution for

Table 2 Summary of percolation durations as a function of volumes to simulate leaching durations

Modelled duration (months)	Duration of percolation (days)	Volume water (mL)/column	Weight of column (kg)
1	2	47.5	1.9
2	4	95	1.9
4	8	190	1.9
10	20	475	1.9
16	32	760	1.9

sediments capable of being used to fill-in terrestrial quarries (for example, pollutant concentrations lower than levels of acceptance in landfills with inert waste).

Sediment Ageing

The objective was to "simulate" the ageing of sediments treated by subjecting sediment samples to given quantities of percolation water (representative of a given time of exposure to rainwater) so as to have available sediments representative of different storage durations under site conditions. Column leaching has been used to study the mobility of toxic elements in industrially contaminated lands and to evaluate the predictive mobility of metals in runoff in urban soils and dredged sediments (Tack et al. 1999; Anderson et al. 2000; Crosnier and Delolme 2002). Several factors can influence the leaching of sediment deposit, such as rain frequency and humidity/dryness alternation (Piou et al. 2009). In the framework of the scenario considered, given an average rainfall of 1 m/year, *i.e.*, 1 m^3/m^2/year, and with an evapotranspiration rate of 70 %, *i.e.*, 30 % of infiltration in the sediment layer, we obtained an annual liquid/solid ratio of 0.3 for the upper soil layer of the deposit supporting the vegetation. Liquid-to-solid ratios were then determined to simulate natural ageing of 1, 2, 4, 10, and 16 months, *i.e.*, 2, 4, 8, 20, and 32 days, respectively, of water percolation in the column (Table 2).

The set-ups designed to allow water to circulate through the sediment were composed of polyvinyl chloride columns [PVC] that were 40 cm high and 10 cm in diameter (Fig. 1). The tops of the columns were sealed with screw-on covers. For each column, a hole was drilled at the base to inject water, and another orifice was drilled in the upper cover to collect the percolate. The water therefore circulated from the bottom upward, thereby optimising contact between all of the sediment and the water driven by the pump. The pumps supplied a continuous flow rate according to the time periods tested in a climate room (25 °C). pH and conductivity were measured in the percolates at the outlets of the columns at the end of the experiment (Table 3). Two replicates of each column were performed (Fig. 1).

Germination Test

Plant organisms were directly exposed to the aged sediments obtained using the column protocol. Given the quantities of matrix available (sediment and control), the bioassays were performed for each microplate from 48 h to 7 days with three types of seeds: *Lolium perenne* sp. (rye grass), *Sinapis alba* (white mustard), and *L. sativum* (watercress).

Germination-inhibition tests were performed on microplates (Phytotoxkit F™; R-Biopharm, France [2004]). This microbiotest measures both the decrease/absence of seed germination and the decrease of root growth after exposing the seeds to sediment/contaminated soils and toxicants. The bioassays were performed with each of the three test plants. Sediment and ISO substrate were placed successively on testing plates and humidified to 70 % of retention capacity. The plates were then covered with filter paper and the seeds of the test plants. Ten seeds were positioned at equal distance on the filter paper. The microplates were closed by a cover to limit evaporation and then incubated vertically in darkness from 48 h to 7 days at 19 °C in a climate culture room. Three dilutions were tested as follows: 100 % (*i.e.* 100 % aged sediment), 50 % (*i.e.* 50 % aged sediment and 50 % ISO substrate), and 10 % (*i.e.* 10 % aged sediment and 90 % ISO substrate). Seed viability and quality were estimated by germination with the same protocol as before but with only ISO substrate in the microplates. Duplicates of germination test were performed for each sediment dilution, *i.e.*, ageing, dilution, and species were performed for each a total of four microplate replications (Fig. 1). The microbiotest procedure was described in detail in the standard procedure (Phytotoxkit [2004]). The reference soil used or ISO substrate (OECD 2006) is a mixture of 10 % peat, 70 % silica (industrial quartz sand; >50 % of particles must be 50–200 µm; and all of the particles must be <2 mm), 20 % clay (<30 % kaolin), and ≤1 % $CaCO_3$.

Concerning the germination rate on only the ISO soil (100 and 0 % sediment ageing), the averages were, respectively, 90 % for *L. perenne*, 91 % for *S. alba* and 88 % for *L. sativum*. The intravariability of seed germination capacity measured did not exceed 7 % (maximum) for the three species tested. We can conclude that the viability of the seeds we used was good and that germination was not inhibited by such "poor/neutral" substrate. The germination of these seeds makes it possible to test them for potential inhibition by the sediments with a minimum variability of 7 %.

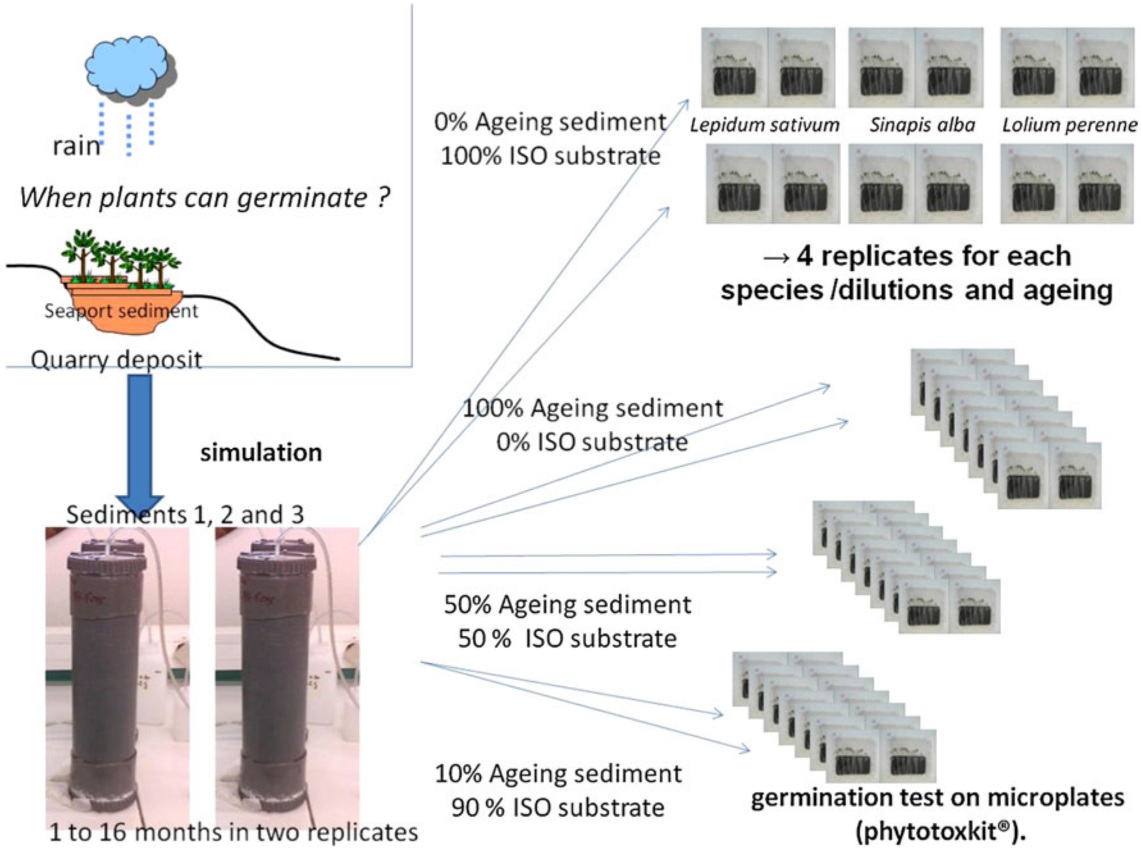

Fig. 1 Schematic representation of the study

Table 3 conductivity obtained in the column percolates of the three sediments ($n = 2$)

Sediment	Duration modelled (months)	Duration of percolation (days)	pH of leachate	Conductivity of leachate (µS/cm)
1 (Toulon 0–20)	1	2	7.56 ± 0.30	17,850 ± 1,378
	2	4	7.55 ± 0.54	17,310 ± 2,029
	4	8	8.32 ± 0.28	17,720 ± 1,025
	10	20	7.39 ± 0.50	17,270 ± 1,305
	16	32	7.88 ± 0.12	14,430 ± 1,063
2 (Toulon fines)	1	2	7.60 ± 0.18	18,270 ± 3,578
	2	4	7.10 ± 0.07	14,600 ± 3,748
	4	8	8.05 ± 0.67	13,550 ± 2,701
	10	20	7.81 ± 0.30	13,480 ± 2,743
	16	32	7.80 ± 0.14	13,000 ± 2,333
3 (Guilvinec)	1	2	12.06 ± 0.6	17,690 ± 3,718
	2	4	11.93 ± 0.95	13,910 ± 2,087
	4	8	11.75 ± 0.41	10,320 ± 1,396
	10	20	11.88 ± 0.36	12,670 ± 1,404
	16	32	12.03 ± 0.34	13,110 ± 1,138

Data Analysis

All of the statistical tests were performed using Statistica version 10©. A value of 10 % for type I errors was chosen (notably with regard seed-germination capacity). To test the influence of the sediment on percentage of germination on one species, we used one-way analysis of variance to show statistical differences in toxicity. *Post hoc* comparisons between samples were made using Fisher test to determine which values differed significantly.

Results

Preamble

Concerning the pH and conductivity values of the percolates, we recorded (1) a stable pH value (±0.95 maximum) and (2) a decrease in conductivity for all three sediments tested when simulating their ageing by leaching in the columns. However, the conductivity values obtained at the column outlet simulated for 16 months were still high (13,000–14,430 µS/cm). Indeed, the conductivity value at the end of the test corresponded to 71–80 % of the initial conductivity value as a function of sediment. Conductivity decreased as a function of the time passed for sediments 1 and 2. However, for sediment 3, the decrease in conductivity was not strictly proportional to the time simulated. We assume that this was certainly related to the treatment (liming) responsible for different chemical reactions through time and leaching in the column. These were linked to carbonates and hydroxides (Peng et al. 2009) but not investigated here.

Inhibition of Germination Obtained for Sediment 1 (Toulon 0–20)

We counted the number of seeds that had not germinated and compared them with the seeds initially deposited to calculate the percentage of inhibition with the different replicates of each test plant and dilution (Figs. 2, 3, 4).

After 16 months of simulated leaching of sediment 1, we observed that *L. perenne* was the most sensitive of the three plant species tested (Fig. 2a–c). After 16 months leaching, inhibition of the germination of this species still reached 40 % for the nondiluted sediment (100 %) (Fig. 2a). For a dilution of half the leached sediment (50 %) in the ISO substrate, inhibition ranged from 40 % for month 1 to 25 % for month 16, but this without significant difference (Fig. 2a). Last, when the sediment was diluted at 10 %, the inhibition of *L. perenne* seed germination did not vary as significantly with the duration of leaching with inhibition remaining at 25 % after 16 months of simulation. The highest inhibition (70 %) observed at 2 months with 100 % sediment showed a significant difference with the ageing simulation and/or proportion of sediment in the box (Fig. 2a).

Whatever the case, inhibition therefore decreased as a function of the proportion of sediment present and also more clearly for the tests performed with sediment alone as a function of age simulated, with the exception of month 2. *L. perenne* was sensitive to germination at the three ratios tested for this sediment.

Except for the significant inhibition of 40 % obtained with sediment only after 1 month of simulated ageing, sediment 1 leached for durations simulated for 1–16 months presented relative low toxicity to the seeds of *S. alba*, and this toxicity decreased significantly with the duration of leaching (from 40 % inhibition of germination for month 1–5 % for month 16; Fig. 2b). We noted that most inhibitions were obtained with sediment alone (Fig. 2b). Dilutions at 50 and 10 % of sediment leached with the ISO substrate showed no significant inhibitory effect on the germination of *S. alba* (Fig. 2b).

The inhibition of *L. sativum* germination by sediment alone was significant (75–50 %) at ≤10 months of simulated leaching (Fig. 2c). At month 16 of simulation, the inhibitory effect decreased again to 20 %, but this was not significant with respect to other inhibition effects observed during the dilution tests. In contrast, whatever the duration of leaching, dilution of the sediment with ISO substrate lessened its toxicity to germination of the seeds of *L. sativum*. However, hardly any significant differences in percentage of germination inhibition were observed as a function of the time of leaching with the diluted sediment tested (Fig. 2c).

To conclude, we note that the three plant species selected presented different sensitivities to sediment 1. Thus, *L. perenne* was particularly sensitive, whatever the mode of attenuation (leaching or dilution with the ISO substrate). *S. alba* was almost insensitive to this sediment, and *L. sativum* was more sensitive than *L. perenne*, although this sensitivity vanished with the dilution of the sediment likewise with its simulated ageing.

Inhibition of Germination Obtained for Sediment 2 (Toulon Fines)

The inhibition of *L. perenne* seeds in the presence of sediment 2 was in the same region of magnitude (from 10 to 30 %) whatever the ratio tested (Fig. 3a). Neither the ISO substrate nor simulated ageing time appeared to have any significant "dilution" effect. However, inhibitions remained lower than those observed for sediment 1 (Figs. 2a, 3a). Regarding *S. alba*, except for significant inhibition (55 %) for sediment alone after 1 month of simulation, all of the other values obtained for this sediment showed no significant inhibitory effect on germination of this species (Fig. 3b). For *L. sativum*, the sediment leached to simulate 1 month of rainfall also led to the highest germination inhibition at 35 % for sediment alone (Fig. 3c). The other values observed were either lower or there was no significant difference between them (Fig. 3c).

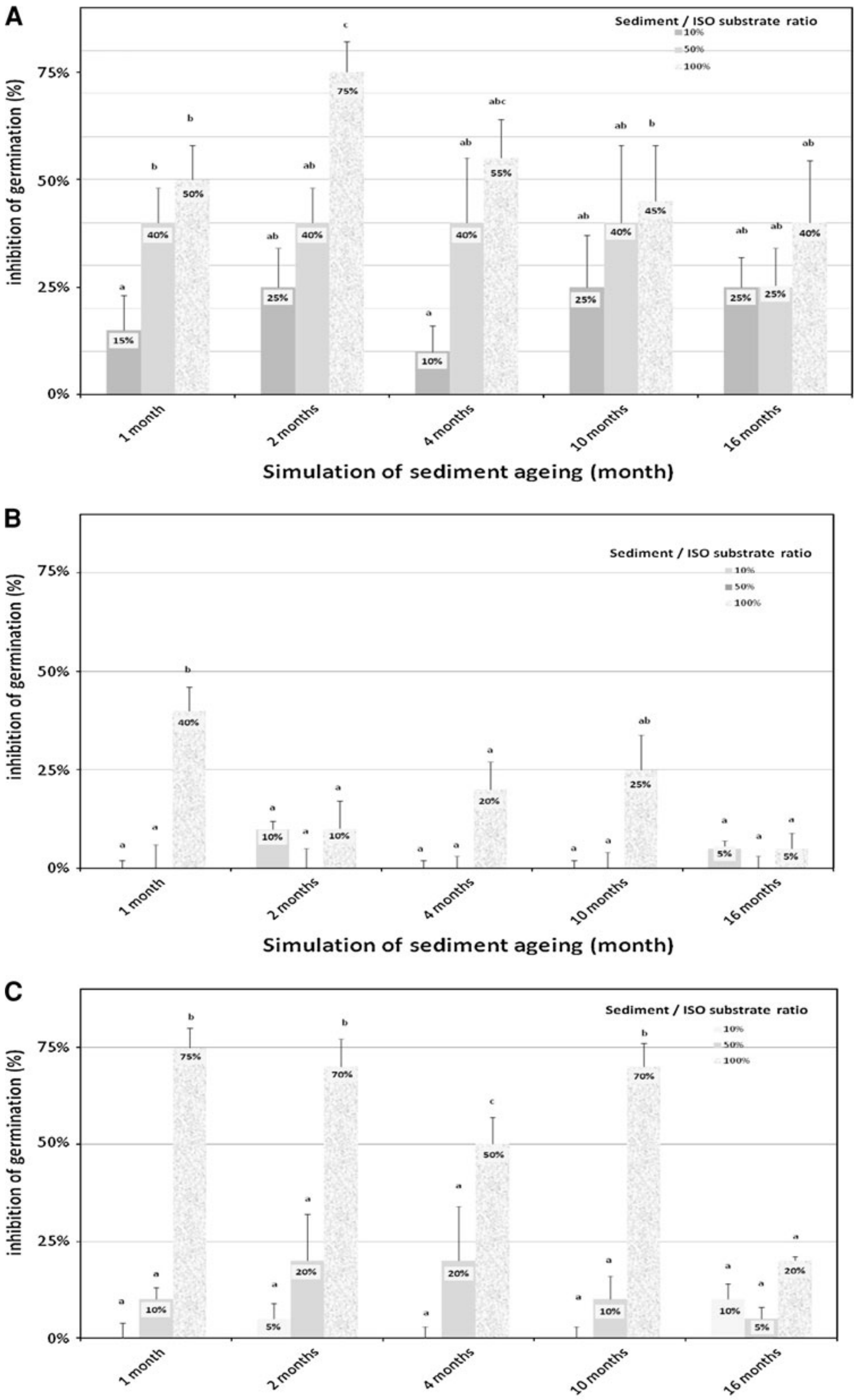

Fig. 2 Inhibition of germination of *L. perenne* (**a**), *S. alba* (**b**), and *L. sativum* (**c**) with sediment 1. Means ($n = 4$) ± SD. *Histograms* with different *superscript lower-case letters* differed significantly (Fisher's test $p < 0.01$)

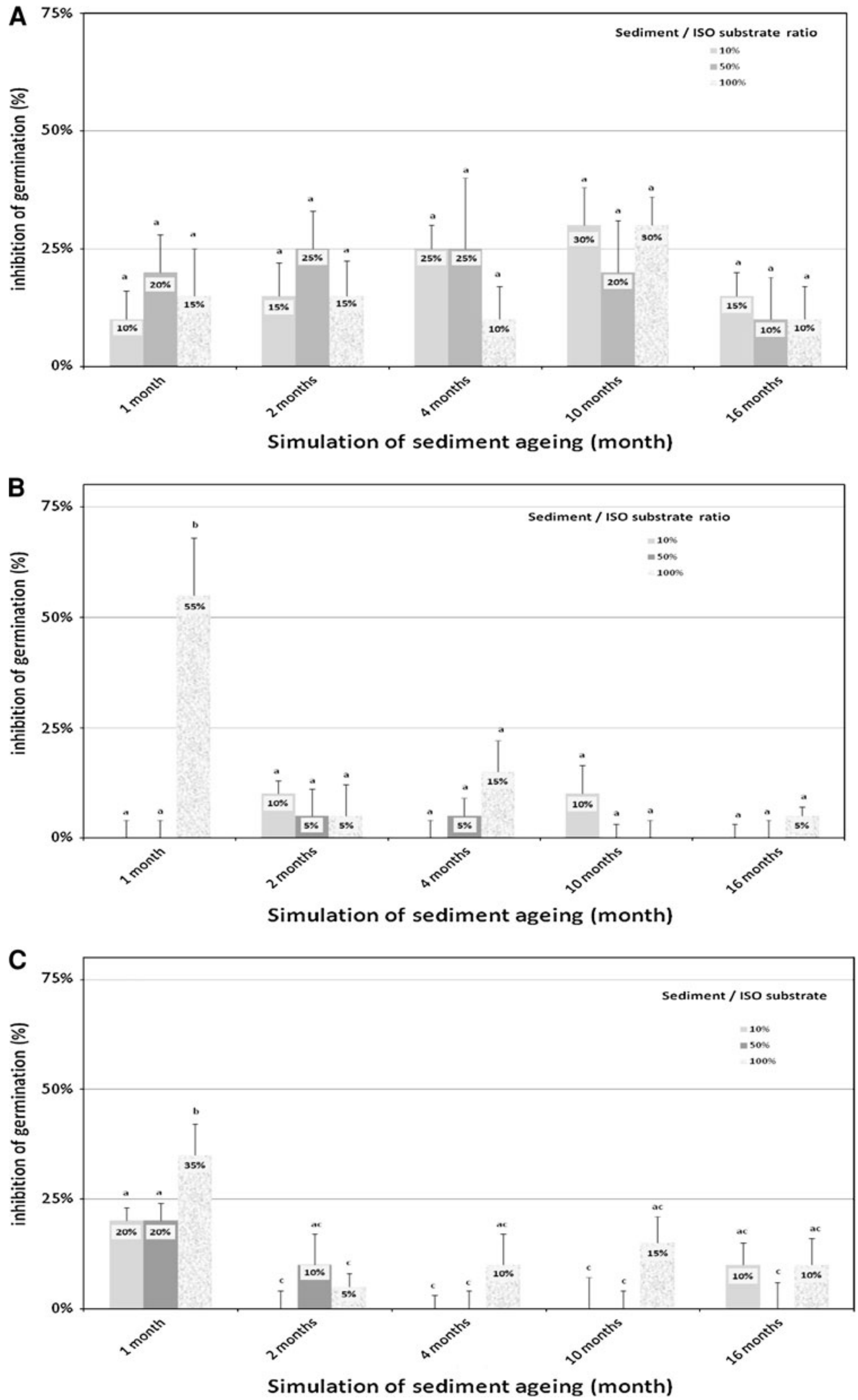

Fig. 3 Inhibition of germination of *L. perenne* (**a**), *S. alba* (**b**), and *L. sativum* (**c**) with sediment 2. Means ($n = 4$) $\pm$ SD. *Histograms* with different *superscript lower-case letters* differed significantly (Fisher's test $p < 0.01$)

To conclude, more or less the same assessment was achieved as for sediment 1. The three plant species selected presented different sensitivities to this sediment. *L. perenne* was especially sensitive $\leq$10 months without toxicity being decreased by the ISO substrate. The germination of *L. sativum* and *S. alba* seeds appeared to be only slightly sensitive to this sediment. Last, for sediment 2, significant decrease of the initial toxicity with simulated ageing was observed.

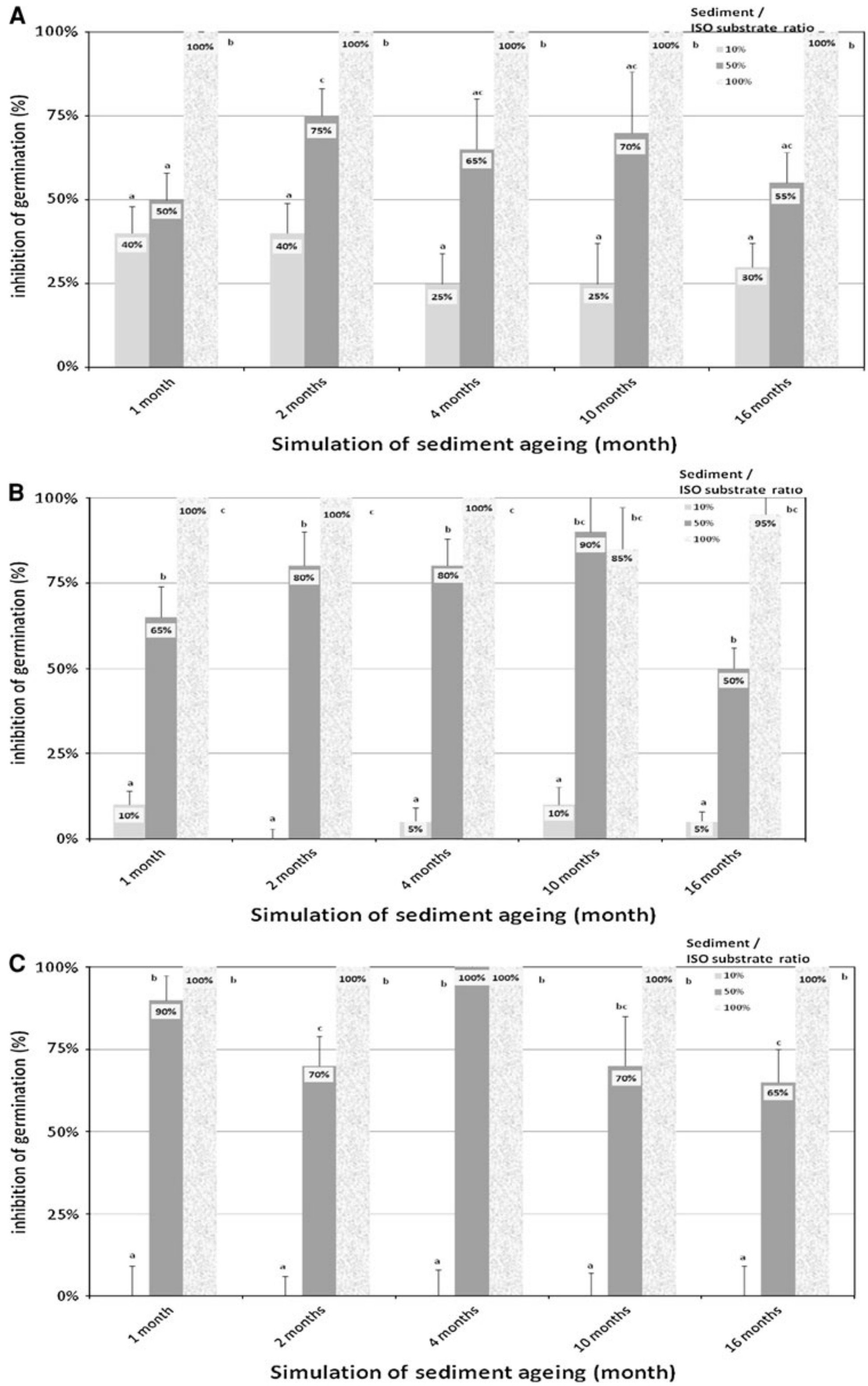

Fig. 4 Inhibition of germination of *L. perenne* (**a**), *S. alba* (**b**), and *L. sativum* (**c**) with sediment 3. Means ($n = 4$) ± SD. *Histograms* with different *superscript lower-case letters* differed significantly (Fisher's test $p < 0.01$)

Inhibition of Germination Obtained for Sediment 3 (Guilvinec)

Sediment 3 inhibited the germination of all of the *L. perenne* seeds deposited, even after 16 months of simulated leaching (Fig. 4). The inhibiting effect on germination was significant whatever the duration simulated or proportion of ISO substrate added. Sediment 3 inhibited all of the germination tests on *L. perenne* seeds (Fig. 4a), doubtless due to its alkaline pH caused by liming. However, we noted that dilution by the ISO substrate attenuated the toxicity of the sediment. Therefore, from months 1 to 16, the rate of germination inhibition decreased from 100 % with the nondiluted sediment, to 50–75 % inhibition with the sediment half diluted by the ISO substrate, and to 25–40 % inhibition with 10 % sediment in 90 % ISO substrate; however, hardly any significant differences due to high variability were obtained (Fig. 4a).

We observed the completely inhibitory effect of the sediment alone on *S. alba* seeds (Fig. 4b). A slight decrease of inhibition was recorded but at values that remained high when this sediment was mixed with 50 % ISO substrate (Fig. 4b). Last, only a low proportion of sediment 3 in mixture (10 %) allowed all of the *S. alba* seeds to germinate (Fig. 4b). The dilution of sediment decreased the inhibition of such seeds more than did ageing.

Sediment 3 inhibited all of the *L. sativum* seeds when they began germinating (Fig. 4c). Even with a mixture of 50 % with the ISO soil, the percentage of inhibition of these seeds remained high (Fig. 4c). Leaching simulated for 16 months appeared to decrease inhibition, although the percentage of the latter remained high, and the difference is as significant as those observed at 2 months. Only the ratio with 10 % sediment in the mixture allowed the germination of *L. sativum* seeds (Fig. 4c). Sediment 3, therefore, did not permit the germination of this species except when it was mixed considerably (in proportions of at least 10 %) with a substrate propitious to germination.

To conclude, for the three species, sediment 3 (Guilevinec) presented the following: (1) complete inhibition of germination when it was tested without dilution, (2) nonsignificant decrease of germination inhibition over time for the three species tested, and (3) significant attenuation of germination inhibition only for the concentration tested at 10 % sediment and 90 % ISO in soil. The high pH of this treated sediment (liming) undoubtedly explains this systematic inhibition of germination for all three species.

Discussion

Germination is a complex physiological phenomenon that occurs during the initial growth stage of plants. For a plant to germinate and a seedling to grow, the seed must be moistened with water (the entry of water into the seed through soaking) and it must be able to respire to fulfill different protein syntheses leading to the formation of the radicle (Bedell et al. 2003b). The main environmental conditions responsible for these phenomena are water content, temperature, oxygen, light, carbon dioxide, and the presence of different chemical substances (Bewley and Black 1994). Generally, solutions extracted from the solid are used to assess the phytotoxicity of a soil or solid residue when performing germination tests (Zucconi et al. 1981; Tiquia and Tam 1998; Bedell et al. 2003b). The results of the germination tests therefore, express the chemical characteristics of the material tested without taking into account its physical properties (notably regarding water and its availability). Therefore, the salinity and content in NH_4^+ ions of sediments can have harmful effects on plants and their development in the same way as can their heavy-metal contents (Tiquia and Tam 1998; Chen et al. 2002). Phytotoxicity assays using Phytotoxkit (2004) with seeds of *L. sativum* and *S. saccharatum* performed on four seaport sediments elutriates have shown no significant difference for seed germination compared with controls with ultrapure water (Mamindy-Pajany et al. 2011).

For our study, however, we were able to classify the three sediments tested as a function of their phytotoxicity potential from most to least effect as follows: sediment 3 > sediment 1 > sediment 2. This classification does not reflect the content of pollutants measured as much as it does the content of chlorides initially present in these three sediments, and the pH value for sediment 3. In their study of seaport sediments, Mamindy-Pajany et al. (2011) performed a principal components analysis (PCA) showing that phytotoxicity is positively involved in the second component of variation of the responses, whereas fines and organic matter are negatively associated with this component. In contrast, both of the toxicity tests (i.e. LuminoTox and the phytotoxicity test on *L. sativum*) and the pollutant contents were positively linked to the first component of variation (64.6 %). These PCAs, which include chemical data and toxicity responses in sediments, show that the relationship between the two kinds of variable are not clear (Mamindy-Pajany et al. 2011). In our study, we found a strong correlation (with the limits of only seven values available in most cases) between conductivity, zinc (Zn) and Pb contents, and PCB101 and PCB118 contents with the different inhibition tests on *L. perenne* germination. Thus, sediment 2, which had high values of such pollutants, highlighted the explanatory parameters for the inhibition of *L. perenne* germination.

However, Czerniawska-Kusza et al. (2006), in tests on contaminated sediments using *Sorghum saccharatum* (sorghum), *Linum sativum* (linen), and *S. alba* (mustard), suggested that sediments rich in organic matter and mainly composed of silt can significantly influence the response of plants. In our study, globally, sediment 2, which was composed of treated fines, presented greater concentrations of contaminants than did sediment 1. Regarding the results of percolation by flows of water rising through columns, and the difference of decrease in conductivity observed between the percolates of sediments 1 and 2, we can assume that in the first case, the water transits through a matrix with coarser and less homogenous elements than in the second case, in which the particles are finer and more homogenous, thus leading to less efficient extraction of soluble elements. Additional measures, in particular of organic and inorganic contaminants, will allow validating or invalidating this hypothesis on hydrodynamic (and/or physical) mechanisms occurring within the columns.

In addition, tests on stabilised WWTP sludges have shown different responses for *Hordeum vulgare* (barley) and *L. sativum* (cress) according to whether a sludge or an aqueous extract is tested (Fuentes et al. 2006). The study of germination with extracts and sediments from canals also highlighted differences of response as a function of plant species and greater sensitivity of *L. perenne* (rye grass), notably to the pollutants present in these sediments (Bedell et al. 2003b). Other investigators have shown stimulated growth in *S. saccharatum* (sorghum) and inhibited growth in *L. sativum* (cress) using the sediments of eutrophised rivers (Czerniawska-Kusza and Kusza 2011). In the study by Mamindy-Pajany et al. (2011), the sensitivity of *L. usitatissimum* (linen), *S. saccharatum* (sorghum), and *L. sativum* (cress) was tested on port sediments. Root growth was assessed after a period of prolonged incubation, and it was seen that two samples of port sediment significantly inhibited the elongation of *S. saccharatum* roots; in contrast, they stimulated the growth of *L. sativum* roots. Czerniawska-Kusza et al. (2006) observed the same sensitivity of these two species to contaminated sediments. For our study, *L. perenne* presented the highest sensitivity to the three sediments followed, in descending order of sensitivity, by *L. sativum* and *S. alba*.

The data obtained in our study indicate that the three species chosen are sensitive to the three sediments in varying degrees. Such inhibition of seed germination can be due to a particular characteristic of the sediment, such as its pH, salinity, or pollutant content (metals, organotins, etc.). One of the latter can have a negative effect on seed soaking, an essential factor at the beginning of germination.

In "Materials and Methods" section, the choice of terrestrial plants is justified by the results of previous studies in which the chloride concentrations acceptable for freshwater organisms and plants were lower than 1.5 g $Cl^- L^{-1}$ (Postma et al. 2002; Mamindy-Pajany et al. 2011). Here we have a maximum of 0.63 g $Cl^- L^{-1}$ in sediment 1 and lower values for the two other sediments. This leads us to assume that our observations on germination are not only related to salinity but are more complex. This is also why tests on these marine sediments with these "inland" species—rather than coastal ones or those of salty environments, also called "glycophytes" (these are not plants naturally encountered on salty substrates although they can tolerate a certain quantity of salt)—show their limits. It would be interesting to pursue these works with halophyte (a plant adapted to salty media or, by extension, to media with high osmotic pressure.) plant species.

In line with the previously mentioned information, Piesschaert et al. (2005) emphasised that "zero" management of dredged port sediment deposits is recommended if water and sediment salt levels permit the installation of spontaneous vegetation by halophytic species. If this is not the case, they advise implementing different management options, such as vegetation with mixtures of herbaceous species on the dikes of the port of Antwerp (Belgium). Thus, with different mixtures of graminacea on these dredged port sediments, Piesschaert et al. (2005) showed that this type of plant could develop with an abundance of 40–60 %. Germination is based on the emergence and growth of the radicle. Seeds differ in their need for temperature, their capacity to eliminate the husk around the seed, their capacity to respond to soaking, and in the disappearance or decrease of their germination in the presence of salts (Mayer and Poljakoff-Mayer 1963). However, the seedlings of halophytic plants can survive and grow in the presence of salts that are toxic for glycophytic plants (Malcolm 1986). Salt-tolerance mechanisms are either low-complexity (involving changes in numerous biochemical pathways) or high-complexity mechanisms (involving water use efficiency, protection of photosynthesis and respiration processes, and preservation of the cytoskeleton, cell wall, and plasma membrane-cell wall interactions) (Parida and Das 2005). Osmond et al. (1980) established that the transition of germination to the installation of the seedling is the most critical phase for plant growth. This is in line with our observations for *L. perenne*, which was only slightly sensitive regarding germination but with atypical early growth with variable aerial and root development in terms of length (results not shown here). The work of Malcolm et al. (2003) focused on measurements of germination and the formation of seedlings for a halophytic plant and a glycophytic plant in a nutritive medium but in the presence of artificial seawater of "low" (160 mS m^{-1}) and "high" salinity (1,900 mS m^{-1}). Their results showed that the soaking phase (entry of water) of the glycophytic plant (*Medicago sativa*; alfalfa) decreases with time at a high level of salinity. Water enters the seed at least a day earlier for the halophytic plant (*Atriplex lentiformis* [quail brush]). In addition, although the radicles of *M. sativa* emerge more quickly than those of *A. lentiformis*, the appearance of real leaves occurs more rapidly for the latter whatever the salinity tested (Malcom et al. 2003). Although our study focused only on germination, the differences of sensitivity observed between the seeds may illustrate these phenomena.

Conclusions

The tests performed with sediments 1 and 2 (Toulon 0–20 and Toulon fines) showed (1) a decrease of their toxicity to the germination of the species selected after percolation with water and (2) that *L. perenne* was the most sensitive species. We can thus conclude that the tests performed showed limited potential for colonisation of the surface of these deposits and varied as a function of plant species. The tests performed with sediment 3 showed that it was improper for colonisation even after leaching simulating 16 months of ageing due to the alkaline pH caused by the treatment undergone. These tests gave a rapid response, whereas the column tests permitted simulating the effect of leaching through time of sediment matrixes to assess whether or not colonisation of these sediments by plants is possible in the long term. Using the three specific sediments tested here, we showed that varying possibilities of vegetation exist after 16 months of leaching. The impact of the marine origin of these sediments (chlorides) was predominant regarding their contamination when assessing the germination of inland seeds. Thus, it is advisable to now consider carrying out these tests with halophytic species. One of the immediate perspectives of this study will be to study whether or not sediments 1 and/or 2 have the same phytotoxic potentials for halophytic species. It is also necessary to simulate longer leaching periods for these matrixes. Last, it is advisable to examine whether or not the percolates resulting from this leaching present a risk for the neighbouring flora in the framework of quarry deposits.

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