

# 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

Brahim Lotmani<sup>1</sup> • Lalia Fatarna<sup>1</sup> • Abdallah Berkani<sup>1</sup> •  
Jacques Rabier<sup>2</sup> • Pascale Prudent<sup>3</sup> • Isabelle Laffont-Schwob<sup>2\*</sup>

<sup>1</sup> Laboratoire Protection des Végétaux, Unité culture *in vitro*, Université A. Ibn Badis de Mostaganem, Algeria

<sup>2</sup> Equipe Biomarqueurs et Bioindicateurs Environnementaux, Institut Méditerranéen d'Ecologie et de Paléocologie, UMR CNRS IRD 6116, Université de Provence, Case 97, 3 pl. V. Hugo, 13331 Marseille Cedex 3, France

<sup>3</sup> Laboratoire Chimie Provence, UMR 6264, Pôle Environnement, Equipe Chimie de l'Environnement Continental, Université de Provence, Case 29, 3 pl. V. Hugo, 13331 Marseille Cedex 3, France

Corresponding author: \* [isabelle.schwob@univ-provence.fr](mailto:isabelle.schwob@univ-provence.fr)

## ABSTRACT

We investigated the effect of three heavy-metal-rich media (Pb, Zn and Cu) on germination and growth of three Algerian populations (Kharouba, Debdaba and El Mactae) of *Atriplex halimus* L. Four concentrations of metals (0, 250, 500 and 1000 ppm) were applied to test the tolerance and metal accumulation of the populations grown in a greenhouse. Germination rates were not decreased by heavy metal treatment. Among the three populations, growth of Kharouba was the least affected, regardless of the metal present as confirmed by the tolerance indices. *Atriplex* from Kharouba originated from a contaminated site that may have led to a better metal tolerance for this population. All three populations showed good metal sorption capabilities in root tissues. Maximal metal accumulation in aerial parts was obtained for Debdaba population with a mean of 30.9, 1.1 and 257 ppm of Cu, Pb and Zn accumulation, respectively. However, metal concentrations were below the level of US Domestic Animal Metal Toxicity Limits and the three *A. halimus* populations, appeared to be good candidates for a phytostabilization strategy without threat to grazing animals.

**Keywords:** germination, growth, halophyte, metal-tolerance, phytoremediation, trace metals

## INTRODUCTION

Lead (Pb), zinc (Zn) and copper (Cu) are elements occurring naturally in the superficial earth strata; however, their concentrations can rise to toxic levels due to industrial activities, including mining and smelting of metalliferous ores, burning of leaded gasoline and the use of fertilizers, pesticides and sewage sludge (He *et al.* 2005). Heavy metals such as Cu and Zn are essential for normal plant growth, although high concentrations of both essential and non-essential metals such as Pb can result in growth inhibition and toxicity symptoms. Some plants appear to possess great tolerance to high levels of heavy metal pollution. However, toxic levels of heavy metals affect a variety of processes in plants (Maksymiec and Krupa 2002) causing different sub-cellular responses, i.e. metabolic reactions, which can cause damage at the cellular level or lead to wider phytotoxic responses (Vangronsveld and Clijsters 1994).

Heavy metal-tolerant plants grown in polluted medium can take up and accumulate metals in their tissues and therefore constitute a health hazard for humans and animals. It is generally assumed that metal tolerance in absolute metallophytes, which grow only on metal-contaminated and naturally metal-rich soil, was gradually acquired, according to a classical evolutionary process, in response to thousands or millions of years of selective pressure (Wild and Bradshaw 1977). However, Remon *et al.* (2007) noted that metal tolerance in metallophytes growing in newly polluted environments does not result from a long and gradual evolutionary process. This basic observation has led to the conclusion that tolerant races or ecotypes can emerge from normal

plant populations after a very short time of exposure to metal, i.e. within a few years (Antonovics *et al.* 1971). In several cases, the evolution of heavy-metal-tolerant populations has been shown to be very rapid (Wu *et al.* 1975; Macnair 1981), and it seems to depend on the occurrence of genes for tolerance in the populations growing on uncontaminated surrounding soil (Macnair 1987; Al-Hiyaly *et al.* 1993).

Halophytes that grow naturally in saline environments, such as salt marshes, salt spans and salt deserts are distinguished from glycophytes by their tolerance of saline conditions. The use of halophytic plants in pasture and fodder production on saline soils is the only economically feasible solution available (Osman *et al.* 2006). Many halophytes have also economic value as ornamentals for arid and coastal regions (Mandák 2003) and as sources of edible oil from the seeds (Weber *et al.* 2001).

*Atriplex* species (saltbushes) are dominant in many arid and semi-arid regions of the world, particularly in habitats that combine relatively high soil salinity with aridity. The studies of the biology of *Atriplex* can be classified in two domains, as fodder and as metal accumulator (both of which are generally considered as antinomic approaches). Halophyte species belonging to the genus *Atriplex* have been recommended for remediation of former mining areas and industrial sites (Salo *et al.* 1996; Glenn *et al.* 2001; Mendez *et al.* 2007; Kachout *et al.* 2010; Eid and Eisa 2010). *Atriplex halimus* L. is a widespread Mediterranean shrub species (Osmond *et al.* 1980; McArthur and Sander-son 1984; Ortíz-Dorda *et al.* 2005) with high resistance to various abiotic stresses such as drought (Le Houérou 1992),

salinity (Bajji *et al.* 1998, 2002), and to heavy metal stress (Lutts *et al.* 2004; Lefèvre *et al.* 2009). In addition to its ability to grow on degraded soils and in very harsh conditions such as soil salinity (Pourrat and Dutuit 1994; Martinez *et al.* 2004), *A. halimus* has the property to produce an abundant foliar biomass even during unfavourable periods of the year (Kessler 1990). These characteristics enable this plant to be used in the phytoremediation of contaminated soils; for example, for phytoextraction of cadmium and zinc in southeastern Spain (Lutts *et al.* 2004). However, since this species showed a high genetic variability (Abbad *et al.* 2004; Ortíz-Dorda *et al.* 2005), phytoextraction capacities may greatly vary from one population to another.

In Algeria, many industrial activities and urban wastes along the coast near Mostaganem have led to heavy metal pollution of the surrounding soils (Guermoud *et al.* 2009; Gürlük 2009) where *A. halimus*, which grows naturally in this area, appears to be a potential species for phytoremediation, provided it does not constitute a health hazard via the food web. Attributes in favour of the use of *A. halimus* include its status as a halophyte (Bajji *et al.* 1998; Nedjimi and Daoud 2009), its deep root system (Belkhouja and Bidai 2004), its metal tolerance (Lutts *et al.* 2004), and the fact that no hyperaccumulation of toxic metals such as Pb has been previously demonstrated. Thus, this species may be useful in phytostabilisation of the degraded coastal areas, given that phytostabilisation, due to lower costs and easier implementation than phytoextraction, is a more realistic strategy of managing polluted land in arid environments. Moreover, since *A. halimus* is an important component of coastal pastures and is a fodder source for many grazing animals along the Algerian coast (Ben Salem and Smith 2008; Nedjimi and Daoud 2009), its use in phytostabilisation is also of higher potential interest than phytoextraction, provided that the metal concentrations in the fodder are low, with proper grazing management.

In this paper, we report on the potential value of Algerian populations of *Atriplex halimus* for phytostabilisation by quantifying, under controlled conditions, their toxic metal accumulation in relation to their growth rates. Three Algerian *A. halimus* populations were evaluated for their tolerance to Cu, Zn and Pb including one that originated from a highly contaminated site.

## MATERIALS AND METHODS

### Plant material and characteristics of the study sites

Mediterranean saltbush (*Atriplex halimus* L.) seeds were collected from two western Algerian micro zones: two populations, from around Mostaganem - Debdaba (DBD) and Kharouba (KHB) - and one from Arzew (an industrial centre) - El Mactae (MCT). Climatically, all three sites occur under semi-desertic conditions. The DBD is in an agricultural zone at around 4 km from the sea, while the KHB is a seaside zone and MCT is on salt marshes. The DBD population was characterized by larger leaves and faster growth than the two other two populations. Plants of this population are used as a hedge, serving as a living horticultural field marker. The MCT and KHB populations occur spontaneously in the region and have small leaves with a red colouration characterizing the young leaves of these two populations; this colouration does not correspond to a stress reaction and is a conserved feature whatever the culture conditions.

The soil is sandy in all three sites. Analysis of the three selec-

ted heavy metals, Cu, Pb and Zn, was carried out on four soil samples taken from the three sites (Table 1). For the three sites, five representative soil sampling points were selected close to seed collecting areas. The site of each soil sample was separated by a distance of 5 m, according to a cross pattern; the top layer (0 to 20 cm, after removing the surface litter) was collected and oven dried for 48 h at 30-35°C. After drying, the soil samples were sieved at 2 mm, ground finer than 0.2 mm and then homogenized for metal analysis. After aqua regia microwave digestion, Cu, Pb and Zn concentrations were determined by ICP-AES (inductively coupled plasma atomic emission spectrometry). Recovery and accuracy of the metal analysis in soils were validated by analyzing certified reference materials (CRM SS-1 contaminated soil, EPA-3050A digestion method).

The soil from KHB was the most polluted of the three but the furthest from the industrial site of Arzew. However this KHB site was previously market gardening land irrigated with water from the watercourse (wadi) flowing through the Tijditt quarter of the city of Mostaganem. Cultivation was forbidden since 1995 when water pollution was noticed.

### Metal solution preparation

CuSO<sub>4</sub>·5H<sub>2</sub>O, PbSO<sub>4</sub> and ZnSO<sub>4</sub>·7H<sub>2</sub>O stock solutions were prepared in deionised water with reagent-grade salts (Fisher Scientific) at three different concentrations i.e. 390, 790, 1570 mM of Cu, 120, 240, 480 mM of Pb or 380, 760, 1530 mM of Zn corresponding to 2.500, 5.000 and 10.000 ppm metal-solutions. The pH of the solutions was adjusted to 7 ± 0.5 for germination experiment. For the greenhouse experiment, the pH of the metal-solutions was adjusted to 5.8 ± 0.5 with KOH.

### Germination conditions

After disinfection in a solution of 95% ethanol and 0.8% formaldehyde during 15 min, *A. halimus* seeds (3 replicates of 30 seeds) of each population were germinated in disposable Petri dishes, 100 mm in diameter, on ashless Whatman filter paper n° 1 moistened with 10 ml of Cu (39, 79, 157 mM), Pb (12, 24, 48 mM) or Zn (38, 76, 153 mM) solutions. Control plants received the same volume of deionised water. Thus, 90 Petri dishes in all were arranged in a completely randomized design with a total of 30 conjugated treatments (three *A. halimus* populations with three metals at three metal concentrations + one control each) in triplicate. The Petri dishes were placed in a growth room at 25°C with continuous illumination of 65 μmol.m<sup>-2</sup>.s<sup>-1</sup> and 85% relative humidity. Seeds were moistened every 3 days to maintain humidity constant. The percentage of germination was calculated after 14 days.

### Growth conditions and biomass production

Growth was measured in a greenhouse at the experimental farm of A. Ibn Badis University. The experiment was a randomized complete block with 9 replicates. After disinfection in 2.5% sodium hypochlorite during 15 min, 90 *A. halimus* seeds were individually sown in plastic pots (150 ml) filled with acid (HCl) rinsed sand used as substrate. Plantlets were kept in the greenhouse for 3 months at 25 ± 5°C and an average PAR of 480 μmol.m<sup>-2</sup>.s<sup>-1</sup> from natural sunlight. During the experiment, each pot was weighed daily and irrigated to maintain 70% relative water content (RWC) with 1/5 Murashige and Skoog (1962) mineral basal medium, free of Zn and Cu. No deficiency of both metals was observed on plants during the experiment. Each pot was placed in a shallow container to recover the solution drained. After two months, 30 ml of stock metal solution were applied per pot to obtain the final

**Table 1** Cu, Pb and Zn contents in soil samples of the three sites.

| Sites | Soil contamination |          |          |          |          |          |          |          |          |
|-------|--------------------|----------|----------|----------|----------|----------|----------|----------|----------|
|       | Mean*              |          |          | Min      |          |          | Max      |          |          |
|       | Cu (ppm)           | Pb (ppm) | Zn (ppm) | Cu (ppm) | Pb (ppm) | Zn (ppm) | Cu (ppm) | Pb (ppm) | Zn (ppm) |
| DBD   | 20 a               | 46 ab    | 38 b     | 11       | 8        | 29       | 24       | 150      | 47       |
| KHB   | 60 a               | 150 a    | 260 a    | 10       | 27       | 90       | 86       | 239      | 416      |
| MCT   | 21 a               | 19 b     | 29 b     | 9        | 4        | 16       | 55       | 52       | 59       |

\* Different letters within a column indicate significant differences according to Tukey's test ( $p \leq 0.05$ ,  $n=5$ )

**Table 2** Germination percentages of the three *A. halimus* populations treated with various Cu, Pb and Zn concentrations.

| Population | Germination % |                    |         |         |                    |        |         |                    |        |        |
|------------|---------------|--------------------|---------|---------|--------------------|--------|---------|--------------------|--------|--------|
|            | Control       | Cu treatment (ppm) |         |         | Pb treatment (ppm) |        |         | Zn treatment (ppm) |        |        |
|            | 0             | 250                | 500     | 1000    | 250                | 500    | 1000    | 250                | 500    | 1000   |
| KHB        | 31 ± 4        | 24 ± 5             | 21 ± 12 | 14 ± 8  | 28 ± 2             | 33 ± 9 | 29 ± 13 | 27 ± 3             | 32 ± 4 | 34 ± 7 |
| DBD        | 100 ± 0       | 89 ± 7             | 86 ± 7  | 83 ± 3  | 91 ± 2             | 93 ± 3 | 98 ± 2  | 94 ± 4             | 97 ± 3 | 98 ± 4 |
| MCT        | 89 ± 11       | 86 ± 4             | 94 ± 10 | 88 ± 10 | 86 ± 5             | 84 ± 7 | 87 ± 9  | 94 ± 4             | 97 ± 3 | 87 ± 6 |

Data are the means of triplicate ± SE. Each replicate corresponds to 30 individuals.

ppm (weight) for each treatment. The test was performed on two months old plants grown in the greenhouse. Plants were then harvested one month after treatment and roots and shoots were analysed separately. Roots were thoroughly washed with tap water, carefully rinsed with deionised water, and then rapidly dried with paper tissue. The length of the shoot and root of each individual was measured. After oven-drying for 72 h at 80°C, the dry weight of each sample was recorded. Metal tolerance indices of populations were expressed as the ratio of either shoot and root length or total dry weight measured in the presence of the metal compared to either shoot and root length or total dry weight under the control condition (Wilkins 1978).

### Metal analyses

Metal concentration was analysed on roots and shoots separately. Tissue (0.2 g of ground material) was dry ashed at 450 °C for 6 h and then dissolved in 10 ml concentrated nitric acid (Zarcinas *et al.* 1987). The three metals were analysed in each sample by atomic absorption spectrometry (PNICAM SP 200).

### Statistical analyses

Germination was analysed using the GLM procedure with population, concentration and metal as main effects. Growth parameters and metal contents were studied with ANOVAs. Prior to the statistical analyses, the Shapiro-Wilk test and the Levene's test were used to check the assumption of normality and homoscedasticity. When the distribution of the data was not linear, data were transformed before statistical analyses. Multiple comparisons of means were performed using Tukey's test. Statistical analyses were performed for all data using JMP 9 statistical software (SAS Institute, Cary, North Carolina, USA). Untransformed data appear in all tables and figure.

## RESULTS

### Effect of copper, lead and zinc treatment on germination

Regardless of the metal treatment, KHB population had a lower germination percentage (ranging from 14 to 34%) than the other two (ranging from 83 to 100%) as shown in **Table 2**. For all three populations, no significant difference was observed between the germination percentages of control and metal treatments, whatever the metal concentration.

### Effect of copper, lead and zinc treatment on shoot and root lengths

Copper treatment (**Fig. 1A**). For KHB population, no difference between control and Cu treatment was measured for both shoot and root lengths. For the DBD and MCT populations, however, Cu significantly reduced the root and the shoot lengths compared to controls.

Lead treatment (**Fig. 1B**). Root lengths did not differ significantly between the various populations or as a function of the Pb treatment excepted for DBD and MCT with 120 and 480 mM of Pb (250ppm and 1000 ppm of Pb), respectively. Shoot lengths were more affected by the Pb treatments than roots. A significant reduction of shoot length was only observed at 480 mM for KHB population. For DBD population, a significant reduction occurred at 240 and 480 mM. For MCT population, no reduction was de-

**Table 3** Cu, Pb and Zn tolerance indices (TI) of *Atriplex halimus* populations.

| Population | Metal treatment | Metal concentration (ppm) | Shoot length TI | Root length TI |
|------------|-----------------|---------------------------|-----------------|----------------|
| KHB        | Cu              | 250                       | 0.77            | 0.79           |
|            |                 | 500                       | 0.86            | 0.93           |
|            |                 | 1000                      | 0.82            | 1.01           |
|            | Pb              | 250                       | 0.73            | 1.04           |
|            |                 | 500                       | 0.91            | 0.85           |
|            |                 | 1000                      | 0.59            | 0.83           |
|            | Zn              | 250                       | 1               | 1              |
|            |                 | 500                       | 0.75            | 0.93           |
|            |                 | 1000                      | 0.8             | 0.9            |
| DBD        | Cu              | 250                       | 0.72            | 0.62           |
|            |                 | 500                       | 0.46            | 0.63           |
|            |                 | 1000                      | 0.59            | 0.68           |
|            | Pb              | 250                       | 1.06            | 0.68           |
|            |                 | 500                       | 0.57            | 0.75           |
|            |                 | 1000                      | 0.44            | 0.73           |
|            | Zn              | 250                       | 0.61            | 0.71           |
|            |                 | 500                       | 0.68            | 0.79           |
|            |                 | 1000                      | 0.67            | 0.64           |
| MCT        | Cu              | 250                       | 0.66            | 0.7            |
|            |                 | 500                       | 0.61            | 0.72           |
|            |                 | 1000                      | 0.59            | 0.67           |
|            | Pb              | 250                       | 0.61            | 0.91           |
|            |                 | 500                       | 0.6             | 0.72           |
|            |                 | 1000                      | 0.74            | 0.7            |
|            | Zn              | 250                       | 0.75            | 0.70           |
|            |                 | 500                       | 0.82            | 0.72           |
|            |                 | 1000                      | 0.72            | 0.85           |

TI expressed as mean of shoot or root length of plant treated/mean of shoot or root length of plant control.

monstrated at 480 mM whereas 120 and 240 mM generated a significant reduction of length compared to control.

Zinc treatment (**Fig. 1C**). No variation of root length was observed for KHB population. For DBD and MCT populations, the reduction was significant for 380 and 760 mM (250 and 500 ppm) but not for 1530 mM (1000 ppm) vs. controls.

No significant difference was observed for Zn treatment on shoot length whatever the treatment concentration for KHB and MCT populations. For DBD, a reduction of shoot length was observed at 380 and 760 mM.

Root and shoot tolerance indices (**Table 3**) indicated that KHB tolerated all metals better than the two other populations.

### Effect of copper, lead and zinc on shoot and root dry weights

For the root dry weight (**Table 4**), no significant difference was noticed between treated and control plants whatever the population and the metal-treatment. Considering each population independently, metal treatments had no effect on shoot dry weight.

However, in respect to the dry biomass tolerance indices, KHB population was more tolerant to Cu, Pb and Zn than the two other populations.

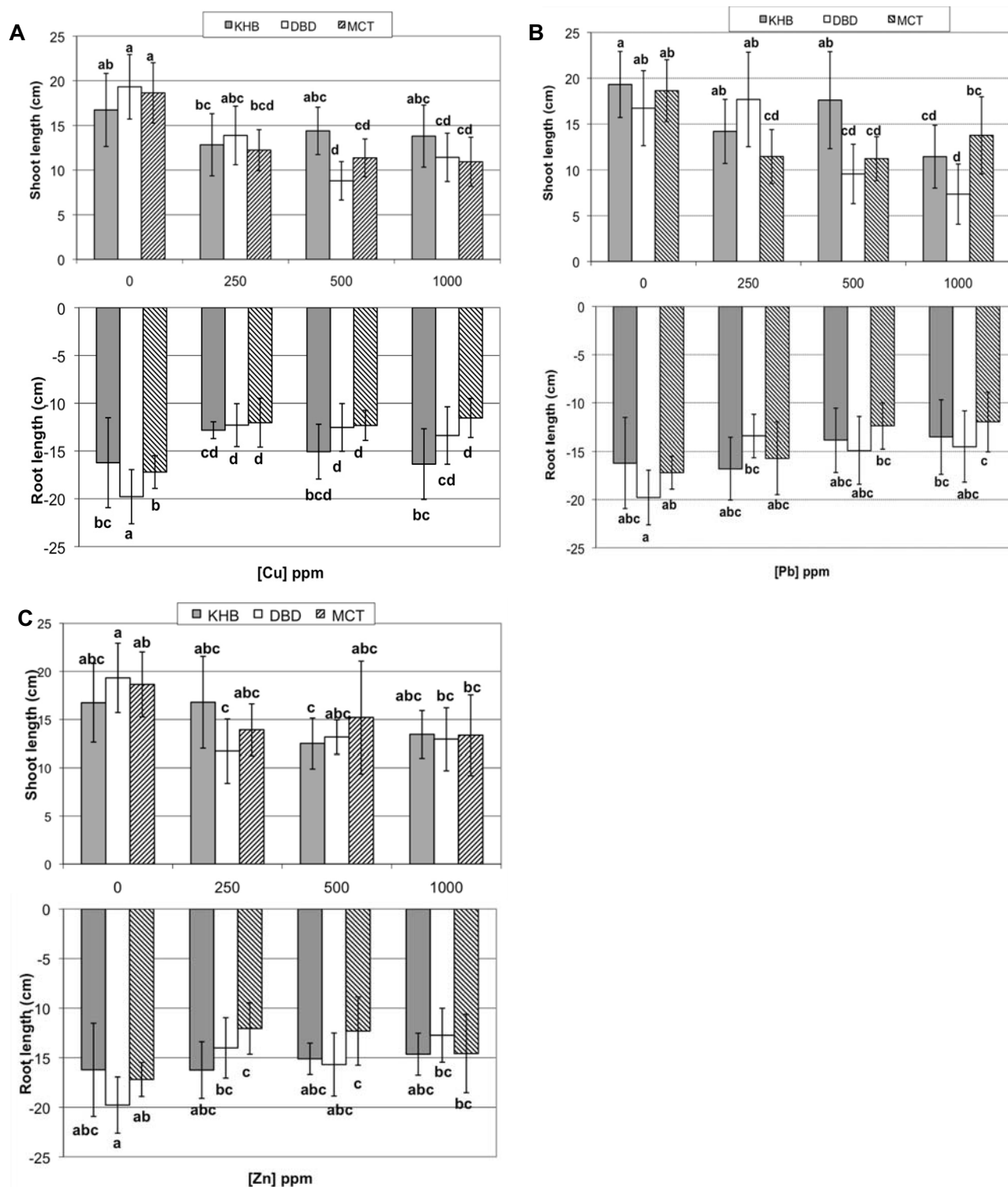


Fig. 1 Shoot and root lengths of *Atriplex halimus* populations (in cm) treated with 250, 500 and 1000 ppm metal: (A): copper (Cu), (B): lead (Pb) and (C): zinc (Zn). Bars denote means  $\pm$  SE. N=9. a b c d: Means with the same letters were not significantly different ( $p \leq 0.05$ ).

## Concentration of copper, lead and zinc in roots and shoots after metal treatment

### 1. Copper treatment

The population had no effect on Cu concentration in roots and shoots (Table 5) while this parameter varied depending on the applied concentration and the conjugate effect of population and concentration. Cu concentrations in shoot and roots were higher for 79 and 157 mM of Cu (i.e. 500 and 1000 ppm) than for control.

### 2. Lead treatment

*A. halimus* plants accumulated Pb (Table 5), although Pb concentrations were low in plant tissues. Regardless to the population, Pb concentrations of roots were higher than those of shoots.

### 3. Zinc treatment

Whatever the population, increasing concentrations of Zn gave increased Zn concentration in roots (Table 5). At 1000 ppm, higher concentrations of Zn were found in shoots of the DBD population compared to the other populations.

**Table 4** Dry biomass and metal tolerance indices (TI) of *Atriplex halimus* populations.

| Population | Metal treatment | Metal concentration (ppm) | RDW (mg)*   | SDW (mg)*    | TDB (mg)     | TI          |      |
|------------|-----------------|---------------------------|-------------|--------------|--------------|-------------|------|
| KHB        | Control         | 0                         | 177 ± 120 a | 428 ± 280 a  | 606          |             |      |
|            |                 | Cu                        | 250         | 238 ± 248 a  | 143 ± 64 ab  | 381         | 1.9  |
|            |                 |                           | 500         | 215 ± 210 a  | 185 ± 141 ab | 400         | 1.24 |
|            |                 |                           | 1000        | 164 ± 108 a  | 354 ± 86 a   | 518         | 0.65 |
|            | Pb              | 250                       | 202 ± 166 a | 410 ± 198 a  | 612          | 1.01        |      |
|            |                 | 500                       | 280 ± 151 a | 288 ± 253 ab | 568          | 0.94        |      |
|            |                 | 1000                      | 219 ± 169 a | 199 ± 125 ab | 418          | 0.69        |      |
|            | Zn              | 250                       | 291 ± 149 a | 393 ± 159 ab | 683          | 1.13        |      |
|            |                 | 500                       | 156 ± 78 a  | 280 ± 113 ab | 436          | 0.72        |      |
|            |                 | 1000                      | 71 ± 29 a   | 181 ± 148 ab | 252          | 0.42        |      |
|            | DBD             | Control                   | 0           | 251 ± 175 a  | 326 ± 184 ab | 576         |      |
|            |                 |                           | Cu          | 250          | 99 ± 47 a    | 177 ± 76 ab | 276  |
| 500        |                 |                           |             | 101 ± 40 a   | 92 ± 22 b    | 192         | 0.33 |
| 1000       |                 |                           |             | 223 ± 100 a  | 149 ± 55 ab  | 373         | 0.65 |
| Pb         |                 | 250                       | 187 ± 134 a | 181 ± 89 ab  | 367          | 0.64        |      |
|            |                 | 500                       | 192 ± 60 a  | 248 ± 122 a  | 439          | 0.76        |      |
|            |                 | 1000                      | 152 ± 84 a  | 200 ± 41 a   | 351          | 0.61        |      |
| Zn         |                 | 250                       | 107 ± 35 a  | 153 ± 93 ab  | 259          | 0.45        |      |
|            |                 | 500                       | 137 ± 67 a  | 160 ± 121 ab | 297          | 0.51        |      |
|            |                 | 1000                      | 103 ± 49 a  | 178 ± 77 ab  | 281          | 0.49        |      |
| MCT        |                 | Control                   | 0           | 239 ± 76 a   | 192 ± 44 ab  | 431         |      |
|            |                 |                           | Cu          | 250          | 231 ± 219 a  | 145 ± 43 ab | 376  |
|            | 500             |                           |             | 103 ± 51 a   | 156 ± 64 ab  | 260         | 0.6  |
|            | 1000            |                           |             | 101 ± 46 a   | 147 ± 83 ab  | 248         | 0.57 |
|            | Pb              | 250                       | 251 ± 143 a | 130 ± 33 ab  | 380          | 0.88        |      |
|            |                 | 500                       | 162 ± 73 a  | 105 ± 23 ab  | 267          | 0.62        |      |
|            |                 | 1000                      | 92 ± 46 a   | 130 ± 69 ab  | 222          | 0.52        |      |
|            | Zn              | 250                       | 154 ± 74 a  | 166 ± 67 ab  | 320          | 0.74        |      |
|            |                 | 500                       | 152 ± 112 a | 286 ± 177 ab | 438          | 1.02        |      |
|            |                 | 1000                      | 161 ± 63 a  | 185 ± 123 ab | 346          | 0.8         |      |

Values are the means ± SE of 9 replicates. \* Different letters within a column indicate significant differences according to Tukey's test ( $p \leq 0.05$ ). RDW: root dry weight; SDW: shoot dry weight; TDB: total dry biomass; TI: total biomass tolerance index

**Table 5** Cu, Pb and Zn accumulation in roots and shoots of three treated *Atriplex halimus* populations vs control.

| Population       | Metal concentration (ppm) | Type of metal* |                |             |               |               |              |
|------------------|---------------------------|----------------|----------------|-------------|---------------|---------------|--------------|
|                  |                           | Cu             |                | Pb          |               | Zn            |              |
|                  |                           | In root        | In shoot       | In root     | In shoot      | In root       | In shoot     |
| KHB              | 0                         | 3.4 ± 0.9 c    | 3.6 ± 0.1 de   | ND          | ND            | 0.42 ± 0.01 d | ND           |
|                  | 250                       | 24.9 ± 0.9 bc  | 1.8 ± 1.7 de   | 4 ± 4 b     | 0.71 ± 0.06 a | 1.7 ± 0.4 cd  | 28 ± 2 e     |
|                  | 500                       | 211 ± 102 a    | 9.1 ± 2.4 abc  | 8 ± 6 ab    | 0.18 ± 0.04 a | 9.8 ± 0.6 b   | 211 ± 102 ab |
|                  | 1000                      | 60 ± 21 ab     | 18.7 ± 5.8 abc | 22 ± 7 ab   | 1.7 ± 0.3 a   | 9 ± 3 b       | 62 ± 19 bc   |
| DBD              | 0                         | 2.7 ± 0.4 c    | 1.9 ± 1.5 e    | ND          | ND            | 1.6 ± 0.7 cd  | ND           |
|                  | 250                       | 11.2 ± 0.9 bc  | 5.8 ± 1.0 bcde | 5.7 ± 0.7 b | 1.1 ± 0.4 a   | 2.3 ± 0.2 c   | 19 ± 4 e     |
|                  | 500                       | 181 ± 42 a     | 17.7 ± 2.0 ab  | 12 ± 2 ab   | 0.8 ± 1 a     | 14 ± 1 b      | 192 ± 14 a   |
|                  | 1000                      | 156 ± 48 a     | 30.9 ± 3.6 a   | 51 ± 1 a    | 0.1 ± 0.01 a  | 37.9 ± 0.3 a  | 257 ± 48 a   |
| MCT              | 0                         | 2.4 ± 2.2 c    | 8.4 ± 6.4 de   | ND          | ND            | 0.8 ± 0.4 cd  | ND           |
|                  | 250                       | 21 ± 8 c       | 3.2 ± 1.1 cde  | 4 ± 3 b     | 0.4 ± 0.1 a   | 2.7 ± 1.3 c   | 21 ± 1 d     |
|                  | 500                       | 68 ± 30 ab     | 4.8 ± 2.7 bcd  | 3.7 ± 2.3 b | 0.13 ± 0.02 a | 9.2 ± 0.7 b   | 68 ± 16 c    |
|                  | 1000                      | 137 ± 60 a     | 18.3 ± 3.3 ab  | 29 ± 24 ab  | 0.9 ± 1 a     | 31 ± 6 a      | 92 ± 6 bc    |
| <i>F</i> -values |                           |                |                |             |               |               |              |
| population       |                           | 0.06 NS        | 0.35 NS        | 0.36 NS     | 2.7 NS        | 0.66 NS       | 0.18 NS      |
| concentration    |                           | 24.3 ***       | 29.4 ***       | 47.5 ***    | 0.09 NS       | 46.7 ***      | 34.8 ***     |
| Pop X conc       |                           | 17.4 ***       | 16.0 ***       | 14.6 ***    | 2.2 NS        | 65.2 ***      | 146 ***      |

Values represent the means ± SE. \* Different letters within a column indicate significant differences according to Tukey's test ( $p \leq 0.05$ ). *F* values followed by \*\*\* mean that the effect of the treatment is significant and, NS, non significant. ND: under the level of detection

## DISCUSSION AND CONCLUSIONS

In our study, Pb, Zn and Cu did not inhibit germination of seed of *Atriplex halimus* even under high concentration of metal (1000 ppm). However, variations in germination capacity were observed among populations. This result is in agreement with the germination rates observed on the field (results not shown) and the fact that this species exhibits a high level of phenotypic and genetic variability with regards to many traits (Haddioui and Baaziz 2001; Abbad *et al.* 2004; Ortiz-Dorda *et al.* 2005; Walker *et al.* 2005).

Growth parameters were differently affected by metal treatments. KHB was the least affected by metal treatment regardless of the metal used. However, KHB population with or without metal had a lower growth rate compared to

the two other populations. Tolerance indices showed that the KHB population was more tolerant to the three metals than the other populations. Analyses of Pb and Zn concentrations in the soils of origin of the three populations showed that for KHB population, the pollutant concentrations were the highest of the three collection sites. We hypothesize that ecotypes of *A. halimus* have evolved in Algeria, confirming the value of a screening for pollution tolerance of various populations for phytoremediation purposes. Moreover, it appeared that *Atriplex* colonizing the site at Kharouba acquired metal tolerance in a short period (i.e. a decade), consistent with the hypothesis of the emergence of tolerant ecotypes after a very short time of exposure to metal (Remon *et al.* 2007).

However, none of the three populations was found to be

a metal-hyperaccumulator. Maximal mean Cu, Pb and Zn accumulation in aerial parts was 30.9, 1.1 and 257 ppm, respectively for DBD population. It has been previously demonstrated that Cu uptake and translocation by plants is strictly regulated, resulting in very low leaf concentrations, in the range of 2-20 ppm (Wallnofer and Engelhardt 1984), whereas Zn is primarily accumulated in leaves, with concentrations in the order of hundreds of ppm (Rosselli *et al.* 2003). Moreover, metal concentrations in shoots were below toxicity set for domestic animals (Mendez and Maier 2008). The three *A. halimus* populations, particularly KHB, appeared to be good candidates for a phytostabilization strategy of the industrial surroundings of Arzew without posing a threat for grazing animals. *A. halimus* can be used for a vegetative cap in combination with methods of urban waste management such as composting and landfill capping (Nagendran *et al.* 2006; Guermoud *et al.* 2009). Since there are few fodder sources in the arid coast around Arzew, *A. halimus* is a shrub of great interest as a sheep food supplement possibly in association with cactus and others feed supplements (Ben Salem and Smith 2008). However, the role of *Atriplex* shrubs as forage is not to increase milk, meat or wool production during the good season, like the herbaceous forages, but to provide green forage, rich in protein, during the dry season, in order to reduce animal weight loss. In this context, heavy metal uptake by *A. halimus* may control polluted dust dissemination and its cultivation may be encouraged. This study also confirms the results obtained by Manousaki and Kalogerakis (2009) and Kachout *et al.* (2009) showing that halophytes are also promising candidates for the removal of heavy metals even on non-saline soils.

## ACKNOWLEDGEMENTS

We thank James Aronson (CEFE/CNRS) for his keen interest in the present study and for providing many useful comments on the manuscript, and Laurent Vassalo for his technical assistance. We are grateful to Andreea Borla for her helpful technical assistance during statistical analysis.

## REFERENCES

- Abbad A, Cherkaoui M, Wahid N, El Hadrami A, Benchaabane A (2004) Variabilités phénotypique et génétique de trois populations naturelles d'*Atriplex halimus*. *Comptes Rendus Biologies* **327**, 371-380
- Al-Hiyaly SAK, McNeilly T, Bradshaw AD, Mortimer AM (1993) The effect of zinc contamination from electricity pylons, genetic constraints on selection for zinc tolerance. *Heredity* **70**, 22-32
- Antonovics J, Bradshaw AD, Turner RG (1971) Heavy metal tolerance in plants. *Advances in Ecological Research* **7**, 1-85
- Bajji M, Kinet JM, Lutts S (1998) Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. *Plant Science* **137**, 131-142
- Bajji M, Kinet JM, Lutts S (2002) Osmotic and ionic effects of NaCl on germination, early seedling growth, and ion content of *Atriplex halimus* (Chenopodiaceae). *Canadian Journal of Botany* **80**, 297-304
- Belkhdja M, Bidai Y (2004) Réponse des graines d'*Atriplex halimus* L. à la salinité au stade de la germination. *Sécheresse* **15**, 331-335
- Ben Salem H, Smith T (2008) Feeding strategies to increase small ruminant production in dry environments. *Small Ruminant Research* **77**, 174-194
- Eid MA, Eisa SS (2010) The use of some halophytic plants to reduce Zn, Cu and Ni in soil. *Australian Journal of Basic and Applied Sciences* **4**, 1590-1596
- Glenn EP, Waugh WJ, Moore D, McKeon C, Nelson SG (2001) Revegetation of an abandoned uranium millsite in the Colorado Plateau, Arizona. *Journal of Environmental Quality* **30**, 1154-1162
- Guermoud N, Ouadjnia F, Abdelmalek F, Taleb F, Addou A (2009) Municipal solid waste in Mostaganem city (Western Algeria). *Waste Management* **29**, 896-902
- Gürlük S (2009) Economic growth, industrial pollution and human development in the Mediterranean Region. *Ecological Economics* **68**, 2327-2335
- Haddioui A, Baaziz M (2001) Genetic diversity of natural populations of *Atriplex halimus* L. in Morocco: An isoenzyme-based overview. *Euphytica* **121**, 99-106
- He ZL, Yang XE, Stoffella P (2005) Trace elements in agroecosystems and impacts on the environment. *Journal of Elements in Medicine and Biology* **19**, 125-140
- Kachout SS, Ben Mansoura A, Jaffel K, Leclerc JC, Rejeb MN, Ouerghi Z (2009) The effect of salinity on the growth of the halophyte *Atriplex hortensis* (Chenopodiaceae). *Applied Ecology and Environmental Research* **7**, 319-332
- Kachout SS, Ben Mansoura A, Leclerc JC, Mechergui R, Rejeb MN, Ouerghi Z (2010) Effects of heavy metals on antioxidant activities of *Atriplex hortensis* and *A. rosea*. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **9**, 444-457
- Kessler JJ (1990) *Atriplex* forage as a dry season supplementation feed for sheep in the Montane Plains of the Yemen Arab Republic. *Journal of Arid Environments* **19**, 225-234
- Lefèvre I, Marchal G, Meerts P, Corréal E, Lutts S (2009) Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. *Environmental and Experimental Botany* **65**, 142-152
- Le Houérou HN (1992) The role of saltbushes (*Atriplex* spp.) in arid lands rehabilitation in the Mediterranean basin: A review. *Agroforestry Systems* **18**, 107-148
- Lutts S, Lefèvre I, Delpérée C, Kivits S, Dechamps C, Robledo A, Correal E (2004) Heavy metal accumulation by the halophyte species Mediterranean saltbush. *Journal of Environmental Quality* **33**, 1271-1279
- Manousaki E, Kalogerakis N (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**, 844-854
- McArthur ED, Sanderson C (1984) Distribution, systematics and evolution of Chenopodiaceae: an overview. In: Tiedemann AR, McArthur ED, Stutz HC, Stevens R, Johnson KL (Eds) *Proceedings of the Symposium on the Biology of Atriplex and Related Chenopods*, US Department of Agriculture, Forest Service, USA, pp 14-24
- Macnair MR (1981) The tolerance of higher plants to toxic materials In: Bishop JA, Cook LM (Eds) *Genetic Consequences of Man Made Change*, Academic Press, London, UK, pp 177-208
- MacNair MR (1987) Heavy metal tolerance in plants: A model evolutionary system. *Trends in Ecology and Evolution* **2**, 354-359
- Maksymiec W, Krupa Z (2002) Jasmonic acid and heavy metals in *Arabidopsis* plants – A similar physiological response to both stressors? *Journal of Plant Physiology* **159**, 509-515
- Mandák B (2003) Germination requirements of invasive and non-invasive *Atriplex* species: A comparative study. *Flora* **198**, 45-54
- Martinez JP, Lutts S, Schanck A, Bajji M, Kinet JM (2004) Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atriplex halimus* L.? *Journal of Plant Physiology* **161**, 1041-1051
- Mendez MO, Glenn EP, Maier RM (2007) Phytostabilization potential of quailbush for mine tailings: Growth, metal accumulation, and microbial community changes. *Journal of Environmental Quality* **36**, 245-253
- Mendez MO, Maier RM (2008) Phytoremediation of mine tailings in temperate and arid environments. *Reviews in Environmental Science and Biotechnology* **7**, 47-59
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497
- Nagendran R, Selvam A, Kurian J (2006) Phytoremediation and rehabilitation of municipal solid waste landfills and dumpsites: A brief review. *Waste Management* **26**, 1357-1369
- Nedjimi B, Daoud Y (2009) Cadmium accumulation in *Atriplex halimus* subsp. *schweinfurthii* and its influence on growth, proline, root hydraulic conductivity and nutrient uptake. *Flora* **204**, 316-324
- Ortiz-Dorda J, Martínez-Mora C, Correal E, Simón B, Cenis JL (2005) Genetic structure of *Atriplex halimus* populations in the Mediterranean Basin. *Annals of Botany* **95**, 827-834
- Osman AE, Bahhady F, Hassan N, Ghassali F, AL Ibrahim T (2006) Livestock production and economic implications from augmenting degraded rangeland with *Atriplex halimus* and *Salsola vermiculata* in northwest Syria. *Journal of Arid Environments* **65**, 474-490
- Osmond CB, Björkman O, Anderson JF (1980) *Physiological Processes in Plant Ecology: Towards a Synthesis with Atriplex*, Springer-Verlag, Berlin, 468 pp
- Pourrat Y, Dutuit P (1994) Etude précoce des effets morphologiques et physiologiques du rapport sodium/calcium *in vitro* sur une population d'*Atriplex halimus*. In: AUPLEF- UREF. (Eds) *Quel Avenir pour l'Amélioration des Plantes?* John Libbey Eurotext, Paris, pp 283-295
- Remon E, Bouchardon JL, Faure O (2007) Multi-tolerance to heavy metals in *Plantago arenaria* Waldst. & Kit.: Adaptative versus constitutive characters. *Chemosphere* **69**, 41-47
- Rosselli W, Keller C, Boschi K (2003) Phytoextraction capacity of trees growing on a metal contaminated soil. *Plant and Soil* **256**, 265-272
- Salo LF, Artiola JF, Goodrich-Mahoney JW (1996) Plant species for revegetation of a saline flue gas desulfurization sludge pond. *Journal of Environmental Quality* **25**, 802-808
- Vangronsveld J, Clijsters H (1994) Toxic effects of metals. In: Farago ME (Eds) *Plants and the Chemical Elements*, VCH Verlagsgesellschaft, Weinheim VCH Publishers, New York, Northwood, pp 149-177
- Walker DJ, Moñino I, González E, Frayssinet N, Correal E (2005) Determination of ploidy and nuclear DNA content in populations of *Atriplex halimus* L. (Chenopodiaceae). *Botanical Journal of the Linnean Society* **147**, 441-448

- Wallnofer PR, Engelhardt G** (1984) Die Schadstoffe aus dem Boden aufgenommen werden. In: Hock B, Elstner EF (Eds) *Pflanzentoxicologie*, Bibliographisches Institut, Mannheim. Germany, pp 95-117
- Weber DJ, Gul B, Khan MA, Williams T, Wayman P, Warner S** (2001) Comparison of vegetable oil from seeds of native halophytic shrubs. In: McArthur ED, Fairbanks DJ (Eds) *Proceedings of a Symposium: Shrubland Ecosystem Genetics and Biodiversity*, RMRS-P-21, USDA Forest Service, Ogden, UT Rocky Mountain Research Station, USA, pp 287-290
- Wild H, Bradshaw AD** (1977) The evolutionary effects of metalliferous and other anomalous soils in South Central Africa. *Evolution* **31**, 282-293
- Wilkins DA** (1978) The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist* **80**, 623-633
- Wu L, Bradshaw AD, Thurman DA** (1975) The potential for evolution of heavy metal tolerance in plants. III. The rapid evolution of copper tolerance in *Agrostis stolonifera*. *Heredity* **34**, 165-178
- Zarcinas BA, Cartwright B, Spouncer LR** (1987) Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* **18**, 131-146