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Growth and mycorrhizal responses to cadmium stress in some halophytic plants

Warda Sidhoum*[1,2] and Zohra Fortas[1]
[1]Laboratoiry of Microorganisms Biology and Biotechnology, University of Oran1 Ahmed Ben Bella, BP 1518 - El Mnaouer - Es-Sénia Oran. Algeria.
[2]Abdelhamid Ibn Badis University, Mostaganem, Algeria.

Abstract

The phytoremedial potential of three halophytes and the role of arbuscular mycorrhizal fungi (AMF) isolated from heavy metal contaminated soil under cadmium stress were studied. The plants were treated with different Cd concentrations (0, 50, 200, and 400 µM) with and without AMF inoculation. On the plant growth, AMF inoculation to all species resulted in increased biomass, shoots and roots length, and leave number compared to control (non-inoculated) plants, especially in Limbarda crithmoides, where no mortality was detected neither in controls nor inoculants. Furthermore, L. crithmoides and Atriplex canescens recorded higher values of relative mycorrhizal dependency and water content. The arbuscular mycorrhizal association was observed in all host plants and was not affected by cadmium. Our results indicate that studied halophytes can tolerate high Cd concentrations due to the support of AMF, particularly in Atriplex halimus association with AMF in pot experiment which was recorded for the first time.

Keywords: Halophytes, arbuscular mycorrhizal fungi, Cd concentrations, plant growth

Introduction

Halophytes are adapted species to saline habitats. Manousaki and Kalogerakis (2011) suggested them to be better adapted to environmental stress compared to salt-sensitive crop plants commonly chosen for phytoremediation purposes. Most recent works recommended them for phytoremediation of heavy metal polluted, saline and non-saline soils (Lotmani *et al.*, 2011; Lutts and Lefèvre, 2015; Mesnoua *et al.*, 2016). Furthermore, some researchers (Bonanno and Lo Giudice, 2010) confirmed their capacity to accumulate important metals concentrations. The majority of these halophytic plants make associations with arbuscular mycorrhizal fungi (Becerra *et al.*, 2016) which represent 30% of the soil microbial biomass (Olsson, 1999). These plants benefit from AM association a better growth (Plenchette and Duponnois, 2005; Hajiboland *et al.*, 2015) an improved plant acclimatization to local soil conditions, increased plants survival and productivity by improving their mineral nutrition and water, especially under drought, extreme temperatures and salinity (Aggarwal *et al.*, 2012). Finally, numerous studies have shown their performance to confer halophytes resistance to metallic trace elements (MTE) (Sánchez-Castro *et al.*, 2017). The AMF behavior in soil remediation should be considered in phytoremediation technologies, seeing as an alternative restoration strategy for ecological and low-cost pollution control of MTE in contaminated soils (Doubková and Sudová, 2016). The success of these technologies is based on the screening of tolerant plants and fungi for soil remediation (Javaid, 2011).

Atriplex halimus, *A. canescens*, and *Limbarda crithmoides* are halophytes, listed in eHALOPH database Version 3.18 (http://www.sussex.ac.uk/affiliates/halophytes/) of salt-tolerant plants-halophytes (Santos *et al*., 2016); they have a wide Mediterranean distribution. *A. halimus* and *L. crithmoides* are Algerian native plant species. *A. canescens* originated from North America, it was introduced to Algerian steppe West region in 1987 (Aouissat *et al.*, 2011). Most surveys focused on their hypertolerance to high salt concentrations (Al Hassan *et al.*, 2016; Suaire *et al.*, 2016) or to elevated soil metal contamination (Mateos-Naranjo *et al.*, 2013; Mesnoua *et al.*, 2016) or even the both (Manousaki and Kalogerakis, 2009). However, no studies are available on AMF effect on their growth under Cd elevated concentrations.

The overall purpose of this research was to assess the tolerance of *A. halimus*, *A. canescens*, and *L. crithmoides* under anthropogenic stress induced by Cd associated or not to mycorrhizal fungi. To complete this aim, we tested the plant's survival, growth, and mycorrhizal potential under metal concentrations.

*Email: sidhoumwarda@yahoo.fr

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Materials and Methods

Plant material, fungal inoculum, and soil substrate

Plant species used in the present experiment are *Limbarda crithmoides*, *Atriplex halimus,* and *A. canescens. Atriplex* spp. seeds were collected from a saline natural wetland (where they were abundant) with serious problems of pollution by heavy metals (35°43'N, 0°23'W and 50 m altitude) located in Oran a city of Algeria North-west: *A. halimus* seeds collected in November 2014; those of *A. canescens* in February 2017, and those of *L. crithmoides* at the same period in Oran university campus (35°38'14.54''N, 0°36'54.31''W). All seeds were surface sterilized in 6% sodium hypochlorite for 10 min and subsequently rinsed with distilled water then sown in brown plastic pots of 400 mL capacity containing a sterile cultivation substrate which was prepared by mixing homogenized peat and sand (v:2v).

The fungal inoculum constituted by native arbuscular mycorrhizal fungi (AMF) was isolated from the saline polluted wetland characterized by a sandy-loamy soil (organic mater=12.87%, K^+= 992.94 mg kg^{-1}, Na^+= 2314.72 mg kg^{-1}, assimilable phosphorous=932.39 mg kg^{-1}, active carbonate=29%, 0.2≤ EC≤9.5 dSm^{-1}, pH=8.09). Briefly, soil samples were taken from the rhizosphere of many halophyte plants at a depth of 10–40 cm. The plants were carefully dug from the substrate and the majority of bulk soil was manually removed from the roots. Trap cultures were prepared in pots (400/mL) as described by Morton *et al.* (2004) and Davidson (2016) by using the rhizospheric soil after homogenization to form a representative sample and sterile sand (1:1 v/v) with (*Pennicum glaucum* and *Trifolium* sp.) as host plants. The cultures were made in glasshouse without air conditioning, under natural light conditions and supplied with tap water 2 or 3 times a week. After six months, shoots were removed; the soil and roots remaining in the pots were used again to repeat the cycle from seeding to shoot removal. After several cycles of pot culture cultivation, soil and root fragments were mixed well and used as a mixed AMF inoculum. The inoculum was composed of approximately 15 species belonging to Acaulosporaceae (≤50%), Archaeosporaceae (≤20%), Glomeraceae (≤15%), Claroideoglomeraceae (<5%), Ambisporaceae (<5%) and Diversisporaceae (<5%).

Inoculation procedure

Each pot received 400mL of the autoclaved substrate (1 hour at 120ºC) (Lawrence, 1956) with 10% (v:v) dry AMF soil inoculum, the same amount of sterilized substrate was added to non-mycorrhizal (C) control pots. In total, 120 pots were made for 3 species with 2 treatments: non-mycorrhized and mycorrhized, each one was replicated 20 times.

Growth conditions

The experiment was carried out under glasshouse without air conditioning and under natural light conditions. Water was supplied 2 or 3 times a week to the entire period of plant growth to avoid any drought effect. After 70 days of seedling culture, the plants were watered once a week with a modified Hoagland solution (0.5M of P) (Hoagland and Arnon, 1950) to allow symbiotic establishment.

Cd treatment

After three months of planting, plants were exposed to four Cd treatments: 0, 50, 200, and 400µM, supplied once a week as $CdCl_2$. $5H_20$ in the nutrient solution cited. Cd concentrations were chosen according to Mesnoua *et al.* (2016) experiment. Each treatment was allocated in one tray, each containing five pots. All measurements were carried out after 15 days of Cd treatment.

Sample collection and growth analysis

At the end of the experiment, plants were harvested, divided into shoot and roots and rinsed with tap water to remove soil particles attached to plant surfaces. Growth parameters (shoots and roots lengths, shoots wet and dry masses and leaves number) were measured. The dry mass was determined after drying shoots and roots at 60°C for 48 h (Vile *et al.*, 2005).

The shoot water content was calculated as previously described (Turner, 1981) using the following formula:

W C (%) = [(F.W – D.W) / F.W] × 100
Where, F.W.: Fresh weight; D.W.: Dry weight.

The mycorrhizal dependency relative index MDRI exhibited plant growth at difference degree associated with (AM) colonization. It was calculated according to the equation described (Plenchette *et al.*, 1983): MDRI (%) = [(DW M – DW NM) / DW M] × 100

Where DW M is the shoot dry weight of mycorrhizal plants and DWNM is those of non-mycorrhizal plants.

AM colonization

Sub-samples of fine roots were clarified in 10% (w/v) KOH at 90°C for 1 h, rinsed three times, bleached with fresh alkaline H_2O_2 solution (10%) for 2 to 3 min, acidified with 10% HCl (1–4 min) and then stained with 0.05% Trypan Blue (w/w) in lactophenol (modified method of Phillips and Hayman (1970)). For each root system, AMF colonization was estimated by optical microscopy from 50 root fragments of approximately 1 cm in length.

Mycorrhizal development was evaluated according to the method of Trouvelot *et al.* (1986) and expressed as mycorrhizal frequency (F%) giving an estimation of the amount of root cortex that became infected by mycorrhiza, mycorrhizal intensity (% M and m%) of colonised root fragments and of all root system respectively, arbuscular richness (A%) and arbuscular richness of colonised root fragments (a%). In the case of other endophytes (Dark Septate Endophytes DSE colonization), the frequency of mycelium occurrence in roots was estimated similarly as it was calculated for the presence of AMF.

Statistical analysis

Statistical analysis was performed using the SPSS software program (SPSS 23.0). The data were analyzed by Two-way analysis of variance (ANOVA) and GLM (Generalized Linear Model). To detect the statistical significance of differences ($p< 0.05$) between means, the Tukey test was performed. The Pearson correlation coefficient was employed to determine the relationships between plant growth parameters, host plants, and Cd treatment.

Results

Growth parameters

We noted that in general, shoot and roots length, leaves number, fresh and dry weight values were higher in mycorrhizal plants than non-mycorrhizal plants (Table 1, Figure 1). Phytotoxicity symptoms were observed exclusively in *A. canescens* at 400 µM, which occurs by leaf rolling, wilting and reduction of its biomass. A mortality rate was recorded in both species of *Atriplex* spp. especially higher in the controls than in inoculants (7.8% for *A. halimus* and 11.4% for *A. canescens*). These losses were recorded mainly in controls at 200 µM of Cd (75% of the *A. canescens* and 60% *A. halimus*), but no mortality was detected for *I. crithmoides* neither in controls nor inoculants.

GLM test showed highly significant differences at ($p<0.0000001$) in inoculation treatment in all growth parameters, and only in root length and dry weight between Cd concentrations at $p<0.05$, and between species in root length, fresh and dry weight at $p<0.0001$. We noted also significant effect of the interaction (Cd treatment * species) only in shoot length at $p<0.05$; as well as shoot and root lengths and dry weight were affected by the interaction (Cd treatment * Inoculation), while, except root length, all growth parameters were significantly affected by the interaction (species* Inoculation). Finally, the interaction (Cd treatment * species * Inoculation) affected root length at $p<0.01$ (Table 1).

L. crithmoides and *A. canescens* recorded higher values of relative mycorrhizal dependency compared to *A. halimus* (Figure 2). Two-way ANOVA showed a highly significant influence of species on MDRI ($p<0.00001$) and WCC ($p<0.00001$), as well as highly significant effects of Cd treatment and the interaction (species * Cd treatment) on control water content (WCC) at ($p<0.00001$) (Table 2).

Species colonization by arbuscular mycorrhizal fungi

The arbuscular mycorrhizal association was observed in all host plants as shown in Table 3. In the absence of Cd treatment, the mean AMF colonization frequency (F) varied with particular species, showing more mycorrhization in *A. canescens* root system (30.55%) than the other species (16.66%), but increased to reach the maximum of mycorrhization rate in moderate Cd concentrations (F=41%) in 200/µM for *L. crithmoides*, 50/µM for *A. canescens* (F=40.47%) and *A. halimus* (F= 30.12%)). Then, it decreased with elevated Cd concentrations. Mean of AMF colonization intensity (M) and arbuscule richness (A) were already low to absent in all treatments. The DSE frequency occurrence in roots was low in the case of all species (0-15.35%).

Table 1: Significance of sources of variation for species, inoculation and Cd treatment effects using Generalized Linear Model (GLM)

variables	Shoot length (cm)	Leaves number	Root length (cm)	Fresh weight (g)	Dry weight (g)
Cd treatment	1.251^{ns}	1.181^{ns}	4.730**	2.316^{ns}	2.776*
species	2.744^{ns}	1.869^{ns}	14.016****	43.552******	17.55*****
Inoculation	75.51******	59.643*****	15.946******	72.909******	75.445******
Cd treatment * species	2.415*	0.184^{ns}	1.025^{ns}	0.836^{ns}	0.732^{ns}
Cd treatment * Inoculation	2.711*	1.242^{ns}	5.96**	1.248^{ns}	2.731*
species * Inoculation	6.864**	15.462*****	1.204ns	32.179*****	17.163*****
Cd treatment*species*Inoculation	1.24^{ns}	2.024^{ns}	3.944**	1.108^{ns}	0.353^{ns}

Significance levels: ns indicates no significant, * $p< 0.05$, ** $p< 0.01$, *** $p<0.001$.

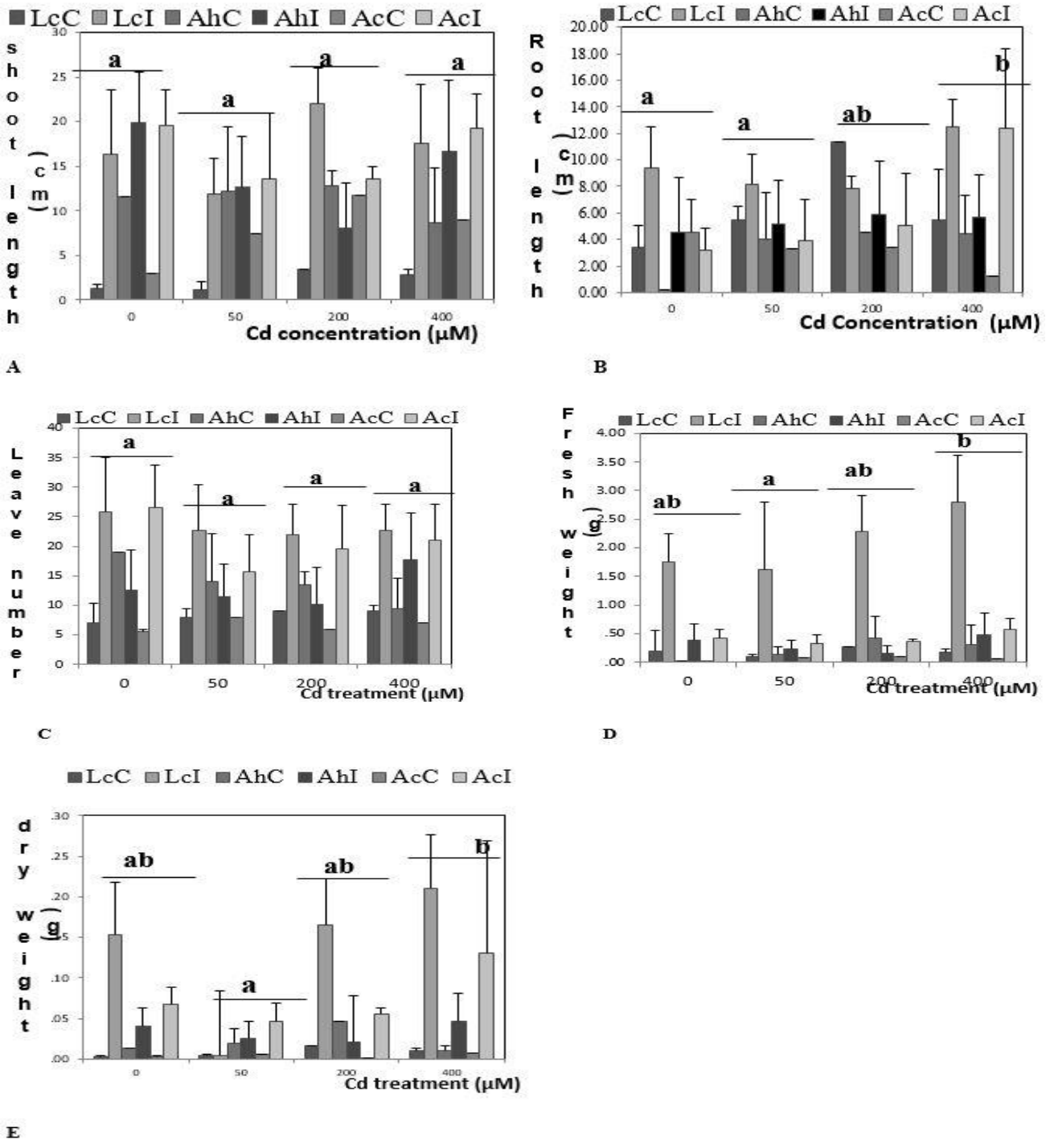

Figure 1: AMF inoculation effect on plant species growth under Cd treatment. Shoot and root length (respectively A and B), leaves number (C), shoot fresh and dry weight (D and E). Lc: *L. crithmoides;* Ah: *A. halimus*; Ac: *A. canescens*; C: control and I: inoculated. Different letters are significantly different from each other. (p<0.05) according to the Tukey test.

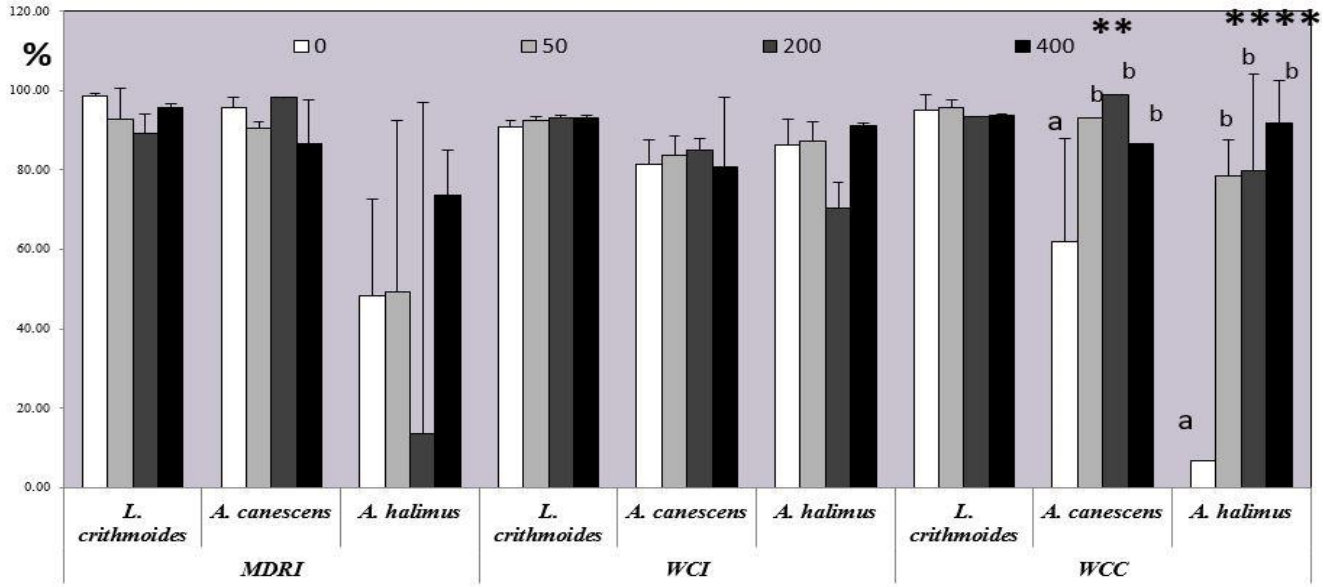

Figure 2: Mycorrhizal dependence relative index (MDRI) and water content (WC) of halophyte examined species exposed to Cd and inoculated with mycorrhizal fungi collected from the metal-contaminated soil. WCI and WCC: respectively water content in inoculated and control plants. Different letters are significantly different from each other (p<0.05) according to the Tukey test. Significance levels: ns indicates no significant, * p< 0.05, ** p< 0.01, *** p<0.001.

Table 2: Significance of sources of variation for both species and Cd treatment effects on plant MD and water content each parameter after two-way ANOVA analyses

Variables	MDRI	WCI	WCC
species	21.18*****	5.22*	38.14*****
Cd traitment	1.18ns	0.814ns	35.95*****
species * Cd traitment	1.61ns	1.53ns	16.13*****

Significance levels: ns indicates no significant, * p< 0.05, ** p< 0.01, *** p<0.001.

Table3: Fungal root endophyte colonization in studied plant species across Cd treatments

Species	Cd treatment	F%	M%	m%	a%	A%	DSE
L. crithmoides	0	16.66±20.81a	0.30±0.43a	1±1a	0.41±0.005a	0.003±0.72a	9.09±15.74a
	50	14.89±19.04a	2.02±3.14a	6.8±7.85a	1.61±0.15a	0.09±2.79a	5.55±9.62a
	200	41.02±16.01a	13.82±14.08a	29.16±22.79a	73.81±26.18b	11.18±11.53a	15.35±13.23a
	400	30.55±12.72a	6.78±1.87a	24.46±9.86a	66.22±33.91cb	4.56±2.47a	11.11±9.62a
A. canescens	0	30.55±17.34a	1.44±1.565a	3.94±2.23a	6.41±5.56a	0.11±0.16a	11.11±12.72a
	50	40.47±16.93a	0.81±0.52a	1.82±0.71a	16.71±22.38a	0.18±0.23a	9.70±10.85a
	200	35.60±16.44a	1.59±1.53a	5.11±6.00a	11.27±4.89a	0.153±0.15a	6.06±8.08a
	400	22.71±20.56a	2.95±4.44a	7.83±8.60a	35.63±32.29a	2.00±3.30a	4.76±8.25a
A. halimus	0	16.66±15.27a	2.46±4.10a	8.33±13.57a	34.16±54.9a	2.34±4.05a	0.00±0.00a
	50	30.12±13.43a	0.52±0.32a	1.60±0.52a	11.34±14.57a	0.08±0.11a	0.00±0.00a
	200	5.12±8.87a	0.05±0.08a	0.33±0.57a	0.00±0.00a	0.00±0.00a	2.56±4.43a
	400	5.35±4.65a	0.15±0.20a	2.00±2.64a	16.66±28.86a	0.01±0.02a	0.00±0.00a
Cd treatment		0.693 ns	1.499 ns	2.107 ns	1.300 ns	2.151 ns	0.184 ns
Species		3.97*	3.91*	6.50**	0.70ns	3.39ns	3.34ns
Cd treatment*species		1.74ns	1.99ns	2.65*	1.017ns	4.37**	0.36ns

Results are expressed as means ±SD (n = 3). Means in the same column with different letters are significantly different from each other (p< 0.05) according to the Tukey test. Significance levels: ns indicates no significant, * p< 0.05, ** p< 0.01, *** p<0.001.

In *A. halimus* root systems, AMF colonization was characterized exclusively by the spread of intercellular running hyphae but rarely arbuscules. This arbuscules scarcity was the reason for our inability to identify general AM mycorrhizal morphology in examined root systems. However, Arum type of AM was the dominant morphology in the other plant hosts (Figure 3).

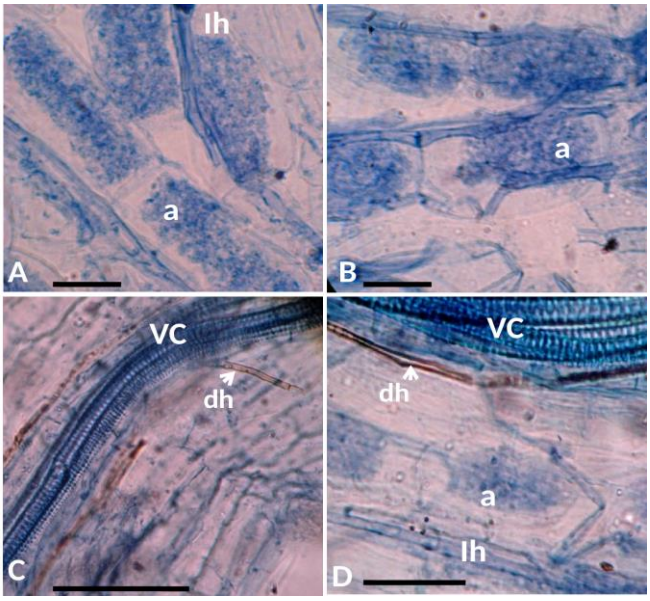

Figure 3 A-D: Fungal endophytes in the roots of investigated plant species. A) *Arum*-type of AM in *L. crithmoides*, (Ih) hyphae growing intercellularly to form terminal arbuscules (a); B) *Intermediate*-type of AM in *A. canescens*, hyphae growing intracellularly from cell to cell to form terminal arbuscules; C-D: DES, melanized hyphae (dh) in *L. crithmoides* root system or in coexistence with AMF (D) in the same species. VC: vascular cylinder. Bar: 20µm

Two-way ANOVA showed a significant effect of species on both mycorrhizal frequency and intensity, in addition to a significant effect of the interaction (Cd treatment*species) on intensity (m%) and arbuscules richness (A%). No effect was noted about Cd treatment on the fungal association.

Person correlation test showed that the inoculation affected positively the shoot ($r=0.539$; $p=7.66\times10^{-11}$) and root ($r=0.290$; $p=0.001$) lengths, leaves number ($r=0.512$; $p=7.43\times10^{-10}$), fresh ($r=0.428$; $p=5.23\times10^{-7}$) and dry ($r=0.500$; $p=2.16\times10^{-9}$) weight. Moreover, all these parameters positively correlated between them at $p<0.01$ but inversely correlated with plant species (leaves number ($r=-0.251$; $p=0.004$), root length ($r=-0.380$; $p=0.000011$), fresh ($r=-0.548$; $p=2.58\times10^{-11}$) dry ($r=-0.464$; $p=3.82\times10^{-8}$). While only root length was induced by Cd concentration ($r=0.251$; $p=0.004$).

Discussion

Mycorrhizal colonization, in the present work, increased at moderate Cd concentrations (50µM for *Atriplex* spp., and 200 µM for *L. crithmoides*). As mentioned in Liu *et al*. (2011) reports, they used 50 mg/Kg of Cd (444µM) as maximum Cd concentration applied to marigold culture and observed similar results for plants inoculated with *G. mosseae* or *Septoglomus constrictum*, but the contrast with those inoculated with *Rhizoglomus intraradices.* Then, we observed decrease in mycorrhizal frequency at higher Cd concentration that is due to the negative metal effect on propagules infectivity. Although, Wei *et al.* (2015) reported that heavy metals reduce, delay, or even eliminate AMF colonization and spore density at heavy metal polluted sites. While no Cd concentration effect was revealed by statistics on mycorrhizal infection as mentioned by Chen *et al.* (2004). This highlights that our inoculum is resistant to Cd>200µM since it was isolated from a polluted soil.

Our results support those of Janoušková *et al.* (2005) who studied AMF effect on the growth of transgenic tobacco in Cd up to 60 mg kg^{-1} and found no significant growth inhibition by Cd in the studied plant. Whereas, we found a significant effect of inoculation on halophytes growth in Cd amended soil. This result highlights the AMF improving effect on plants grown in contaminated soils. This may be due to better plants mineral nutrition induced by mycorrhizal infection since mycorrhiza is often considered as a biomass stimulator or nutrient uptake enhancer and a health and stability enhancer as confirmed in

Table 4: Pearson correlation test between growth parameters

	Shoot length	Leaves number	Root length	Fresh weight	Dry weight	Species	Inoculation
Cd treatment	0.087ns	-0.075ns	0.250**	0.128ns	0.105ns	0.129ns	-0.012ns
Shoot length		0.623**	0.252**	0.456**	0.568**	0.071ns	0.539**
Leaves number			0.292**	0.608**	0.700**	-0.251**	0.512**
Root length				0.567**	0.572**	-0.380**	0.290**
Fresh weight					0.895**	-0.548**	0.428**
Dry weight						-0.464**	0.500**

Significance levels: ns indicates no significant, * $p< 0.05$, ** $p< 0.01$

several works (Liu *et al.*, 2011; Hajiboland *et al.*, 2015). The AMF also enhance plant development by reducing Cd translocation to aerial plants parts, in order to prevent them from Cd toxicity in the photosynthetic apparatus as suggested by other authors (Clemente *et al.*, 2012). However, these suggestions must be confirmed by mineral and physiological plant analysis.

These plant species are known to be naturally adapted to metallic stress, as mentioned by several authors (Clemente *et al.*, 2012; Mesnoua *et al.*, 2016). This was confirmed in our results by stability in plant growth, and in water content and confirmed in our results by their stability in plant growth and water content. Whereas, *A. canescens* exhibited a great sensibility, compared to the other species since we detected more than 70% of mortality in 200 µM in the absence of AMF inoculation.

In the light of our results, *L. crithmoides* was more resistant to Cd treatment than *Atriplex* spp., attesting its metallophyte properties. *L. crithmoides* behavior to Cd resistance was reported by Zurayk *et al.* (2001); they confirmed the species multi-tolerance to Ni, Pb, and Cr without affecting its biomass.

The Mycorrhizal dependency index was greater in *A. canescens* and *L. crithmoides* than in *A. halimus.* This is due to the fact that *A. halimus* is naturally weakly mycotrophic; few works reported its AM association in field but at little frequency. Berliner *et al.* (1989) showed its AM occurrence in sandy soils (South Africa) without mentioning the frequency, later He *et al*. (2002) and Rabier *et al.* (2014) confirmed that the species was mycorrhizal but slightly where the F<20% at different depth horizons in a desert environment and F<42% in saline polluted sites. In contrast, Maremmani *et al.* (2003) demonstrated that it was nonmycorrhizal in coastal nature reserves of the Mediterranean basin; these results suggested that this species associates with AMF in the extremely stressed environment. On the other hand, *A. halimus* AM association in the experimental field is mentioned for the first time. Moreover, *A. canescens* was previously reported to be mycorrhizal by several authors (Barrow and Aaltonen, 2001); they reported mycorrhizal benefits on the species growth as confirmed by our results. Our results corroborate with previous works (Maciá-Vicente *et al.*, 2012) who reported average to high *L. crithmoide*s AM frequency even in highly polluted and saline habitats.

Compared to *L. crithmoides*, arbuscular richness in *Atriplex* spp. was very poor; this arbuscules laking was reported by many authors (Barrow and Aaltonen, 2001; Becerra *et al.*, 2016), Plenchette and Duponnois (2005) who hypothesized about the existence of a third AM morphological type with no arbuscules in the Amaranthaceae family.

DSE occurrence in plant species was affected neither by Cd treatment nor by plant host, which is puzzling taking into account that DSE occurred in highly stressed environments as described by literature (Wang *et al.*, 2016). In the field, native populations of examined species have been found colonized by DSE (Maciá-Vicente *et al.*, 2012; Rabier *et al.*, 2014); *Atriplex* spp. were reported more colonized by DSE than by AMF (Barrow and Aaltonen, 2001; Plenchette and Duponnois, 2005).

Conclusion

Plant species showed in the present study high tolerance to Cd-induced stress, especially *L. crithmoides* as proved by the fact that all plants were able to survive and did not show any visible Cd toxicity symptoms, such as chlorosis, necrosis or growth inhibition at concentrations up to 400µM. Likewise, the importance of native AMF inoculation in halophyte establishment and survival under Cd stress is confirmed. In the light of these results, we assume that inoculation with AMF offers a protective effect on host plants from the potential toxicity caused by Cd, while the degree of protection varies according to the plant species and its mycorrhizal dependency. Moreover, *L. crithmoides* are the species most appropriate for use in phytoremediation. Further studies on plants physiological and biochemical Cd impact are needed such as photosynthesis rate and antioxidant enzymes activity to understand the mechanisms of Cd tolerance used by these plant species.

References

Aggarwal, A., N. Kadian, A. Tanwar and K.K. Gupta. 2012. Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *Journal of Applied and Natural Science* 4(1): 144–155.

Al Hassan, M., J.Chaura, M.P. López-Gresa, O. Borsai, E. Daniso, M. P. Donat-Torres, O. Mayoral, O. Vicente and M. Boscaiu. 2016. Native-invasive plants vs. Halophytes in Mediterranean salt marshes: Stress tolerance mechanisms in two related species. *Frontiers in Plant Science* 7: 1–21. doi: 10.3389/fpls.2016.00473.

Aouissat, M., D. J.Walker, K. Hcini, M. Belkhodja and E. Correal. 2011. Osmolyte concentrations in *Atriplex halimus* L. and *Atriplex canescens* (Pursh) Nutt. adapted to salinity and low temperature (Chenopodiaceae). *Anales de Biología* 33(33): 117–126.

Barrow, J.R. and R.E. Aaltonen. 2001. Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and the influence of

host physiological activity. *Mycorrhiza* 11(4) :199–205. doi : 10.1007/s005720100111.

Becerra, A., N. Bartoloni, N. Cofré, F. Soteras and M. Cabello. 2016. Hongos micorrícico-arbusculares asociados a Chenopodiaceae en dos ambientes salinos de Córdoba. *Boletin de la Sociedad Argentina de Botanica* 51(1): 5–13.

Berliner, R., D.T. Mitchell and N. Allsopp. 1989. The vesicular-arbuscular mycorrhizal infectivity of sandy soils in the south-western Cape, South Africa. *South African Journal of Botany* 55(3): 310–313.doi: 10.1016/S0254-6299(16)31181-4.

Bonanno, G. and R. Lo Giudice. 2010. Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators'. *Ecological Indicators* 10 (3): 639–645. doi: 10.1016/j.ecolind.2009.11.002.

Chen, B.D., Y. Liu, X.L. Li, H. Shen and P. Christie. 2004. Uptake of cadmium from an experimentally contaminated calcareous soil by arbuscular mycorrhizal maize (*Zea mays* L.). *Mycorrhiza* 14: 347–354. doi: 10.1007/s00572-003-0281-2.

Clemente, R., D.J. Walker, T. Pardo, D. Martínez-Fernández and M.P. Bernal. 2012. The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions. *Journal of Hazardous Materials* 223–224: 63–71. doi: 10.1016/j.jhazmat.2012.04.048.

Davidson B.E., J.N. Stephen and D.S. Marcelo. 2016. Consequences of inoculation with native arbuscular mycorrhizal fungi for root colonization and survival of *Artemisia tridentata* spp. *wyomingensis* seedlings after transplanting. *Mycorrhiza* 26(6): 595–608. doi: 10.1007/s00572-016-0696-1.

Doubková, P. and R. Sudová. 2016. Limited impact of arbuscular mycorrhizal fungi on clones of Agrostis capillaris with different heavy metal tolerance. *Applied Soil Ecology* 99: 78–88. doi: 10.1016/j.apsoil.2015.11.004.

He, X., S. Mouratov and Y. Steinberger. 2002. Spatial distribution and colonization of arbuscular mycorrhizal fungi under the canopies of desert halophytes. *Arid Land Research and Management* 16(2): 149–160. doi: 10.1080/153249802317304440.

Hajiboland, R., F. Dashtebani and N. Aliasgharzad. Physiological responses of halophytic C_4 grass *Aeluropus littoralis* to salinity and arbuscular mycorrhizal fungi colonization. *Photosynthetica* 53 (4): 572–584. doi.org/10.1007/s11099-015-0131-4.

Hoagland, D.R. and D.I. Arnon, 1950. The Water-Culture Method for Growing Plants without Soil. Circular. *California Agricultural Experiment Station* 347 (2nd edit).

Janoušková, M., D. Pavlíková, T. Macek and M. Vosátka. 2005. Arbuscular mycorrhiza decreases cadmium phytoextraction by transgenic tobacco with inserted metallothionein. *Plant and Soil* 272:29-40. doi: 10.1007/s11104-004-3847-7.

Javaid, A. 2011. Importance of Arbuscular Mycorrhizal Fungi in Phytoremediation of Heavy Metal Contaminated Soils. p.125–141. *In: Environmental Pollution*. M. Al., S.K. et (ed). doi: 10.1007/978-94-007-1914-9_5.

Lawrence, W. J. C. 1956. Soil Sterilization. George Allen and Unwin Ltd, London. 171p.

Liu, L.Z., Z.Q. Gong, Y.L. Zhang and P.J. Li. 2011. Growth, cadmium accumulation and physiology of marigold (*Tagetes erecta* L.) as affected by Arbuscular Mycorrhizal Fungi. *Pedosphere* 21(3):319–327. doi: 10.1016/S1002-0160(11)60132-X.

Lotmani, B., L. Fatarna, A. Berkani, J. Rabier, P. Prudent and I. Laffont-Schwob. 2011. Selection of Algerian populations of the mediterranean saltbush, *Atriplex halimus*, tolerant to high concentrations of Lead, Zinc, and Copper for phytostabilization of heavy metal-Contaminated Soils. *The European Journal of Plant Science and Biotechnology* 5(2): 20–26.

Lutts, S. and I. Lefèvre. 2015. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? *Annals of Botany* 115(3): 509–528. doi: 10.1093/aob/mcu264.

Maciá-Vicente, J.G., V. Ferraro, S. Burruano and L.V. Lopez-Llorca. 2012. Fungal assemblages associated with roots of halophytic and non-halophytic plant species vary differentially along a salinity gradient. *Microbial Ecology* 64(3): 668–679. doi: 10.1007/s00248-012-0066-2.

Manousaki, E. and N. Kalogerakis. 2009. Phytoextraction of Pb and Cd by the mediterranean saltbush (Atriplex halimus L.): Metal uptake in relation to salinity. *Environmental Science and Pollution Research* 16(7): 844–854. doi: 10.1007/s11356-009-0224-3.

Manousaki E. and N. Kalogerakis. 2011. Halophytes—An emerging trend in phytoremediation. *International Journal of Phytoremediation* 13(10): 959–969. doi: 10.1080/15226514.2010.532241.

Maremmani, A., S. Bedini, I. Matoševic, P.E. Tomei and M. Giovannetti. 2003. Type of mycorrhizal associations in two coastal nature reserves of the Mediterranean basin. *Mycorrhiza* 13(1): 33–40. doi: 10.1007/s00572-002-0194-5.

Mateos-Naranjo, E., L. Andrades-Moreno, J. Cambrollé and A. Perez-martin. 2013. Assessing the effect of copper on growth, copper accumulation and physiological

responses of grazing species Atriplex halimus: Ecotoxicological implications. *Ecotoxicology and Environmental Safety* 90:136–142. doi: 10.1016/j.ecoenv.2012.12.020.

Mesnoua, M., E. Mateos-Naranjo, J.M. Barcia-Piedras, J. A. Pérez-Romero, B. Lotmani and S. Redondo-Gómez. 2016. Physiological and biochemical mechanisms preventing Cd-toxicity in the hyperaccumulator *Atriplex halimus* L. *Plant Physiology and Biochemistry* 106: 30–38. doi: 10.1016/j.plaphy.2016.04.041.

Morton, J., R. Koske, S. Stürmer, and S. Bentivenga, 2004. Mutualistic arbuscular endomycorrhizal fungi. Biodiversity of Fungi: *Inventory and Monitoring Methods*: 317–336.

Olsson, P.A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiology Ecology* 29(4): 303–310. doi: 10.1016/S0168-6496(99)00021-5.

Phillips, J. M. and D. S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55(1): 158–161. doi: 10.1016/S0007-1536(70)80110-3.

Plenchette, C. and R. Duponnois. 2005. Growth response of the saltbush *Atriplex nummularia* L. to inoculation with the arbuscular mycorrhizal fungus *Glomus intraradices*. *Journal of Arid Environments* 61(4): 535–540. doi: 10.1016/j.jaridenv.2004.10.003.

Plenchette, C., A. Fortin and V. Furlan.1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. *Plant and Soil*, 70: 199–209.

Rabier, J., I. Laffont-Schwob, A. Pricop, A. Ellili, G. D'Enjoy-Weinkammerer, M.D. Salducci, P. Prudent, B. Lotmani, A. Tonetto and V. Masotti. 2014. Heavy metal and arsenic resistance of the halophyte *Atriplex halimu*s L. along a gradient of contamination in a French Mediterranean spray zone. *Water, Air, and Soil Pollution* 225:1993. doi: 10.1007/s11270-014-1993-y.

Sánchez-Castro, I., V. Gianinazzi-Pearson, J.C. Cleyet-Marel, E. Baudoin and D. van Tuinen. 2017. Glomeromycota communities survive extreme levels of metal toxicity in an orphan mining site. *Science of the Total Environment* 598:121–128. doi: 10.1016/j.scitotenv.2017.04.084.

Santos, J., M. Al-Azzawi, J. Aronson and T.J. Flowers. 2016. eHALOPH a Database of Salt-Tolerant Plants: Helping put Halophytes to Work. *Plant and Cell Physiology* 57 (1): e10. doi.org/10.1093/pcp/pcv155.

Suaire, R., I. Durickovic, L. Framont-Terrasse, J.Y. Leblain, A.C. De Rouck and M.O. Simonnot. 2016. Phytoextraction of Na^+ and Cl^- by *Atriplex halimus* L. and *Atriplex hortensis* L.: A promising solution for remediation of road runoff contaminated with deicing salts. *Ecological Engineering* 94: 182–189. doi: 10.1016/j.ecoleng.2016.05.055.

Trouvelot, A., J. Kough and V. Gianinazzi-Pearson.1986. Measuring the rate of VA mycorrhization of root systems. Research methods for estimating having a functional significance. p. 217-222. *In:* V. Gianinazzi-Pearson and S. Gianinazzi, (eds) *1st European Symposium on Mycorrhizae: Physiological and Genetical Aspects of Mycorrhizae*. Dijón, INRA, Paris.

Turner, C.T. 1981. Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil* 58: 339–366.

Vile, D., É. Garnier, B. Shipley, G. Laurent, M. L. Navas, C. Roumet, S. Lavorel, S. Díaz, J. G. Hodgson, F. Lloret, G. F. Midgley, H. Poorter, M. C. Rutherford, P. J. Wilson and I. J. Wright. 2005. Specific leaf area and dry matter content estimate thickness in laminar leaves. *Annals of Botany* 96(6): 1129–1136. doi: 10.1093/aob/mci264.

Wang, J. L., T. Li, G.Y. Liu, J.M. Smith and Z. W. Zhao. 2016. Unraveling the role of dark septate endophyte (DSE) colonizing maize (*Zea mays*) under cadmium stress: Physiological, cytological and genic aspects. *Scientific Reports* 6: 1–12. doi: 10.1038/srep22028.

Wei, Y., Z. Chen, F.Wu, H. Hou, J. Li, Y. Shangguan, J. Zhang, F. Li and Q. Zeng. 2015. Molecular diversity of arbuscular mycorrhizal fungi at a large-scale antimony mining area in southern China. *Journal of Environmental Sciences* 29: 18–26. doi: 10.1016/j.jes.2014.10.002.

Zurayk, R.A., N.F. Khoury, S.N. Talhouk, and R.Z. Baalbaki. 2001. Salinity-heavy metal interactions in four salt-tolerant plant species. *Journal of Plant Nutrition* 24(11): 1773–1786. doi:10.1081/PLN-100107311.