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Bacterial inoculants for enhanced seed germination of *Spartina densiflora*: Implications for restoration of metal polluted areas

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ABSTRACT

The design of effective phytoremediation programs is severely hindered by poor seed germination on metal polluted soils. The possibility that inoculation with plant growth promoting rhizobacteria (PGPR) could help overcoming this problem is hypothesized. Our aim was investigating the role of PGPR in *Spartina densiflora* seed germination on sediments with different physicochemical characteristics and metal pollution degrees. Gram negative *Pantoea agglomerans* RSO6 and RSO7, and gram positive *Bacillus aryabhattai* RSO25, together with the *consortium* of the three strains, were used for independent inoculation experiments. The presence of metals (As, Cu, Pb and Zn) in sediments reduced seed germination by 80%. Inoculation with *Bacillus aryabhattai* RSO25 or *Pantoea agglomerans* RSO6 and RSO7 enhanced up to 2.5 fold the germination rate of *S. densiflora* in polluted sediments regarding non-inoculated controls. Moreover, the germination process was accelerated and the germination period was extended. The *consortium* did not achieve further improvements in seed germination.

1. Introduction

Environmental pollution by heavy metals and trace elements is one of the most serious problems worldwide, increasing in parallel with human technology development (Wuana and Okieimen, 2011). Deleterious effects of metal pollution are especially significant in estuarine systems, such as salt marshes, since the widespread and diverse usage from industry activity has elevated metal concentrations in sediments and water above permitted levels in many estuaries and marine environments worldwide (Xia et al., 2011; Gao et al., 2015; Soliman et al., 2015).

Literature is full of strategies designed to counterbalance the harmful effects of heavy metals, including physical, chemical and biological methods that immobilize or remove metals from the environment (Bentley et al., 2005; Peng et al., 2009). But recently phytoremediation, the use of plants to act upon the pollutants, by extracting, degrading or immobilizing them, is gaining *momentum*, due to its cost-effectiveness, long-term applicability and ecological compatibility (Tangahu et al., 2011; Laghlimi et al., 2015). Among plant species with phytoremediation potential, the halophyte cordgrass *Spartina densiflora* possesses a high capacity for accumulating heavy metals in its tissues, particularly in roots (Mateos-Naranjo et al., 2008a, 2008b, 2015a), as well as for phytostabilizing them in its rhizosphere (Cambrollé et al., 2008; Redondo-Gómez et al., 2011; Redondo-Gómez, 2013).

As an adjunct to make phytoremediation more efficacious, a number of scientists have begun to explore the possibility of using soil bacteria together with plants, in order to alleviate plant stress in the presence of pollutants (Glick, 2010; De-Bashan et al., 2012; Phieler et al., 2013; Pajuelo et al., 2014; Nadeem et al., 2015). It has recently been found that the bioaugmentation of *S. densiflora* adult plants with a selected bacterial *consortium* isolated from its rhizosphere can be claimed to enhance plant growth response and tolerance to the physicochemical properties of marshes soils (Andrades-Moreno et al., 2014; Mateos-Naranjo et al., 2015b). However, little is known about the response of bacteria-plant interactions in terms of seed germination (Andrades-Moreno et al., 2014; Faisal, 2013; Ndeddy and Babalola, 2016), despite that *S. densiflora* is characterized by its prolific seed production (Mateos-Naranjo et al., 2008c).

Seed germination is strongly inhibited by the presence of excess metals in soils (Sethy and Ghosh, 2013). Several processes are involved, with differences depending on the metal, including, inhibition of enzyme activities (Sfaxi-Bousbih et al., 2010; Singh et al., 2011), disruption of metabolism (Zhang et al., 2009), increased ROS production and activation of antioxidant system (Sharma and Dietz, 2009; Hossain et al., 2012), damage to DNA (Lin et al., 2008), DNA methylation (Rancelis et al., 2012), damage to membranes and electrolyte leakage (Rahoui et al., 2010), etc. In this particular, the negative effect of heavy metals on seed germination is a handicap for phytoremediation projects, since the germination and initial establishment of the plants on metal polluted soils are severely hindered (Singh et al., 2007; Mateos-Naranjo et al.,

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2011; Ndeddy and Babalola, 2016). Thus we hypothesize that inoculation with PGPR could facilitate the germination of *S. densiflora* in sediments with different physicochemical properties, being this information essential in order to improve the design of restoration programs. The aim of this study was to investigate the role of bacteria isolated from the rhizosphere of *Spartina maritima* in the germination of *S. densiflora* seeds on sediments with contrasting physicochemical properties and different degrees of metal pollution. Comparison between inoculants based on gram positive and gram negative bacteria is also approached.

2. Materials and methods

2.1. Seed and sediment source

Ripe spikes of *S. densiflora* were collected in December 2014 in the Odiel marshes (37°15′N, 6°58′W; SW Spain) from 30 different clumps chosen randomly located in a well-drained gently sloping intertidal lagoon (mean sea level + 1.85 m relative to SHZ). Caryopses were stripped from the spikes and those with seeds were selected and stored in the dark for 4 months at 4 °C until the beginning of the experiment.

In addition, samples of the first 10 cm of sediment were collected from two different natural areas at Odiel and Piedras marshes (Gulf of Cadiz, SW Spain), and subsequently transported to the laboratory for their physic-chemical characterization. Measurements of sediment texture, conductivity, pH, redox potential, sediment metal concentrations (n = 5) were determined by using the method employed by Mateos-Naranjo et al. (2011). The physicochemical properties of the sediments are given in Table 1. Moreover, in order to eliminate any bacteria present, sediments were sterilized three times at 121 °C for 30 min, separated by 24 h, and stored in sterile plastic bags until the beginning of the germination experiment.

2.2. Bacterial inocula and seed germination experiment

Bacteria used in this work were isolated from the rhizosphere of *S. maritima* grown in the Odiel marshes and identified as the gram negative *Pantoea agglomerans* strains RSO6 and RSO7 and the gram positive *Bacillus aryabhattai* strain RSO25 (Paredes-Páliz et al., in revision). They were selected due to their high resistance to several metals and metal biosorption, together with plant growth promoting (PGP) properties and the capacity for biofilms' formation (Paredes-Páliz et al., in revision).

In May 2015 a glasshouse experiment was performed to assess the effect of individual bacterial and a bacterial *consortium* on seed germination of *S. densiflora*. The three bacterial strains were cultivated separately in 100 ml of Tryptic Soy Broth (TSB) medium at 28 °C and continuous shaking (200 rpm) for 24–48 h depending on the bacterium. Cultures were centrifuged at 8.000 ×g and pellets were suspended in sterile distilled water for the inoculation process. The absorbance of the cultures at 600 nm was determined and adjusted to 1.5 for all of them with sterile distilled water. Moreover, in order to guarantee that there were not antagonistic effects between the strains in the *consortium*, an antagonist test was done as follows: cultures of the three strains with equal absorbance at 600 nm were mixed. The mix was diluted up to 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}. One hundred microliters of the diluted samples was plated on TSA medium and plates were incubated at 28 °C for 24 h (n = 3). Our results indicated that there were not antagonistic effects between the components of the *consortium* (data not shown).

Seeds used for experiment were previously surface-sterilized by vigorous shaking in sodium hypochlorite solution (5%, v/v) for 5 min and then washed with sterilized water. Five 10-seed replicates were sown 1 cm deep in individual plastic pots of 100 ml filled with sterile homogenates of either, previously collected sediments (Odiel and Piedras) mixed with perlite in order to increase the hydraulic conductivity (proportion 3:1).

Pots were kept at controlled temperature between 21 and 25 °C, 40–60% relative humidity, natural daylight of 250 as minimum and 1000 μmol m^{-2} s^{-1} as maximum light flux and were randomly assigned to four inoculation treatments (without inoculation, inoculation with strain RSO25, with strains RSO6 and RSO7 together and with the *consortium* integrated by the strains RSO6, RSO7 and RSO25) in combination with the two collected sediments (n = 50, one seed per pot and 50 pots per sediment and inoculation treatment). Inoculation process was done at the beginning of the experiment and was repeated once a week during the experiment period. Also at the beginning of the experiment 1 l of tap water was placed in each of the trays down to a depth of 1 cm, and water levels were monitored and controlled during the experiment.

Pots were daily inspected for 50 days and seed germination considered after cotyledon appearance. Seven germination characteristic parameters were determined at the end of the experiment: final germination percentage, number of days to first germination (FGD), number of days to final germination (LGD), mean time to germination (MTG), coefficient of velocity of germination (CVG) and mean daily germination (MDG) (Curado et al., 2010; Mateos-Naranjo et al., 2011; Jahanian et al., 2012).

2.3. Statistical analysis

Statistical analyses were done using 'Statistica' v. 6.0 (Statsoft Inc.). Comparisons between means of germination parameters in relation with the different sediments and inoculation treatments were tested using two-way ANOVA (F-test) analyses. Data were first tested for normality with the Kolmogorov–Smirnov test and for homogeneity of variance with the Brown–Forsythe test. Significant test results were followed by Tukey tests for identification of important contrasts.

Table 1
Physicochemical properties and concentration of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), zinc (Zn) and iron (Fe) of sediments from Piedras and Odiel marshes.

Sediment	Physico-chemical properties		Metal (mg Kg^{-1})	
Piedras	Texture (%)	20/14/66	As	6.5 ± 0.1
	pH	7.6 ± 0.3	Cd	0.2 ± 0.1
	Redox potential (mV)	150 ± 15	Cu	19.0 ± 0.2
	Conductivity (mS cm^{-1})	1.2 ± 0.4	Pb	16.0 ± 0.3
	Organic matter (%)	11.4 ± 0.5	Zn	78.0 ± 0.2
	Nitrogen (%)	0.2 ± 0.1	Fe	12,669 ± 229
Odiel	Texture (%)	60/16/24	As	339.8 ± 12.0
	pH	6.9 ± 0.1	Cd	4.0 ± 0.13
	Redox potential (mV)	262 ± 10	Cu	1318.4 ± 26.8
	Conductivity (mS cm^{-1})	15.6 ± 0.5	Pb	406.7 ± 29.4
	Organic matter (%)	13.5 ± 0.2	Zn	2522.7 ± 65.3
	Nitrogen (%)	0.3 ± 0.1	Fe	71026 ± 343

Data are means ± SE of five replicates. Texture (silt/clay/sand percentage).

3. Results and discussion

There are increasing reports indicating that PGP bacteria can diminish the stress of plants when grown on sediments with different physicochemical properties and/or with high metal pollution levels (Yang et al., 2008; Rajkumar et al., 2012; Mateos-Naranjo et al., 2015a, 2015b; Ullah et al., 2015; Choudhary et al., 2016) although not so much information is available on the effect at the stage of seed germination (Miransari and Smith, 2014). Thus, it has been reported that the phase of germination is more sensitive to environmental factors and/or metal contamination than other phases of plants life cycle by the absence of some defense mechanisms to cope with these stressful conditions (Liu et al., 2005; Xiong and Wang, 2005; Mateos-Naranjo et al., 2008c). However, there is not a clear pattern of germination response to these conditions, since it has been described species in which there was a clear inhibition (Kjær et al., 1998; Ahsan et al., 2007), while in others the germination was not affected in response to environmental factors or to the presence of metals (Mahmood et al., 2005; Street et al., 2007; Mateos-Naranjo et al., 2008c, 2011).

The capacity of *S. densiflora* to germinate in polluted sediments had been previously reported by Curado et al. (2010) and Mateos-Naranjo et al. (2011), which found that high metal bioavailability, may inhibit or alter the germination response of *S. densiflora*. Thus our results indicated that the kinetic of germination, final percentage of germination and germination characteristics of *S. densiflora* were highly influenced by the sediment characteristics, according to previous results. But this is the first study that demonstrated that this capacity could be also influenced by bacteria from its rhizosphere. Thus in Piedras sediments, the seed germination started quickly between the days 7th and 14th (FGD and LGD, respectively), with a MTG of 10.0 ± 0.8, CVG of 0.1 ± 0.01 and MDG of 1.8 ± 0.2 (Table 2), and also the final germination percentage reached up to 90% in non-inoculated treatment (Figs. 1A and 2). By contrast, in the Odiel sediments the germination of *S. densiflora* was impaired, as indicated the mean delay in germination period between the days 24th and 30th, (FGD and LGD, respectively), with a MTG 26.9 ± 4.7, CVG of 0.05 ± 0.03 and MDG of 0.35 ± 0.2 (Table 2), and also the final germination percentage decreased to 18% (Fig. 2). These differences could be ascribed to the different physicochemical characteristics between both contrasting sediments, and particularly to metal content, as it have been previously indicated by Sethy and Ghosh (2013). Thus the physicochemical analysis of the sediments (Table 1) showed that metal/metalloid concentrations were lower in Piedras sediments, whereas in Odiel metal levels were over the toxicity threshold for plants, and also metal bioavailability would be greater due to the lower pH values (Table 1), which could reduce the germination capacity of *S. densiflora*. However bacterial inoculation contributed to counterbalance the negative impact of metal excess on *S. densiflora* germination capacity. Thus our results showed that inoculation with the gram positive strain *Bacillus aryabhattai* RSO25, and with gram negative bacterial strains RSO6 and RSO7 together, increased the percentage of germination from 18% to 46% and 42%, respectively in polluted sediments (Fig. 2). Furthermore, the germination kinetics was also affected; for instance, the FGD was advanced from day 24th to days 13th and 10th, and MDG increased from 0.35 to 0.9 and 0.8 seeds per day for the inoculations with the strains RSO25, and with the strains RSO6 and RSO7, respectively (Fig. 2). Contrarily, in non-polluted sediment, the inoculation with the strain RSO25 or with the strains RSO6 and RSO7 together did not significantly affect the germination response of *S. densiflora* respect to non-inoculation treatment, except in LGD, which was extended from the day 14th until the day 24th (Figs. 1 and 2, Table 2).

The amelioration effect of individual bacterial strains inoculation under metal excess could be explained by its biological characteristics. Thus *Bacillus aryabhattai* RSO25 and *Pantoea agglomerans* RSO6 and RSO7 showed elevated tolerance to metal excess and a great biosorption capacity; i.e. RSO7 is able to bind up to 26,000 μg Pb g^{-1} dry weight (Paredes-Páliz et al., in revision). Furthermore, the bacterial strains, in particular the gram negative had the ability to form biofilms (Paredes-Páliz et al., in revision). So it is possible that these characteristics could contribute to seed germination, by adsorbing metal onto the bacterial surface and decrease metal availability in the seed vicinity. There is abundant literature showing that metal resistant bacteria with high biosorption capacity enhance plant growth (Rodríguez-Llorente et al., 2010; Dary et al., 2010) but the information is scarce on the stage of germination. In this regard, genetic engineering of the bacterial cell surface has been carried out in order to enhance metal biosorption and increase plant protection against metals (Valls et al., 2000; Biondo et al., 2012). Furthermore, it is well known that different types of microorganisms (gram positive and gram negative bacteria, yeast, mycorrhizal fungi, algae, cyanobacteria, etc.) with cell walls of different chemical nature can display distinct metal biosorption capacities depending on the chemical groups onto the surface (Wang and Chen, 2009; Das, 2010). Furthermore microbial populations can affect heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes (Gadd, 2004; Ullah et al., 2015), so it is possible the these mechanisms could contribute to enhance germination capacity of *S. densiflora* under metal excess. On the other hand, the three selected strains showed good PGP properties (such as nitrogen fixation, phosphate solubilization and siderophores and auxin production), even in the presence of As, Cu, Zn and Pb *in vitro* (Paredes-Páliz et al., in revision). These properties could help seed germination. For instance, auxins are clearly involved in maintaining seed dormancy (Miransari and Smith, 2014; Shu et al., 2016). But, although auxins seem not to be directly related to seed germination, they become crucial once the radicle has emerged (Kucera et al., 2005; Miransari and Smith, 2014). These plant phytohormones can either be produced by rhizosphere microorganisms or plant-produced upon alteration of plant gene expression by the bacteria. In both cases, the role of rhizosphere populations in seed germination is envisioned as a

Table 2
Effect of different bacterial inoculation treatment (without inoculation, inoculation with gram positive *Bacillus aryabhattai* RSO25, with gram negative *Pantoea agglomerans* RSO6, RSO7 and with the *consortium* integrated by RSO6, RSO7, RSO25) on days to first germination (FGD), days to last germination (LGD), mean time to germinate (MTG), coefficient of velocity of germination (CVG) and mean daily germination (MDG) of *Spartina densiflora* in non-polluted (Piedras) and polluted (Odiel) sediment for 50 days.

		Germination characteristics				
	Inoculation treatment	FGD (days)	LGD (days)	MTG	CVG	MDG
Piedras	Non-inoculated	6.4 ± 1.9	13.8 ± 2.1	10.0 ± 0.8	0.1 ± 0.01	1.8 ± 0.2
	RSO25	5.6 ± 1.9	24.0 ± 6.8	11.8 ± 1.9	0.09 ± 0.01	1.5 ± 0.3
	RSO6, RSO7	6.8 ± 1.3	22.8 ± 8.8	11.6 ± 1.7	0.1 ± 0.01	1.8 ± 0.1
	RSO25, RSO6, RSO7	7.4 ± 1.5	17.8 ± 4.5	11.5 ± 1.9	0.09 ± 0.01	1.3 ± 0.4
Odiel	Non-inoculated	23.6 ± 13.1	29.6 ± 12.2	26.9 ± 10.1	0.05 ± 0.03	0.35 ± 0.2
	RSO25	13.4 ± 3.6	23.6 ± 7.7	19.6 ± 5.6	0.05 ± 0.01	0.9 ± 0.2
	RSO6, RSO7	9.7 ± 2.2	38.2 ± 9.9	25.8 ± 11.6	0.05 ± 0.02	0.8 ± 0.3
	RSO25, RSO6, RSO7	13.6 ± 5.9	29.0 ± 7.7	20.5 ± 3.4	0.05 ± 0.01	0.7 ± 0.3

Values are means ± ES of five replicates, with 10 seeds each (total = 50 seeds per treatment).

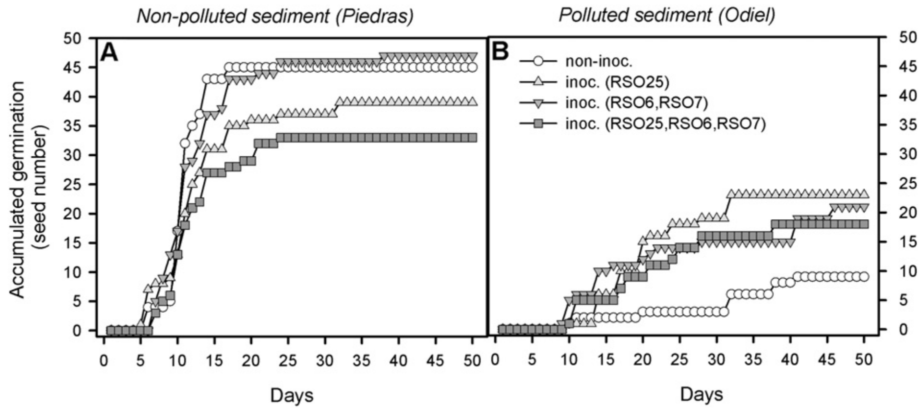

Fig. 1. Effect of different bacterial inoculation treatment (without inoculation, inoculation with gram positive *Bacillus aryabhattai* RSO25, with gram negative *Pantoea agglomerans* RSO6, RSO7 and with the *consortium* integrated by RSO6, RSO7, RSO25) on the kinetics of germination of *Spartina densiflora* seeds in non-polluted (Piedras) A, and polluted (Odiel) B sediments.

very effective tool for improving seed germination, particularly under stress conditions (Miransari and Smith, 2014).

Finally in relation with the inoculation with the bacterial *consortium* integrated by all individual strains, although we could expect a greater positive effect on germination capacity of *S. densiflora*, since antagonistic effects between the bacterial strains in vitro were not found, we did not record additional improvements in the germination response of *S. densiflora*, neither in contaminated or in non-contaminated sediments, respect to the inoculations with individual strains (Figs. 1 and 2, Table 2). Several studies have shown that bacterial communication between the members of a specific bacterial population in the rhizosphere could be intercepted by other microbes, particularly *Bacillus* strains, in natural systems, which could affect PGP properties depending upon *quorum sensing* (Boyer and Wisniewski-Dyé, 2009). Thus it is possible that this behaviour between the components of our *consortium* could explain the absence of a higher positive effect on germination response of *S. densiflora*.

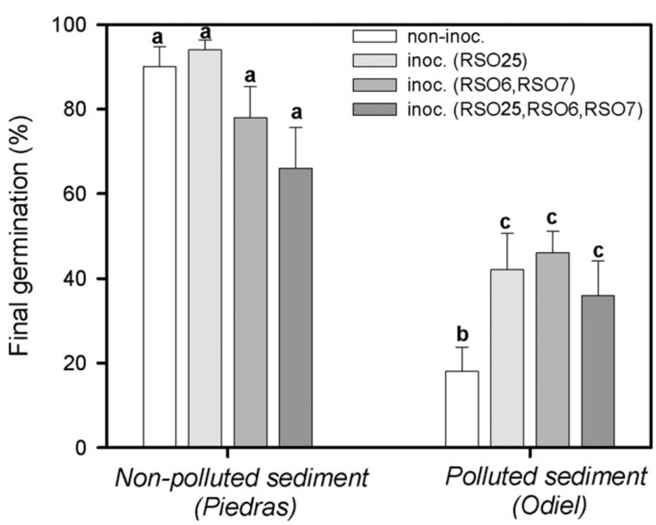

Fig. 2. Effect of different bacterial inoculation treatment (without inoculation, inoculation with gram positive *Bacillus aryabhattai* RSO25, with gram negative *Pantoea agglomerans* RSO6, RSO7 and with the *consortium* integrated by RSO6, RSO7, RSO25) on the final germination percentage of *Spartina densiflora* seeds in non-polluted (Piedras), and polluted (Odiel) sediments. Values are mean ± SE of five replicates with 10 seeds each one. Different letters indicate means that are significantly different from each other ($p < 0.05$).

4. Conclusions

We can conclude that the inoculation with appropriate selected bacterial isolates from the rhizosphere of *S. densiflora* could contribute to improve its seed germination rate and extend the germination period, which could be crucial in the design of restoration projects of metal polluted areas. In particular, in our study, the gram positive strain *Bacillus aryabhattai* RSO25, as well as the gram negative bacterial strains *Pantoea agglomerans* RSO6 and RSO7 have proved to be efficient in protecting seed germination against metal excess, increasing seed germination up to 2.5 fold with regard to non-inoculated seeds, while the inoculation with the *consortium* of the three strains did not achieve additional improvements. Several factors could contribute to explain the positive effect of bacterial inoculation, such as, their high metal resistance, metal biosorption and biofilms forming ability, which could decrease metal availability in the seed vicinity, together with PGP properties, which are maintained in the presence of metals.

Conflict of interest

The authors declare no conflicts of interests.

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References

Ahsan, N., Lee, D., Lee, S., Kang, K.Y., Lee, J.J., Kim, P.J., Yoon, H., Kim, J., Lee, B., 2007. Excess copper induced physiological and proteomic changes in germination rice seeds. Chemosphere 67, 1182–1193.

Andrades-Moreno, L., del Castillo, I., Parra, R., Doukkali, B., Redondo-Gómez, S., Pérez–Palacios, P., Caviedes, M.A., Pajuelo, E., Rodríguez-Llorente, I.D., 2014. Prospecting metal-resistant plant-growth promoting rhizobacteria for rhizoremediation of metal contaminated estuaries using *Spartina densiflora*. Environ. Sci. Pollut. Res. 21, 3713–3721.

Bentley, S., Thibodeaux, L., Adriaens, P., Li, M.Y., Romero-González, M., Banwart, S.A., Filip, Z., Demnerova, K., Reible, D., 2005. Physicochemical and biological assessment and characterization of contaminated sediments. In: Reible, D., Lanczos, T. (Eds.), Assessment and Remediation of Contaminated Sediments. Springer, Netherlands, pp. 83–136.

Biondo, R., Almeida da Silva, F., Vicente, E.J., Souza, J.E., Guerrini-Schenberg, A.C., 2012. Synthetic phytochelatin surface display in *Cupriavidus metallidurans* CH34 for enhanced metals bioremediation. Environ. Sci. Technol. 46, 8325–8332.

Boyer, M., Wisniewski-Dyé, F., 2009. Cell–cell signaling in bacteria: not simply a matter of quorum. FEMS Microbiol. Ecol. 70, 1–19.

Cambrollé, J., Redondo-Gómez, S., Mateos-Naranjo, E., Figueroa, M.E., 2008. Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment. Mar. Pollut. Bull. 56, 2037–2042.

Choudhary, D.K., Kasotia, A., Jain, S., Vaishnav, A., Kumari, S., Sharma, K.P., Varma, A., 2016. Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J. Plant Growth Regul. 35, 276–300.

Curado, G., Rubio-Casal, A.E., Figueroa, E., Castillo, J.M., 2010. Germination and establishment of the invasive cordgrass *Spartina densiflora* in acidic and metal polluted sediments of the Tinto River. Mar. Pollut. Bull. 60, 1842–1848.

Dary, M., Chamber-Pérez, M.A., Palomares, A.J., Pajuelo, E., 2010. "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J. Hazard. Mater. 177, 323–330.

Das, N., 2010. Recovery of precious metals through biosorption—a review. Hydrometallurgy 103, 180–189.

De-Bashan, L.E., Hernandez, J.P., Bashan, Y., 2012. The potential contribution of plant growth promoting bacteria to reduce environmental degradation: a comprehensive evaluation. Appl. Soil Ecol. 61, 171–189.

Faisal, M., 2013. Inoculation of plant growth promoting bacteria *Ochrobactrum intermedium*, *Brevibacterium* sp. and *Bacillus cereus* induce plant growth parameters. J. Appl. Biotechnol. 1, 45–53.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122, 109–119.

Gao, W., Du, Y., Gao, S., Ingels, J., Wang, D., 2015. Heavy metal accumulation reflecting natural sedimentary processes and anthropogenic activities in two contrasting coastal wetland ecosystems, eastern China. J. Soils Sediments 16, 1093–1108.

Glick, B.R., 2010. Using soil bacteria to facilitate phytoremediation. Biotechnol. Adv. 28, 367–374.

Hossain, M.A., Piyatida, P., Teixeira da Silva, J., Fujita, M., 2012. Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J. Bot. 1–37.

Jahanian, A., Chaichi, M.R., Rezaei, K., Rezayazdi, K., Khavazi, K., 2012. The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). Int. J. Agric. Crop Sci. 4, 923–929.

Kjær, C., Pedersen, M.B., Elmegaard, N., 1998. Effects of soil copper on black bindweed (*Fallopia convolvulus*) in the laboratory and in the field. Arch. Environ. Contam. Toxicol. 35, 14–19.

Kucera, B., Cohn, M.A., Leubner-Metzger, G., 2005. Plant hormone interactions during seed dormancy release and germination. Seed Sci. Res. 15, 281–307.

Laghlimi, M., Baghdad, B., Hadi, H., Bouabdli, A., 2015. Phytoremediation mechanisms of heavy metal contaminated soils: a review. Open J. Ecol. 5, 375–388.

Lin, A., Zhang, X., Zhu, Y.G., Zhao, F.J., 2008. Arsenate-induced toxicity: effects on antioxidative enzymes and DNA damage in *Vicia faba*. Environ. Toxicol. Chem. 27, 413–419.

Liu, D.Y., Li, Y., Chu, L., 2005. Study of the tolerance of *Hippochaete ramosissimum* to Cu stress. Sci. China Ser. C Life Sci. 48, 150–155.

Mahmood, S., Hussain, A., Saeed, Z., Athar, M., 2005. Germination and seedling growth of corn (*Zea mays* L.) under varying levels of copper and zinc. Int. J. Environ. Sci. Technol. 2, 269–274.

Mateos-Naranjo, E., Redondo-Gómez, S., Cambrollé, J., Figueroa, M.E., 2008a. Growth and photosynthetic responses to copper stress of an invasive cordgrass, *Spartina densiflora*. Mar. Environ. Res. 66, 459–465.

Mateos-Naranjo, E., Redondo-Gómez, S., Cambrollé, J., Luque, T., Figueroa, M.E., 2008b. Growth and photosynthetic responses to zinc stress of an invasive cordgrass, *Spartina densiflora*. Plant Biol. 10, 754–762.

Mateos-Naranjo, E., Redondo-Gómez, S., Luque, C.J., Castellanos, E.M., Davy, A.J., Figuero, M.E., 2008c. Environmental limitations recruitment from seed in invasive *Spartina densiflora* on a southern European salt marsh. Estuar. Coast. Shelf Sci. 79, 727–732.

Mateos-Naranjo, E., Andrades-Moreno, L., Redondo-Gómez, S., 2011. Comparison of germination, growth, photosynthetic responses and metal uptake between three populations of *Spartina densiflora* under different soil pollution conditions. Ecotoxicol. Environ. Saf. 74, 2040–2049.

Mateos-Naranjo, E., Gallé, A., Flórez-Sarasa, I., Perdomo, J.A., Galmés, J., Ribas-Carbó, M., Flexas, J., 2015a. Assessment of the role of silicon in the Cu-tolerance of the C4 grass *Spartina densiflora*. J. Plant Physiol. 178, 74–83.

Mateos-Naranjo, E., Mesa, J., Pajuelo, E., Pérez-Martín, A., Caviedes, M.A., Rodríguez-Llorente, 2015b. Deciphering the role of plant growth-promoting rhizobacteria in the tolerance of the invasive cordgrass *Spartina densiflora* to physicochemical properties of salt-marsh soils. Plant Soil 394, 45–55.

Miransari, M., Smith, D.L., 2014. Plant hormones and seed germination. Environ. Exp. Bot. 99, 110–121.

Nadeem, S.M., Naveed, M., Ahmad, M., Zahir, Z.A., 2015. Rhizosphere bacteria for crop production and improvement of stress tolerance: mechanisms of action, applications, and future prospects. In: Arora, N.K. (Ed.), Plant Microbes Symbiosis: Applied Facets. Springer, India, pp. 1–36.

Ndeddy, R.J., Babalola, O.O., 2016. Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes feacalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. Int. J. Phytoremediation 18, 200–209.

Pajuelo, E., Rodríguez-Llorente, I.D., Lafuente, A., Pérez-Palacios, P., Doukkali, B., Caviedes, M.A., 2014. Engineering the rhizosphere for the purpose of bioremediation: an overview. CAB Rev. 9, 1.

Paredes-Páliz, K.I., Caviedes, M.A., Doukkali, B., Mateos-Naranjo, E., Rodríguez-Llorente, I.D., Pajuelo, E., 2016. Screening beneficial rhizobacteria from *Spartina maritima* for phytoremediation of metal polluted salt marshes: comparison of gram positive and gram negative strains. Environ. Sci. Pollut. Res. (in revision).

Peng, J.F., Song, Y.H., Yuan, P., Cui, X.Y., Qiu, G.L., 2009. The remediation of heavy metals contaminated sediment. J. Hazard. Mater. 161, 633–640.

Phieler, R., Voit, A., Kothe, E., 2013. Microbially supported phytoremediation of heavy metal contaminated soils: strategies and applications. Geobiotechnology 1, 211–235.

Rahoui, S., Chaoui, A., El Ferjani, E., 2010. Membrane damage and solute leakage from germinating pea seed under cadmium stress. J. Hazard. Mater. 178, 1128–1131.

Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnol. Adv. 30, 1562–1574.

Rancelis, V., Cesniene, T., Kleizaite, V., Zvingila, D., Balciuniene, L., 2012. Influence of cobalt uptake by *Vicia faba* seeds on chlorophyll morphosis induction, SOD polymorphism, and DNA methylation. Environ. Toxicol. 27, 32–41.

Redondo-Gómez, S., 2013. Bioaccumulation of heavy metals in *Spartina*. Funct. Plant Biol. 40, 913–921.

Redondo-Gómez, S., Andrades-Moreno, L., Mateos-Naranjo, E., Parra, R., Valera-Burgos, J., Aroca, R., 2011. Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass *Spartina densiflora*. J. Exp. Bot. 62, 5521–5530.

Rodríguez-Llorente, I.D., Dary, M., Gamane, D., El Hamdaoui, A., Doukkali, B., Lafuente, A., Delgadillo, J., Caviedes, M.A., Pajuelo, E., 2010. Cadmium biosorption properties of the metal resistant *Ochrobactrum cytisi* Azn6.2. Eng. Life Sci. 10, 49–56.

Sethy, S.K., Ghosh, S., 2013. Effect of heavy metals on germination of seeds. J. Nat. Sci. Biol. Med. 4, 272–275.

Sfaxi-Bousbih, A., Chaoui, A., El Ferjani, E., 2010. Copper affects the cotyledonary carbohydrate status during the germination of bean seed. Biol. Trace Elem. Res. 137, 110–116.

Sharma, S., Dietz, K.J., 2009. The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci. 14, 43–50.

Shu, K., Liu, X.D., Xie, Q., He, Z.H., 2016. Two faces of one seed: hormonal regulation of dormancy and germination. Mol. Plant 9, 34–45.

Singh, D., Nath, K., Sharma, Y.K., 2007. Response of wheat seed germination and seedling growth under copper stress. J. Environ. Biol. 28, 409–414.

Singh, H.P., Kaur, G., Batish, D.R., Kohli, R.K., 2011. Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biol. Trace Elem. Res. 144, 1295–1301.

Soliman, N.F., Nasr, S.M., Okbah, A.A., 2015. Potential ecological risk of heavy metals in sediments from the Mediterranean coast. Egypt. J. Env. Health Sci. Eng. 13, 70.

Street, R.A., Kulkarni, M.G., Stirk, W.A., Southway, C., Van Staden, J., 2007. Toxicity of metal elements on germination and seedling growth of widely used medicinal plants belonging to Hyacinthaceae. Bull. Environ. Contam. Toxicol. 79, 371–376.

Tangahu, B.V., Abdullah, S.R.S., Basri, H., Idris, M., Anuar, N., Mukhlisin, M., 2011. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. Int. J. Chem. Eng. 1–33.

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S., Yang, X., 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ. Exp. Bot. 117, 28–40.

Valls, M., Atrian, S., Lorenzo, V., Fernández, L.A., 2000. Engineering a mouse metallothionein on the cell surface of *Ralstonia eutropha* CH34 for immobilization of heavy metals in soil. Nat. Biotechnol. 18, 661–665.

Wang, J., Chen, C., 2009. Biosorbents for heavy metal removal and their future. Biotechnol. Adv. 27, 195–226.

Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecol. 1, 20.

Xia, P., Meng, X., Yin, P., Cao, Z., Wang, X., 2011. Eighty-year sedimentary record of heavy metal inputs in the intertidal sediments from the Nanliu River estuary, Beibu Gulf of South China Sea. Environ. Pollut. 159, 92–99.

Xiong, Z.T., Wang, H., 2005. Copper toxicity and bioaccumulation in Chinese cabbage (*Brassica pekinensis* Rupr.). Environ. Toxicol. 20, 188–194.

Yang, J., Kloepper, J.W., Ryu, C., 2008. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci. 14, 1–4.

Zhang, H., Lian, C., Shen, Z., 2009. Proteomic identification of small, copper-responsive proteins in germinating embryos of *Oryza sativa*. Ann. Bot. 103, 923–930.