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The beneficial role of indigenous arbuscular mycorrhizal fungi in phytoremediation of wetland plants and tolerance to metal stress

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Abstract: The potential of five plants namely *Atriplex halimus* L., *A. canescens* (Pursh) Nutt., *Suaeda fruticosa* (*Forssk. ex J.F. Gmel.*), *Marrubium vulgare* L. and *Dittrichia viscosa* (L.) Greuter from two selected wetlands in northwest Algeria subjected to house and industrial effluents were examined to assess their arbuscular mycorrhizal fungal (AMF) diversity and colonization, as well as to determine their tolerance and ability in accumulating metallic trace elements (MTEs). The purpose was to investigate whether, or not, these fungi are related to metallic uptake. Arbuscular mycorrhizal association was observed in all plant species, since the dual association between AMF and dark septate endophytes (DSE) was found in roots of 80% plants species. Hence, the decreasing trend of metal accumulation in most plant organs was Zn>Cu>Pb, and the most efficient species were *M. vulgare*> *S. fruticosa*> *A. canescens*> *D. viscosa*> *A. halimus*. The bioaccumulator factors exceeded the critical value (1.0) and the transport factors indicated that all these species were phytoremediators. Pearson correlation showed that Cd bioaccumulation and translocation were inhibited by AMF infection; meanwhile Zn, Pb and Cd accumulation were affected by AMF spore density and species richness, DSE frequency, pH, AMF and plant host. Native halophytes showed a multi-metallic resistance capacity in polluted wetlands. *M. vulgare* was the most efficient in metal accumulation and the best host for mycorrhizal fungi. AMF played a major role in metal accumulation and translocation.

Introduction

Among 300 wetlands of Algeria harbors, 50 are known as internationally important similar to Ramsar sites. They have caught attention of several researchers due to their distinct diversity of flora and fauna (Samraoui et al. 2015, Chenchouni 2017). The selected study site, Oran city, is located in the northwest part of Algeria which is characterized by a semi-arid Mediterranean climate and comprised of a wetland complex of eight classified Ramsar zones of ecological interest. It serves as important winter grounds for several world populations of the endangered bird species (Samraoui et al. 2015). These grounds are characterized by soil salt gradient covered by plant communities distributed inversely to the presence of salts, whilst most of the characteristic plant community are halophytic, succulent vegetation and well-adapted perennial species, such as Amaranthaceae: *Atriplex* spp., *Suaeda* spp., *Salsola* spp. and *Salicornia* spp. (Megharbi et al. 2016). Despite the international importance of wetlands, they tend to be heavily contaminated with metallic trace elements (MTEs), including Pb, Cu, Zn and Cd, due to human activities such as grazing of animals, urbanization and pollution from industrial liquid and solid waste, wastewater discharges and dumps. MTE pollution in wetland soils makes a highly complex disruption of ecological equilibrium (Belabed et al. 2017). MTEs are found to be extremely persistent in the environment, non-biodegradable and non-thermodegradable, with high accumulation to toxic levels. Consequently they could be transferred from plants into the food chain, leading to serious systemic health problems.

The utilization of Arbuscular mycorrhizal fungai (AMF) in phytoremediation technologies is considered as a potential alternative strategy for ecologic and low-cost cleanup of MTEs in contaminated soils (Doubková and Sudová 2016). The success of these technologies is basically related to tolerance of plants and fungi to soil restoration. Phytostabilization leads to the reduction of pollutant spread, and may have an advantage of exudates released by arbuscular mycorrhizal fungi (AMF), thus reducing pollutant availability (Lutts and Lefèvre 2015).

Recent researches are typically concentrated on indigenous phytodiversity and AMF in local contaminated sites for an extended period and featuring environmental stress adaptive strategies. Because these spontaneously colonizing polluted soil plants and fungi have been well adapted to the particular site conditions, their characterization provoked scientists to understand the adaptive strategies of these sites, and be useful

for soil remediation engineer to establish a novel ecosystem in polluted soils (Yang et al. 2014). As reported by (Wójcik et al. 2015), the communities of the indigenous metallophyte of abandoned metalliferous waste sites are considered as an important source of species, seed banks and gene pools for the environmental phytotechnologies.

Two strategies are probably used by plants in order to use to transact with high metal concentrations in the rhizosphere: exclusion (avoidance) mechanisms, where the uptake and/or root-to-shoot transport of metals are restricted, phytostabilization process (reduction of the mobility, bioavailability and/or toxicity of pollutants in the rhizosphere), or in contrast, sequestration of MTE contaminants by plant roots, and then translocation to their aerial parts internally. Here, the aim of phytoextraction or phytoaccumulation is to increase the accumulation of metal in plant tissues, and thus the mechanisms of internal tolerance could be important (Padmavathiamma and Li 2007).

The AMF are ubiquitous soil inhabitants associated symbiotically with most plants roots, and constitute a major component of the soil microbial biomass. They promote the penetration of nutrients in ecosystems, enhance plant establishment and growth, soil aggregation, and mineral uptake (Luginbuehl and Oldroyd 2017). In view of above background, the present study was aimed at to 1) determine the concentrations of Cu, Zn, Pb, Cd, Ni and Cr in some plants growing on contaminated saline wetland soils; 2) evaluate the root colonization by AMF and dark septate endophytes (DSE); 3) examine AMF diversity naturally related with the studied plants; 4) identify the hyperaccumulator plants with several established criteria, and thus assess the feasibility of using these plants for phytoremediation purpose, and 5) highlight principle factors in association with plant rhizosphere, affecting plant metal accumulation.

Materials and methods

Sampling area

The study was undertaken in two wetlands: Telamine Lake (LT) (35°42’50” N; 0° 23’30” W) which is listed under Ramsar convention from 2004, and Dayet Morsli (DM) (35°39’58” N, 0°36’27” W) known as the subject of interest due to its Ramsar classification. Their altitude varies between 50 and 87 m.a.s.l., along with a semi-arid Mediterranean regional climate model, characterized by cold and rainy winters followed by dry summers, lasting for about 4–6 months: with 250 m<precipitation<400 mm and 9<T<32°C. These wetlands belong to a group of Oran’s wetlands located in the northwest of Algeria, making an important complex for the wintering and passage of migratory birds. These habitats are under a persistent risk due to industrial pollution and they house wastewater discharges of neighboring villages. DM and LT are located at 2 and 7 km distance from the industrial Zones (I and II) in the south and southwest of Oran. Importantly, the most characteristic plant communities in the studied wetlands are halophytic, succulent and well-adapted perennial species dominated by Amaranthaceae and Asteraceae followed by Poaceae, Solanaceae Malvaceae and Liliaceae.

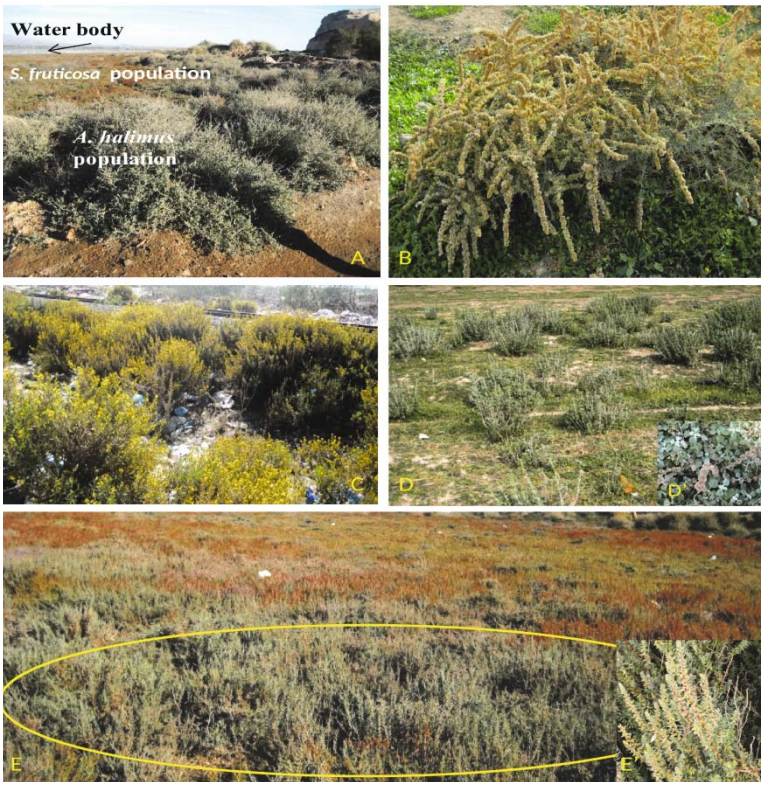

Fig. 1. General view of halophyte landscape near edges of the two wetlands (western Algeria). A: *A. halimus* distributed around the water edge forming a halophytic belt, general aspect of *A. canescens* (B), *D. viscosa* (C), *M. vulgare population* (D), *D’: plant aspect and S. fruticosa* (surrounded by a circle) with *Sarcoconia fruticosa* inhabiting the same biotope (*E*), E*’: plant aspect.* (by W. Sidhoum).

Sample collection procedure

Rhizospheric soil and plant samples (*Atriplex halimus* L., *A. canescens* (Pursh) Nutt., *Suaeda fruticosa* (syn. *S.vera Forssk. ex J.F. Gmel.*) (Amaranthaceae), *Marrubium vulgare* L. (Lamiaceae) in LT, and *Dittrichia viscosa* (L.) Greuter (syn. *Inula viscosa* (L.) Aiton) (Asteraceae) in DM, were collected in March and September 2015, respectively in LT and DM sites (Fig. 1, A–E). The plant specimens collected from the selected and marked rectangles (50×100 m) were grouped according to the size of the natural population; they included from 5 to 9 specimens in order to avoid drastic depletion of plant populations. The surface soil samples were taken from 10 to 30 cm depth. The determination of herbarium specimens for floristic inventory was carried out using classical data from different Flora of North Africa (Maire 1958–1976). Plant nomenclature was then actualized using the Synonymic Index proposed by (Dobignard and Chatelain 2013).

Sample preparation

Soil samples were obtained from every plant rhizosphere. The plants were softly dug and removed from the substrate manually with a bulk soil from the roots for chemical analysis, as well as the substrate closely attached to the root system was used for AMF spore isolation. In addition, soil samples were dried at room temperature, and sieved (2 mm diameter) (Mathieu and Pieltain 2003). Thereafter, the samples were chemically analyzed for pH, electrical conductivity (1:2.5) and soil to water suspension ratio (Mathieu and Pieltain 2003). The analysis of available phosphorus, K^+ and Na^+ concentrations were performed in Matmar regional laboratory (Ghelizane Department) of National Institute of Soils, Irrigation and Drainage, since, trace elements were extracted according to aqua regia method (Bradl 2005). In brief, 0.5 g dried soil at 105°C was treated with 2.5 mL/7.5 mL of HNO_3 (≥65%, d = 1.37–1.41 g/mL at 20°C Sigma-Aldrich) and HCl (36–38%, d=1.2 g/mL at 25°C (lit.) Sigma-Aldrich), heated at 100°C for 2 h, and then filtered and diluted to 25 mL. Afterwards, the solution was stored at 4°C until being used. Whole plants collected were divided into roots, stems and leaves, washed twice in tap water, and then in distilled water for 2 min, later the samples were dried at 60°C during 48 h. 0.5 g of dry weight was combusted in a muffle furnace at 450°C for 3 h, and grey white ash was obtained at the completion of the aching. The ash samples were allowed to cool with addition of 2 mL of HNO_3 (1N), and evaporate to near dryness on a hot plate, and then put in muffle furnace for 1 h. The content was treated alike to the soil. The solutions were then filtered into 25 mL volumetric flasks. The plant and soil solution extracts were measured by Atomic Absorption Flame Emission Spectrophotometer AAS Shimadzu AA-7000.

The potential plants for phytoextraction and phytostabilization

The translocation factor (TF), bioconcentration factor (BCF) and bioaccumulation factor (BAF) values were used to evaluate the potential of plants for phytoextraction and phytostabilization of metals in soil. According to Baker (1981), BAF<1 (plant species are excluders), TF<1 and BCF>1 (root accumulation, high efficiency in phytostabilization) and TF›1 (high efficiency for metal translocation used for phytoextraction). The factors are calculated by the following formula:

BCF = Croot⁄Csoil

BAF = Cleaf⁄Csoil

TF = Cleaf⁄Croot

Where, Cleaf, Croot, and Csoil are MTE concentrations successively in leaves, in roots, and in soil.

Mycorrhizal colonization analysis

Young roots of the plant species (with root tips) washed in tap water to remove soil particles and fixed in FAA (formalin, glacial acetic acid and ethanol) were taken out from the fixation solution, washed several times in tap water, 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 afterwards they were stained with 0.1% Trypan Blue (w/w) in lactophenol according to the modified method of (Phillips and Hayman 1970). For each root system, AMF colonization was examined by optical microscopy (Olympus CX22) for 50 root fragments of roughly 1 cm in length. The mycorrhizal development was evaluated as described by Trouvelot et al. (1986) and expressed as mycorrhizal frequency (F%, percentage of root cortex infected by mycorrhiza), mycorrhizal intensity (amount of root cortex that became mycorrhized and it is referred either to the whole root system (M%) or only to the mycorrhizal root fraction (m%)), arbuscular richness (A%) in the whole root system or in the colonized root fragments (a%). In the case of other endophytes (DSE colonization or other fungal endophytes), the frequency of mycelium occurrence in roots was estimated likewise to that calculated in the presence of AMF.

Isolation and taxonomic identification of AM fungal spores

AM fungal spores were isolated from rhizospheric soil by using wet sieving method (Gerdemann and Nicolson 1963) followed by soil centrifugation in 50% sucrose solution and filtration (mesh size 40 µm) (Brundrett et al. 1996). The samples were counted under a stereomicroscope (Leica EZ4HD) and identified following the morphological and subcellular spore characteristics compared to the descriptions of Oehl et al. (2011) and Redecker et al. (2013). In addition, other descriptions are available on the sites http://www.agro.ar.szczecin.pl/ jblaszkowski and http://invam.caf.wvu. edu., while the nomenclature employed follows that used by the Mycobank (www.mycobank.org).

Statistical analysis

Some ecological measurements were used to describe structures of AMF communities, including spore density (number of spore in 100g of soil), species richness (number of identified AMF species per soil sample), relative abundance (RA) and isolation frequency (IF) (Franke-Snyder et al. 2001). We determined the dominant AMF species with respect to the IF >50% and the RA>5%.

$$IF=\frac{number\ of\ soil\ samples\ where\ a\ species\ (genus)}{total\ number\ of\ soil\ samples}$$

$$RA=\frac{spore\ number\ of\ a\ species\ (genus)}{total\ number\ of\ identified\ spore\ samples}\times 100$$

Comparisons of multiple groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Statistical test was performed using the SPSS software (SPSS 23.0), where $p< 0.05$ was considered significant. Also, the Pearson correlation coefficient was employed to determine the relationships between metal accumulation in plant organs and AMF and some soil parameters (pH and salinity).

Results and discussion

Soil chemical properties

Table 1 shows the analyses of physicochemical properties of the studied plant rhizospheric soils. Here, we noted a slight alkaline pH varying from 7.41 to 8.14 along with high soil salinity (6.61 to 7.98 dS/m) for *A. halimus* and *S. fruticosa* respectively, medium salinity for *A. canescens* and *M. vulgare* (3.83 and 1.75 dS/m), but the lowest soil salinity was noticed for *D. viscosa* (0.36 dS/m). In addition, high levels of exchangeable K^+ (505.44–838.89 mg/L), Mg^{2+} (1028.5–2133.05 mg/L), Ca^{2+}(1970–3170.6 mg/L) and Na^+ (3540.62–46759 mg/L) were remarkably noticed. The solutions resulting from the analyzed samples of rhizopheric soil were dominated by chlorides (15080.4–49914 mg/L) followed by sulfates (1402.48–2225.75 mg/L) with the presence of very low levels of HCO^-_3 (30.5–97.6 mg/L).

Colonization of plant species collected from fields by AMF and DSE

Arbuscular mycorrhizal association was observed in all plant species, since the dual association between AMF and DSE was found in roots of 80% plants species (Table 2; Fig. 2). DSE incidence and co-occurrence with AMF under stressful conditions was previously reported (Liu et al. 2017). The mean AMF colonization frequency (F) varied with particular species, ranging from 24% (*S. fruticosa*) to up to 98% (*M. vulgare*). The highest AMF frequency was detected in *D. viscosa* and *M. vulgare* roots (>94%), alike to what has been confirmed by several studies carried out on high salinity and

Table 1. Values of soil physicochemical parameters measured in different species rhizospheric soils

parameters	*A. halimus*	*A. canescens*	*M. vulgare*	*S. fruticosa*	*D. viscosa*
pH	7.49±0.37	7.54±0.17	8.14±0.06	7.41±0.00	7.97±0.1
EC(dS/m)	6.61±0.00	3.83±0.00	1.75±0.56	7.98±0.00	0.36±0.15
P_2O_5(ppm)	146	108.47	nd	197.18	nd
Ca^{++}(mg/L)	3170.6	2138.6	nd	1970	nd
Mg^{++}(mg/L)	1649.48	1028.5	nd	2133.05	nd
K^+(mg/L)	505.44	765.44	nd	838.89	nd
Na^+(mg/L)	46759	3540.62	nd	6457.94	nd
Cl^-(mg/L)	49914	15080.4	nd	47577.6	nd
CO_3^{2-}(mg/L)	0	0	nd	0	nd
HCO_3^-(mg/L)	61	30.5	nd	97.6	nd
SO_4^{2-}(mg/L)	1725.6	1402.48	nd	2225.75	nd

nd not determined.

Table 2. Fungal root endophyte colonization of wetlands plant species

Plant species	Plant family	AM type	F%	M%	m%	a%	A%	DSE%	SD	SR
Suaeda fruticosa	Amaranthaceae	*Arum*	24.00 ±20.78b	5.44 ±4.71b	15.11 ±13.08bcd	14.46 ±12.52b	1.16 ±1.02b	4.00 ±0.00a	975.44 ±0.00a	10 ±0.00a
Atriplex halimus	Amaranthaceae	*Arum*	58.08 ±0.70cd	16.82 ±3.31cb	26.91 ±8.86cd	7.90 ±6.30cb	1.04 ±1.20cb	19.73 ±12.36ac	1443.33 ±715.14ac	10 ±1.00a
Atriplex canescens	Amaranthaceae	*Arum*	49.68 ±2.9db	10.29 ±5.45db	20.15 ±9.12dc	28.91 ±27.44db	3.94 ±5.21bd	11.66 ±4.04a	1180 ±190.78a	8.66 ±3.05a
Dittrichia viscosa	Asteraceae	*Paris /Arum*	94.14 ± 3.90a	59.19 ±8.31a	57.33 ±12.98a	86.72 ±4.32a	57.90 ±8.86a	6.04 ±8.78a	690 ±216.50a	13 ±2.00a
Marrubium vulgare	Lamiaceae	*Arum*	98.66 ± 1.15a	49.85 ±2.04a	42.80 ±0.00ad	88.14 ±0.00a	49.63 ±0.00a	0.00 ±0.00ab	500 ±0.00ab	12 ±0.00a
one way Anova			32.33****	65.99****	9.03**	23.88***	109.62****	3.56*	3.57*	0.16ns

F%:mycorrhizal frequency; m% and M%: mycorrhizal intensity of colonized root fragments and all root system respectively; A: arbuscular richness in all root system, and a%:arbuscular richness of colonized root fragments; DSE%: dark septate endophyte frequency of mycelium occurrence in roots. 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 significance, * p<0.05, **p<0.01, *** p<0.001, ****p<0.0001.

heavy metal contaminant-soils (Roda et al. 2008). In contrast, average AMF colonization (24<F<58.08%) was recorded in Amaranthaceae. Similar results were reported in *Atriplex* spp. in polluted and saline soils (Rabier et al. 2014). Chaudhry et al. (2005) identified *S. fruticosa* mycorrhizal frequency as low to moderated (15–30%), which concords with our results (24%).

However, mean of AMF colonization intensity (M) was slightly changed in Amaranthaceae (5.44–16.82%), and was average to high in *M. vulgare* and *D. viscosa* roots. The mean arbuscule richness (A) also varied and was the lowest in all Amaranthaceae, in particular, *A. halimus* (1.04%) and highest in *D. viscosa* (57.9%) (Table 2). The lack of arbuscule rates in Amaranthaceae root systems (A<5%) has been well documented (Becerra et al. 2016), although, (Plenchette and Duponnois 2005) hypothesized about the existence in this family of a third AM morphological type with no arbuscules. *Arum* type morphology was present in all plant species, while Paris type was found in some roots of *D. viscosa* (Fig. 2C–D). Amaranthaceae mycorrhization was differently and exclusively characterized by spread of intercellular running hyphae, rarely arbuscules, intraradical spores (Fig. 2A).

One way ANOVA showed a highly significant difference in percentage of total AMF colonization (p<0.0001) among plant species, relative mycorrhizal root length (p<0.0001) and mycorrhizal intensity in colonized root fragments (m%) (p<0.01), root length of arbuscules A (P<0.0001) and arbuscular richness in colonized root fragments (a%) (p<0.001) and DSE ($p < 0.05$). DSE were not found in *M. vulgare,* while a frequency occurrence in roots was found to be slight in the case of other species (4–19.73%) (Table 2). DSE were also detected in *D. viscosa* (<10%) and *A. halimus* (<20%) accordingly to what has been described by several authors (Maciá-Vicente et al. 2012). DSE rate was represented in *D. viscosa* by the dark melanized hyphae along the cortex (Fig. 2F), placed more inside the cortex, with an infrequent microsclerotia and extraradical hyphae. Similarly, single resting spores and zoospore cysts of *Olpidium* spp. (Chytridiomycota) were found in roots of *A. halimus* (Fig. 2E).

Spore density and species richness

Spore density varied in the rhizosphere soils of the selected plant species, along with maximum values for *A. halimus* (1443.33 spores 100 g^{-1} soil) and minimum ones for *M. vulgare* (500 spores 100 g^{-1} soil) (Table 2). *D. viscosa* supported maximum species richness with 13 AM fungal species (Table 2; Fig. 3B). One way ANOVA showed a significant difference in spore density (p<0.05) among plant species. No significant variations were observed in species richness between expected plants. Pearson correlation test showed that mycorrhizal frequency was significantly correlated with pH (r=0.9; p<0.001), mycorrhizal intensity (r=0.92; p<0.001) and species richness (r=0.66; p<0.01), along with inverse correlation for salinity (r=-0.82; p<0.001). Negative correlation was also recorded between pH and salinity

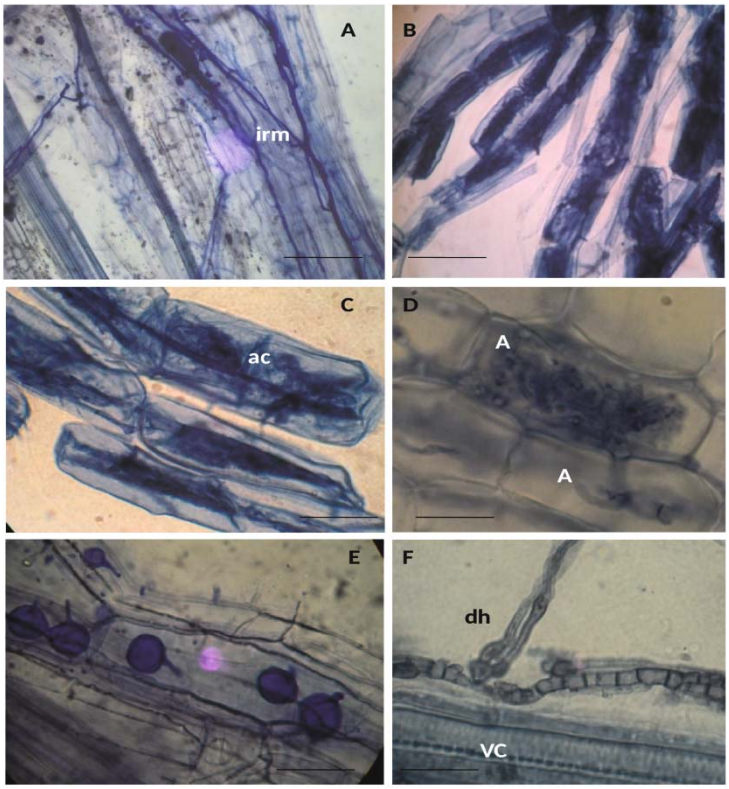

Fig. 2. A–F: Fungal endophytes in the roots of investigated plant species.
A) spread of intercellulary AM running hyphae in *Atriplex halimus* roots system(irm: intraradical mycelium);
B–C) AM *Paris* type in *D. viscosa* root system, coarse AMF hyphae growing intracellularly from cell to cell
to form arbuscular coils (ac); D). Terminally formed arbuscules (A) of AM *Arum-type* in *M. vulgare* root system;
E) *Olpidium* germinated zoospore cysts in *A. halimus* root system; F) DSE melanized hyphae (dh)
in the outer cortex of *D. viscosa* (VC) vascular cylinder. Bar = 100 µm (A and B); 20 µm (C–F).

(r=-0.76; p<0.01), spore density (r=-0.58; p<0.05), since the positive interaction was noticed between mycorrhizal intensity (r=0.82; p<0.001) and species richness (r=0.71; p<0.05). Moreover, salinity was correlated positively with spore density (r=0.52; p<0.05), and negatively with species richness (r=-0.51; p<0.05). Nevertheless, spore density was not correlated with mycorrhizal frequency, positively correlated with DSE frequency (r=0.57; p<0.05), and inversely with mycorrhizal intensity (r=-0.61; p<0.05). Species richness was positively affected by AMF intensity (r=0.75; p<0.01), while DSE colonization was affected neither by soil parameters (salinity and pH) nor by AMF colonization.

According to Pearson correlation test, the insufficiency in any relationship between mycorrhizal frequency and DSE confirms the independence of these fungal endophytes, probably owed to their occupation of different niches in the same root system (Nagaraj et al. 2015). The remarkable positive correlation between mycorrhizal frequency (F) and species richness (SR) and its absence with spore density suggests that the colonization may be due to great AMF diversity in root systems, not automatically derived from species producing more spores, because, AMF root infection could be involved also by mycorrhizal roots and soil extraradical mycelia, as confirmed by de Marins et al. (2009), who showed an important spore occurrence in both macrophytes rhizosphere of whose roots were internally colonized by AMF and in non-colonized macrophytes. A significant correlation between spore number and salinity is likely related to the potential adaptation of indigenous AMF species to high stress salinity, and thus they become able to survive and naturally occur in such environments and could be stimulated by it (Hammer et al. 2011). Soil alkalinity increases AMF infection and proliferation in roots, as well as AMF diversity along with a significant decrease of AMF sporulation as confirmed by Pearson correlation, and supported by the results obtained in alkaline sandy soil (Ouzounidou et al. 2015).

AMF species diversity

Thirty one species samples were distinguished following the morphological criteria, i.e. morphospecies (Fig. 3B). The community of AMF was obviously found to be overcome by taxa relating to the families of Glomeraceae (11 taxa) represented by five genera *Glomus* (1 spp.), *Funneliformis* (4 spp.) *Rhizoglomus* (3 spp.), *Sclerocystis* (2 spp.) and *Septoglomus* (1 sp.). Acaulosporaceae with *Acaulospora* (11 spp.). A few morphospecies were detected in Diversisporaceae (*Diversispora* 1 spp. and *Tricispora* 1 sp.), Archaeosporaceae (*Archaeospora* 2 spp.), Claroideoglomeraceae (*Claroideoglomus* 2 sp.), Paraglomeraceae (*Paraglomus* 2 sp.), Ambisporaceae (*Ambispora* 1 sp.), and overall, these appeared rarely. Eleven morphospecies are still unidentified due to the incomparable morphological characteristics of these species with any of

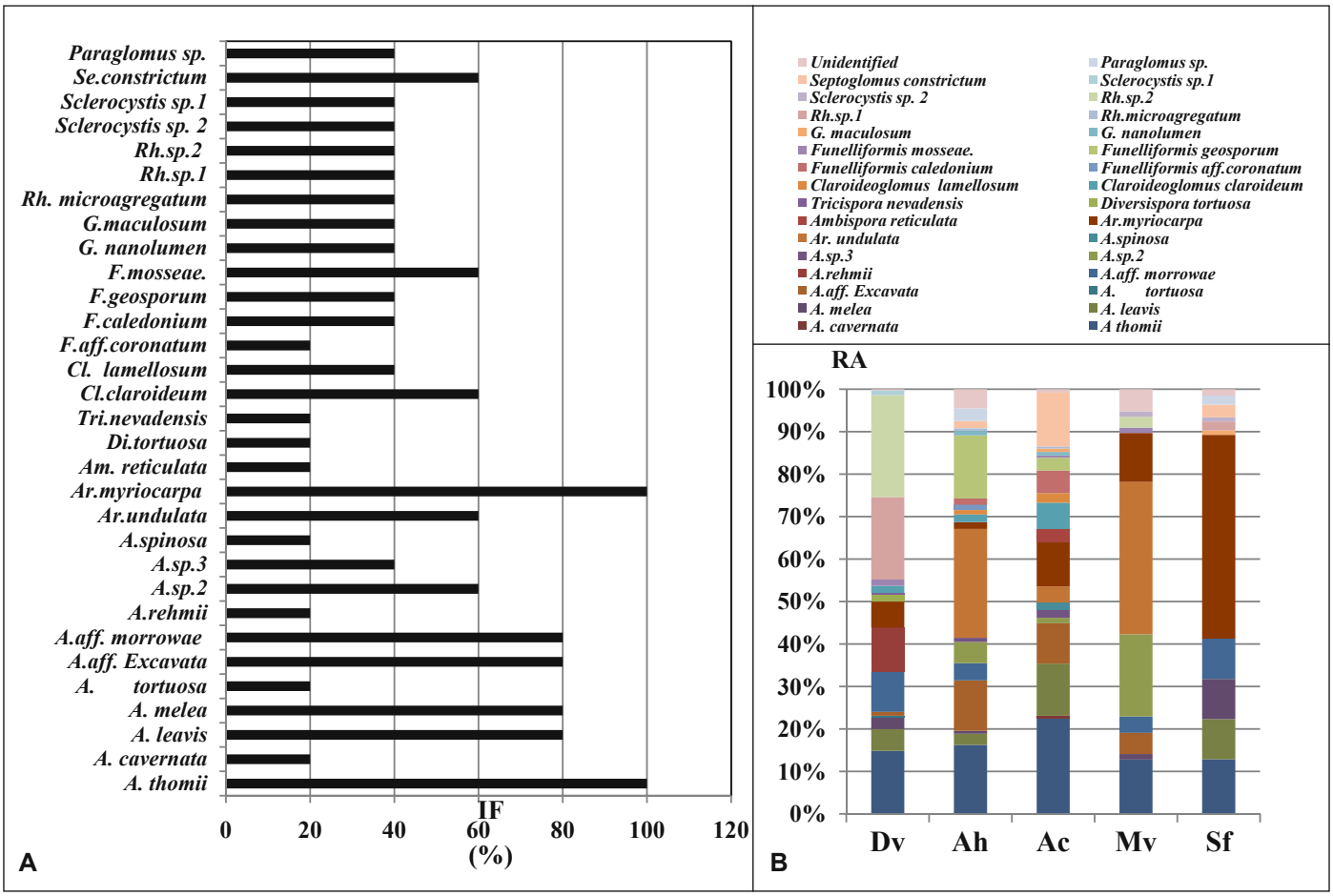

Fig. 3. AMF community dynamics in rhizospheric soil of host plants. A: isolation frequency (IF) at each plant species rhizosphere and B: relative abundance of AMF species. Dv: *Dittrichia viscosa* , Mv: *Marrubium vulgare*; Sf: *Suaeda fruticosa*; Ah: *Atriplex halimus*; Ac: *Atriplex canescens*.

those previously described for the known species. *A. thomii* and *Ar. myriocarpa* were classified as generalists (IF=100%) (Fig. 3A), whereas eight species were classified as exclusives (IF=20%): 4 species found only in *D. viscosa* (*A. rehmii, Di. tortuosa, Tr. nevadensis and A. tortuosa),* 3 restricted to *A. canescens (A. cavernata, Ambispora reticulata* and *A. tortuosa*), and *Funelliformis* aff. *coronatum* to *A. halimus,* and 21 species were classified as intermediate (40<IF<80). The presence of AMF family varied in the rhizosphere soils of the selected plant species (Fig. 4), where *Atriplex* spp. were generally characterized by the dominance of Acaulosporaceae (41 and 50%), followed by Glomeraceae, Archeosporaceae (14 to 27%), Diversisporaceae (8%) and the rest of families, whereas *M. vulgare* and *S. fruticosa* were distinguished by the codominance of Archeosporaceae and Acaulosporaceae and slight occurrence of Glomeraceae (5–7%). Also, the codominance was found in *D. viscosa* rhizosphere by Acaulosporaceae and Glomeraceae (44–46%) followed by low percentages of other families (2–6%). By focusing on relative abundance and isolation frequency, the 7 dominant species were *A. thomii, A. leavis, A*. aff. *excavata*, *A.* aff. *morrowae*, *A.* sp. 2*, Ar. undulata* and *Ar. myriocarpa* (Fig. 3 A–B).

Several studies have investigated the dominance of Acaulosporaceae (41–50%) and Glomeraceae (21–46% in *D. viscosa* and *Atriplex* spp.) evocated in extremely stressed soils by salinity and heavy metals (Yang et al. 2015). Nevertheless, the dominance of Archaeosporaceae (48%) in *M. vulgare* and *S. fruticosa* rhizosphere was evocated for the first time, and conversely this ancestral family dominates in submerged roots of some macrophytes (Moora et al. 2016). The most abundant AMF species recorded in stressed soils by several authors were the same as those found in our investigation and isolated from saline or heavy metal pollutant areas by several works, including *Se. constrictum, Fu. mosseae, Fu. geosporum, Fu. coronatum, Cl. claroideum, A. Koskii,* and *A. mellea* (Lenoir et al. 2016).

Heavy metals concentration in plant tissues and rhizospheric soil

No marked toxicity symptoms were observed in the sampled plants grown in contaminated soils. The mean concentrations of metallic trace elements in plant parts are presented in Table 3. Also, metallic trace element levels in plant organs were either lower or greater than their adjacent soils, despite high concentrations in some soil samples exceeded threshold values for the corresponding environmental soil quality guidelines "AFNOR NFU 44–041" (Table 3), including Cd in *A. halimus* soil rhizospheric (3.24 mg/kg) and lead in *M. vulgare* and *S. fruticosa* representing, respectively, 473.60 and 114.52 mg/kg. Various plants accumulate heavy metals in different ways and concentrations. The variations in large metal concentrations existed among plant species, with concentrations of Cu ranging from 9 to 152.83 mg/kg, Zn 121.33–178 mg/kg, Pb 12.19–41.52 mg/kg, Cr 9.27–49.30 mg/kg, Ni 2.67–27.51 mg/kg and Cd 0.55–1.90 mg/kg.

Importantly, the ability to accumulate Zn was strongest in all tested plants and lowest for Cd. The decreasing trend of metal levels *in S. fruticosa*, *A. canescens* and *D. viscosa*was: Zn>Cu>Pb>Cr>Ni>Cd, in *M. vulgare* leaves: Zn>Cr>Pb>Cu>Ni>Cd, but in roots: Zn> Pb >Ni > Cu >Cr >Cd, in *A. halimus* leaves: Zn> Pb> Cr> Cu>Ni>Cd and roots Zn> Pb> Cu>Cr> Ni>Cd. Also, the decreasing Zn>Cu>Pb was detected in the majority of plant organs.

In spite of the soil pollution levels, Ni and Zn were retained mainly in all plant roots, since Pb was retained in leaves. Plants from *M. vulgare* populations deposited Cr into leaves, with comparable concentrations in roots and leaves of the other plants, although Cd was retained in *M. vulgare*, *D. viscosa* and *S. fruticosa* leaves, comparable to that found in *A. canescens* organes and higher in *A. halimus* roots. ANOVA followed by the Tukey's test indicated highly significant differences between soil and metal in plant contents at (p<0.05) (Table 3). As previously reported, salt marsh plants accumulate more MTEs in the roots rather than in the shoots (Shahid et al. 2014), which could be explained by the necessity of these plants to prevent toxicity to the photosynthetic apparatus, which leads to reduce MTE translocation to aerial plant parts. At present, there is no available data showing concentrations in leaves higher than 1.0 g kg^{-1} for Cu, Pb or As, and higher than 10.0 g kg^{-1} for Zn, consequently the studied species cannot be believed as hyperaccumulator species of any of these elements following the criteria given by (Baker and Brooks 1989).

Translocation (TF), bioconcentration and bioaccumulation factors (BCF and BAF) studied for each metal (Cr, Zn, Pb,

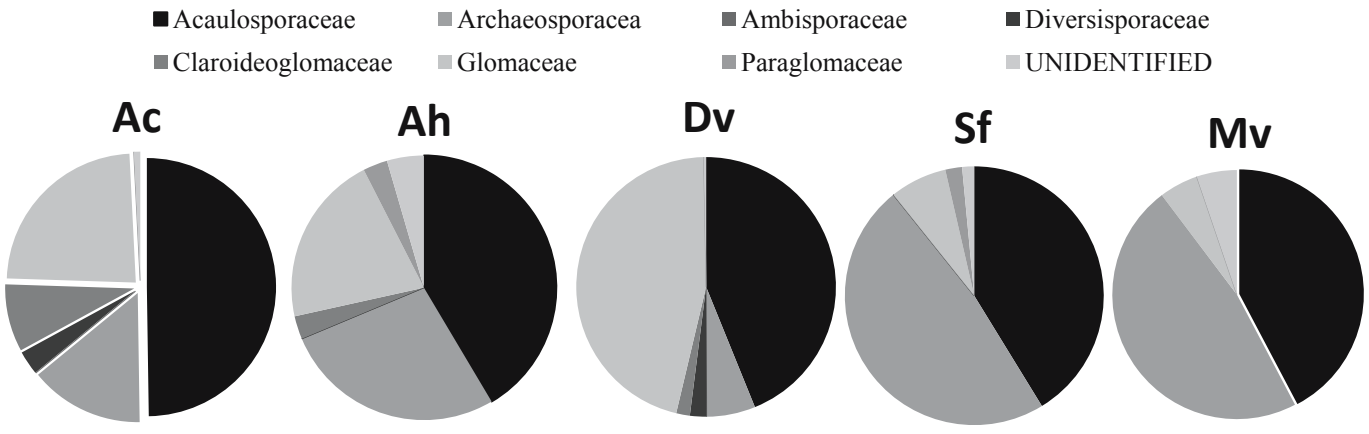

Fig. 4. Occurrence of AMF families in rhizospheric soil of investigated plants. (Ac: *Atriplex canescens;* Ah: *Atriplex halimus;* Dv: *Dittrichia viscosa;* Sf: *Suaeda fruticosa;* Mv: *Marrubium vulgare*)

Cu, Cd and Ni) are recorded in Figure 5. Our data showed that the slightest TF was recorded in *D. viscosa* for Cr, suggesting that high concentrations of metal are stored in the roots instead of being translocated to plant aerial parts, and conversely, *A. halimus* showed the highest TF values for Ni, Zn, Pb and Cu, while Cr and Cd are, respectively, presented in *M. vulgare* and *S. fruticosa*. In some cases, the BCF>6 were found as very high (Fig. 5). Overall, plants exhibit a great concentration of Cd and Cu in roots and leaves, although the slight concentration was noticed for Pb, in *M. vulgare* which concentrates Zn in leaves and Ni and Cu in roots. According to average mean of plant accumulation factors, the most efficient plant in metal accumulation is *M. vulgare*>*S. fruticosa*>*A. canescens*> *D. viscosa*>*A. halimus*. One way ANOVA showed significant differences between plants showing bioaccumulation in leaves of Zn, Pb, Ni and Cd. BAF (Zn) (F=4.57; p=0.023), BAF (Pb) (F=5.18; p=0.016), BAF (Ni) (F=6.94; p=0.006); BAF (Cd) (F=4.62; p=0.023). As given by (Baker 1981), all species except *A. canescens* could be used in both phytoextraction and phystabilization. Previous studies focused on the phytoremediation potential of halophytic plants, like *Atriplex halimus* L. on field and under controlled conditions for Zn, Cu, Cd and Pb (Rabier et al. 2014), *A. canescens* for Pb, Cu, and Zn uptake and accumulation in leaves (Sai Kachout et al. 2012), as well as *S. fruticosa* in Cr accumulation (Bareen and Tahira 2011). However, the present study showed that *S. fruticosa* exhibited low capacity in Cr accumulation and shoot translocation as compared to those of the main report, meanwhile great copper and cadmium accumulations (BCF and TF(Cu, Cd)>2; BAF(Cu, Cd)>4) were noticed., Other results revealed that *S. fruticosa* could have the basic characteristics of a tolerant plant with high capacity for phytostabilization of Cu, Cd, Zn and Pb in its below-ground structures (Bankaji et al. 2016). *D. viscosa* was examined for a long time and described as useful species for phytoremediation technologies (Martínez--Fernández et al. 2014). It is characterized by high As, Cd and Pb accumulations in higher parts (Martínez-Fernández et al. 2014). Moreover, it shows the highest metal concentrations in above-ground biomass (mean average of Zn: 1680 mg/kg, Pb: 420 mg/kg, Cd: 28 mg/kg), and was categorized as good candidate for a phytoextraction procedure (Barbafieri et al. 2011), which is consistent with our findings, except for Cu (BAF,BCF,TF>2), Pb and Ni (TF>1) phytoextraction. However, other authors found that this species can change phytoremediation procedure following metal soil concentration (Jiménez et al. 2011), and therefore this could explain our results with moderate soil metal concentrations. Our results for Zn, Ni, Cu and Cr phytoextraction behavior of *M. vulgare* are in good agreement with previous studies, indicating its effectiveness in MTEs uptake, root concentration and shoot transfer in field, and thus making it an important candidate for phytoextraction of contaminated soils (Moreno-Jiménez et al. 2007).

Table 3. Metal concentrations (mg/kg) in investigated plant species and their respective rhizospheric soil in Telamine Lake and Dayet Morsli

Species	Cu	Zn	Pb	Cr	Ni	Cd
D. viscosa						
leave	36.69±18.29be	126.71±62.14a	21.16±13.30b	9.27±4.02ad	3.83±0.83a	0.65±0.56a
root	16.24±4.17b	178.83±32.84a	13.09±4.02b	12.02±3.15ad	2.67±0.32ac	1.36±0.68a
Soil	9.1±2.97b	164.50±2.78a	91.13±8.75a	59.03±2.76bdc	17.62±3.71ab	0.52±0.23a
M. vulgare						
leave	25.79±0.98b	121.33±6.11a	30.94±6.08b	49.30±43.09adc	15.26± 4.58ab	0.55±0.31a
root	23.9±5.01b	135.16±46.60a	41.52±11.80b	12.54±1.81ad	27.51±27.85b	1.75±0.83a
Soil	4.80±0.25bc	84.5±19.91a	473.60±70.84c	17.52±0.00adc	15.05±0.93ab	0.85±0.10a
S. fruticosa						
leave	94.84±14.71a	142.66±34.26a	24.75±2.13b	8.75±1.36ad	6.76±2.56ab	1.90±0.76a
root	152.83±201.70bd	156.16±29.19a	17.33±3.91b	12.71±6.10ad	6.94±1.50ab	0.97±0.33a
Soil	16.15±1.94b	162.50±42.56a	114.52±18.75a	65.22±17.27c	20.15±2.13ab	0.33±0.05a
A. halimus						
leave	9.09±2.59b	143.66±36.83a	14.87±3.57b	12.88±0.51ad	4.70±2.08adb	0.95±0.47a
root	12.11±2.83b	139.00±62.93a	12.19±2.61b	9.27±0.89ad	5.68±.68ab	0.80±0.16a
Soil	6.52±5.08b	135.76±40.27a	17.31±3.46ba	24.08±8.86adc	11.05±2.25ab	3.24±5.58a
A. canescens						
leave	24.31±18.29b	156.66±30.73a	12.82±1.24b	10.82±5.80ad	7.89±3.7ab	1.03±0.49a
root	22.37±19.99b	159.50±6.87a	12.46±4.85b	10.13±7.47ad	7.78±3.79ab	0.66±0.05a
Soil	14.09±5.18b	188.16±31.50a	37.22±24.64ab	27.19±7.81adc	14.51±6.63ab	0.46±0.59a
Criteria in soil qualitya	<100	<300	<100	<150	<50	<2
One way Anova	18.964****	1.371ns	64.518****	3.976***	2.727**	0.827ns

Values are 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 significance, * p < 0.05, ** p < 0.01, *** p <0.001, ****p <0.0001. [a] quality Normes AFNOR NFU 44-041

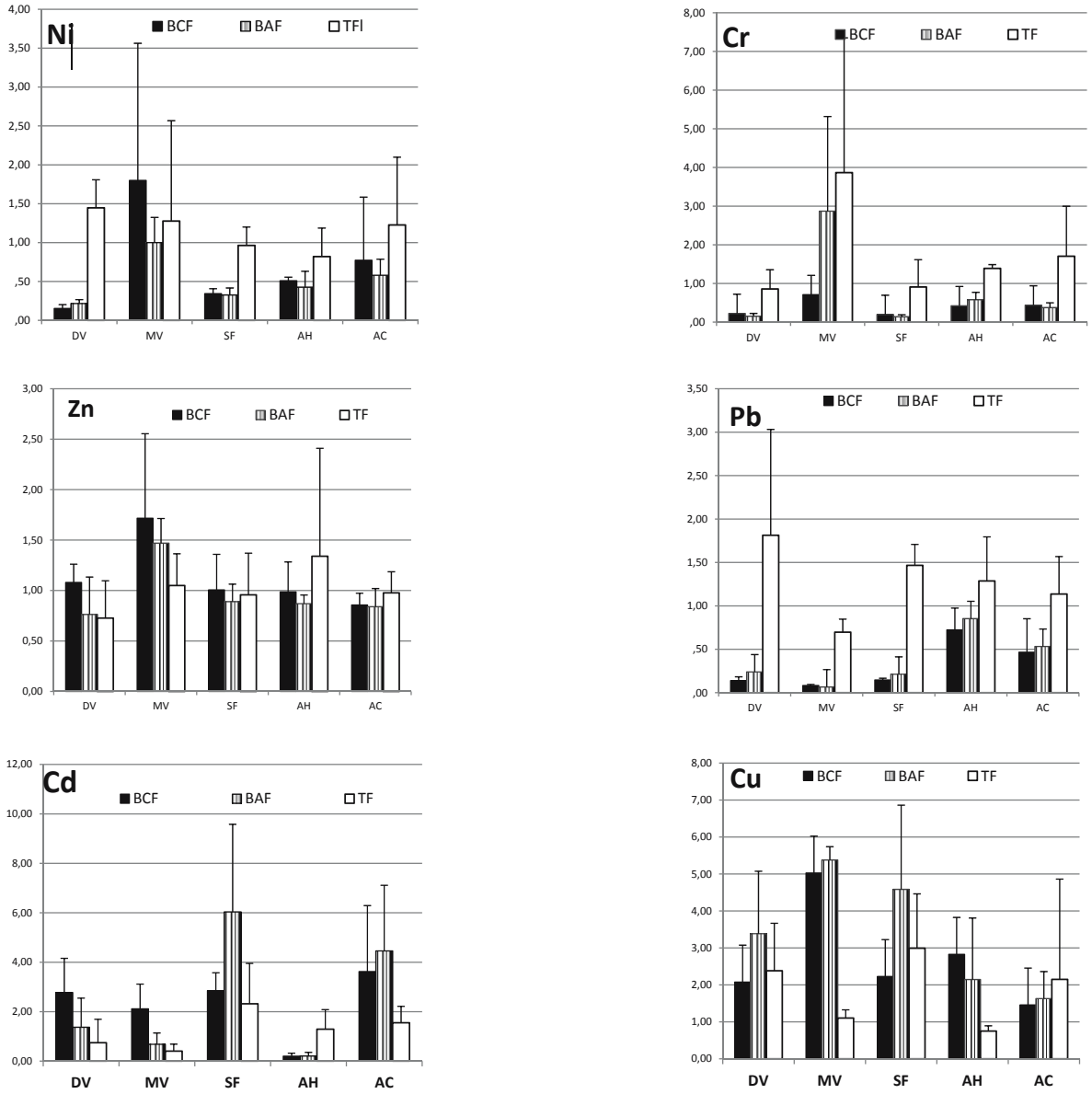

Fig. 5. Bioconcentration(BCF), bioaccumulation (BAF) and translocation factors (TF) of Cd, Cu, Zn, Pb, Ni and Cr in wetland plants. (DV: *Dittrichia viscosa* , MV: *Marrubium vulgare*; SF: *Suaeda fruticosa*; AH: *Atriplex halimus*; AC: *Atriplex canescens*)

Relationship between bioaccumulation factors, fungal infection and some soil properties

Pearson correlation showed a different interaction between bioaccumulation of metals, translocation factors and soil parameters, including pH and EC, fungal infection, AMF spore density and richness at $p<0.05$ (Table 4). Negative correlation was observed between pH, TF (Cd) (r=-0.666; p=0.007), TF (Pb) (r=-0.515; p=0.05), and BCF (Pb) (r=-0.554; p=0.032).

Salinity favoring TF (Cd) (r=0.535; p=0.04) and host plant (r=0.581; p=0.023). BCF (Pb) and BAF (Pb) are significantly correlated with host plant (r=0.613, p=0.015) and (r=0.569, p =0.027). We also noticed that AMF infection rate is negatively associated with Cd bioaccumulation (r=-0.647; p=0.009) and translocation (r=-0.673; p=0.006), however, species richness shows a negative correlation with BCF (Pb) (r=-0.619*; p =0.014) and Cd translocation-(r=0.546; p =0.035). DSE infection is positively correlated with Pb bioaccumulation (r=0.554; p=0.032), Zn translocation factor (r=0.623; p=0.013), along with negative correlation with BAF (Cu) (r=-0.547; p=0.035). Host plant also had a remarkable effect on the total AMF spores (r=0.620; p=0.014) produced in plant species rhizosphere indicating the effective role of plant species as trap plants in proliferation of AMF spores. In contrast, plants influence negatively species richness (r=-0.725; p=0.002) due to their nature (halophytes to halotolerant) which inhibits or restrains AMF species number to most specific and resistant. Thus, AMF species that is unable to multiply and to infect root systems would be successfully eliminated. Only AMF species shows an effective adaptation to the environmental problems, following addition of metals or salinity, and subsequently it could overcome the stress condition and complete their life cycles (Kamal et al. 2010), mycorrhizal frequency (r=-0.685; p=0.005) and intensity (r=-0.848; p=0.000066) and soil pH (r=-0.650; p=0.009).

As found in our study, soil salinity promotes Cd translocation factor into plant leaves, along with bioaccumulation decrease

Table 4. Effect of soil parameters and fungal colonization and diversity on accumulation factors

		pH	F	M	EC	SD	SR	DSE
Cu	BCF	ns	ns	ns	ns	ns	ns	ns
	BAF	ns	ns	ns	ns	ns	ns	-0.547*
	TF	ns	ns	ns	ns	ns	ns	ns
Zn	BCF	ns	ns	ns	ns	ns	ns	ns
	BAF	ns	ns	ns	ns	ns	ns	ns
	TF	-0.515*	ns	ns	ns	ns	Ns	0.623*
Pb	BCF	-0.554*	ns	ns	ns	ns	-0.619*	ns
	BAF	ns	ns	ns	ns	ns	ns	ns
	TF	ns	ns	ns	ns	ns	ns	ns
Cr	BCF	ns	ns	ns	ns	ns	ns	ns
	BAF	ns	ns	ns	ns	ns	ns	ns
	TF	ns	ns	ns	ns	ns	ns	ns
Ni	BCF	ns	ns	ns	ns	ns	ns	ns
	BAF	ns	ns	ns	ns	ns	ns	ns
	TFI	ns	ns	ns	ns	ns	ns	ns
Cd	BCF	ns	ns	ns	ns	ns	ns	ns
	BAF	ns	-0.647**	ns	ns	ns	ns	ns
	TF	-0.666**	-0.673**	-0.559*	0.535*	ns	-0.546*	ns
	Host plant	-0.650**	-0.685**	-0.848**	0.581*	0.620*	-0.725**	ns

Pearsons's correlation coefficients and their significance are given. Significance levels: ns indicates no significant, *p<0.05, **p<0.01, ***p<0.001.

of Cu in leaves, and with no effect on other MTEs uptake and accumulation. These results are in line with those of (Wali et al. 2014), who observed that the presence of salt facilitated the translocation of Cd to the shoot through enhancement of Cd flow in the xylem, somewhere else more than 30% of the accumulated Cd was found within *A. halimus* trichomes at the leaf surface (Lefèvre et al. 2009). Cd uptake enhancement by NaCl treatment has been also reported (Manousaki and Kalogerakis 2009), whereas Pb uptake was not affected. In fact, the negative correlation between pH, Pb, Zn root concentration and leaves translocation obtained in the present study was previously discussed by several authors on the basis of Cr mobility in *A. canescens* (Sawalha et al. 2005), showing that the maximum accumulation was in acidic pH (3–5), then it decreases at high pH value, and thus becomes comparable to our results where soil was slightly alkaline. AMF infection rate significantly inhibited Cd bioaccumulation and translocation in higher plant parts. This is due to reason that the contribution of AMF in phytoremediation is associated with fungal role in phytostabilization or phytoextraction (Meier et al. 2012). In our case, mycorrhizal colonization promoted MTE stabilization in plant rhizospheres, whether by MTE sequestration in root apoplasm or by spore metal retention favoring phytostabilization. In contrast, well-developed mycorrhization could promote bioconcentration (BCF) and translocation (TF) factors and increase the metal content in plant shoots, promoting phytoextraction (Wei et al. 2016).

Conclusion

This first report on Algerian AMF diversity in the rhizosphere of MTE accumulator plant species in saline metal-polluted wetlands revealed an environmental stress, including high salinity and average pollution by trace elements. The investigated plants in the current study are defined as important candidates for phytoremediation of saline wetlands. Interestingly, AMF diversity developed mechanisms to resist or tolerate heavy metal stress, and could accordingly develop more efficient symbiosis with their host plants, and, as it has been suggested, for use in disturbed or polluted saline land remediation.

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