



Behavior of some plant species used as alternatives for salt-affected soil reclamation and treated wastewater valorization

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Abstract

Jatropha curcas, *Aloe vera* and some forest plants were planted in an experimental plot in the Kalaât Landelous region (Tunisia) to evaluate the change of morphological properties, yield and adaptation to the new environmental conditions. The salinity of the soil exceeded 16 dS m⁻¹ due to a shallow and saline water table. *J. curcas* and *A. vera* could not be recommended as alternative plants, at least under the local conditions in Kalaât Landelous. The short-term treated wastewater (TWW) irrigation generated no harmful effect on forest plants. The most promising species were *Atriplex nummularia*, *Casuarina glauca*, *Acacia cyanophylla* and *Eucalyptus gomphocephala*, which showed some resistance to the difficult conditions in the plot.

Keywords: Alternative plants, Heavy metals, Hydromorphic soil, Salinity, Treated wastewater

Introduction

Tunisia has a semi-arid climate, few renewable natural resources and a freshwater availability per capita below 450 m³. The water quality, especially salinity, is a serious constraint in the country and only 50% of the water resources have salinity levels lower than 1.5 g/l. Tunisia is one of the most drought-stressed countries in the Middle East and North Africa regions. Since the severe drought in 1989, the use of the treated wastewater (TWW) in irrigation has become a part of the government overall water resource management strategy. Water quality criteria for TWW use in irrigation have been developed on the basis of the World Health Organization (WHO) guidelines and a list of crops that can be irrigated has been specified. The effluents can be used after the secondary treatment for irrigating all types of crops except vegetables, *i.e.*, of fruit trees, cereals, fodder and industrial crops. Despite the high available volume of TWW (260 Million m³) (ONAS, 2017), the percentage of

reuse for irrigation is still low. More than 80% is discharged into the sea. Because of the low fraction of TWW used for agronomic crops, some actions and alternatives should be undertaken to increase TWW reuse. Another option to reuse TWW is the introduction of new plants, especially because many of the wastewater treatment plants in Tunisia are located in waterlogged and saline floodplains. This is also the case for the wastewater treatment plant of Kalaât Landelous.

Despite their attractive environmental and socio-economic benefits with regard to water and nutrient supply and the reduction of effluent release in the environment, TWW can carry significant amounts of salts and potentially toxic metals usually found at trace concentrations. Both salts and heavy metals are considered as major problems for the farmers who are afraid of the long-term impacts of TWW on soils and crops.

Besides water scarcity, Tunisia suffers from an increase of salt-affected soils which cover now about 1.5 million hectares (around 10% of the total country's area) (Hachicha, 2007). Thus, one of the strategies to use a part of these salt-affected soils is the search for alternative plants with high economic values. This would also help to use more TWW in agriculture and reduce the pressure on the limited fresh water resources. In our study, *Jatropha curcas*, *Aloe vera*, and six forest species were tested as alternative plants. *J. curcas* belongs to the Euphorbiaceae family, and is an oil producing species. Different parts of *J. curcas* have been used for various purposes such as animal feeding, medicine and ecosystem restoration (Ogunwole *et al.*, 2008; Carels, 2009). This plant is a promising new crop in arid and semi-arid regions, thanks to its high tolerance to drought, heat and salinity (Silva *et al.*, 2010; Chary *et al.*, 2014). *A. vera*, a CAM desert plant, is used in medicine, food

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industry, as surfactant detergent, in cosmetics and also in health care. It is a xerophyte with a strong drought resistance and a certain degree of salt tolerance (Souguir *et al.*, 2015). The proposed forest plants are species with a varying degree of salt tolerance that can survive on marginal lands (Hussin *et al.*, 2013; Miah, 2013; Roy, 2016). Among them are *Atriplex nummularia*, *Acacia cyanophylla*, *Casuarina glauca*, *Eucalyptus gomphocephala*, and *Pinus halepensis*. These plants are also used for afforestation, erosion fighting, and as animal feeding.

The objectives of this study were to evaluate the change of morphological properties, yield and adaptation to the new environmental conditions of *J. curcas*, *A. vera*, and six forest species to irrigation with TWW on the hydromorphic and saline soils in the region of Kalaât Landelous.

Materials and Methods

Study site and experimental design: The experimental plot with a high salt content was chosen next to a treatment plant in the city of Kalaât Landelous, in the northern part of Tunisia (35 kms north of the capital Tunis), close to the Mediterranean Sea. The climate is Mediterranean semi-arid with an average rainfall of 450 mm/year and an average potential evaporation of 1400 mm/year. The soil is an alluvial formation of the Medjerda river characterized by a fine texture (silty clay to clay). The organic matter content is very low (about 0.70%) and the total carbonate content is high (43%). During the rainy season, the experimental plot (60 m x 40 m) becomes inaccessible because of the water stagnation and a shallow and saline water table. To reclaim this salt-affected soil, the experiment was carried out in a parcel raised by 1 m above the soil surface (block). The water table characteristics (depth, pH and salinity) of the plot were monitored with three piezometers at 2 meters. The average depth of the water table was 83 cm from the soil surface. During the rainy season, the level approached the surface up to 30 cm from the soil surface in December 2009. During the dry season, the water table moved down to a maximal depth of 142 cm in July 2014. The pH was neutral to basic and ranged from 6.38 to 7.51. The average electrical conductivity (EC) was 79 dS m⁻¹, it varied with depth from one season to another.

Jatropha curcas plants were cultivated on both the original soil surface and the elevated part, while *A. vera* was only cultivated on the elevated part. In total, 44 plants of *J. curcas* and 60 plants of *A. vera* were used. Plants

were irrigated during summer with the TWW from the aerated lagoons of the wastewater treatment plant of Kalaât Landelous. The pH of the TWW was basic (7.73) and the salinity 4.78 dS m⁻¹ with dominant Cl and Na ions. The TWW contained low concentrations of heavy metals below the Tunisian Standard for the reuse of TWW in agriculture (TN106.03).

On July 2012, *J. curcas* and *A. vera* were removed. The plot was replanted with 153 plants of six forest species provided by the Regional Commission for Agricultural Development of Ariana, namely: *Atriplex nummularia* (20 plants), *Acacia cyanophylla* (27 plants), *Casuarina glauca* (31 plants), *Cupressus sempervirens* (28 plants), *Eucalyptus gomphocephala* (31 plants), and *Pinus halepensis* (16 plants). They were randomly planted in 3 lines, each line with 17 plants.

Sampling and data recording: Soil samples were collected before and after the irrigation cycle at different points of the plot. Samples were collected from at least nine locations in each sampling period from depths of 0-30, 30-60, 60-90, and 90-120 cm. The electrical conductivity of the saturate paste extract (ECe) of each sample was measured according to the method outlined by the US Salinity Laboratory Staff (1954). For the genotoxic assay, 4 kg of surface soil (0-20 cm) were collected from different locations in the experimental plot. Dry soil samples were mixed with distilled water (1 kg: 1 l) and stirred for 24 hours at room temperature. Subsequently, the suspensions were well mixed and filtered. Finally, the aqueous extract obtained was stored at 4°C. To test the genotoxicity, *Vicia faba* seeds (local variety Chahbi) were used. Seeds were grown in distilled water for 3 days and then the micronucleus (MCN) test was applied according to Souguir *et al.* (2008). The mitotic index (MI) was calculated as a percentage of the ratio of the dividing cells and the number of the scored cells. The MCN frequency was expressed as the number of interphase cells with MCN per 1,000 scored cells. The treatment was carried out with three replicates which makes an average of 9,000 cells per treatment (control and soil aqueous extract).

The growth of each plant was measured in length and diameter. The mortality of each species was monitored, too. At the end of the experiment, the leaves, the shoots and the roots were collected and used to determine the chemical contents in the different plant parts. The samples were thoroughly washed in tap water, rinsed with distilled water, dried at 50°C to constant weight and,

finally, ground up to pass through a 2-mm sieve. After digestion with hot HNO_3 , the samples were analysed with flame emission spectrophotometry (Jenway, PFP7) and atomic absorption spectrometry (Perkin Elmer).

Statistical analysis: The results are presented as means \pm standard deviation (SD) obtained from at least 9 replicas. Means were compared by Tukey's test at the 0.05 confidence level using the SPSS program (IBM SPSS statistics, v20). Heavy metal concentrations were compared to the WHO limits and the ranges given by Adriano (2001) and Pendias (2000).

Results and Discussion

Soil characterization: The soil salinity of the experimental plot varied with soil depth, season, and irrigation. Generally, the soil had a high salinity level ($\text{EC}_e > 16 \text{ dS m}^{-1}$) (Fig 1A). After the TWW irrigation cycle, salinity was lower in the top soil layers, in comparison to the deepest layers, suggesting positive effects of TWW on soil salinity. This salinity was essentially caused by the shallow and highly saline water table. It appeared that the soil elevation 1 m above the surface reduces the soil salinity, but it remains very strongly saline and with genotoxic effects. In fact, as shown in Fig 1B, the roots exposed to the aqueous extract showed a depressive effect on the cells at different stages of division compared to the control, the MI did not exceed 6 cells. The frequency of MCN (Fig 1B) in the soil extract was significantly enhanced.

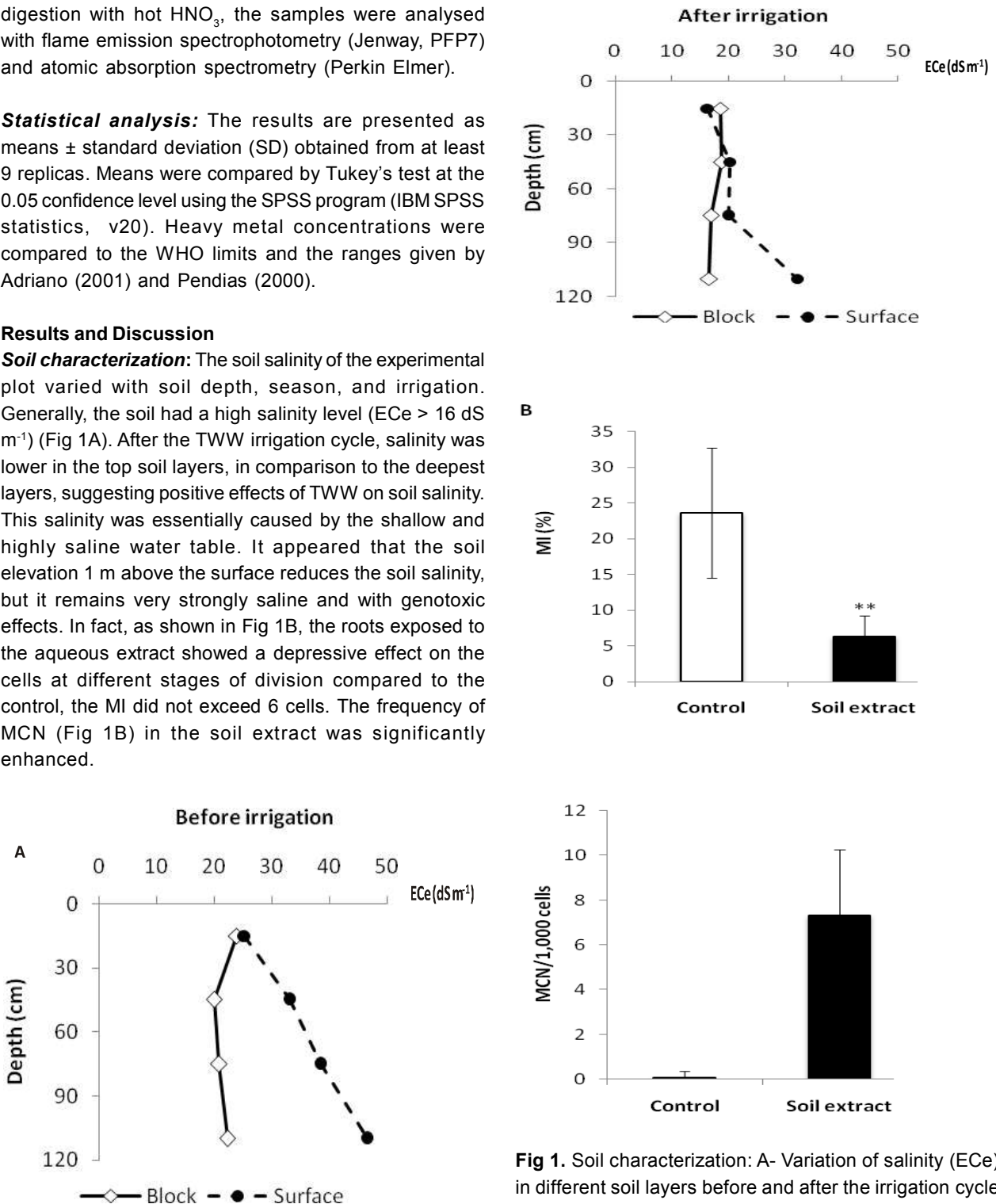


Fig 1. Soil characterization: A- Variation of salinity (EC_e) in different soil layers before and after the irrigation cycle (the values are the means of 12 measures analyzed at each depth). B- Genotoxicity parameters in *V. faba* roots exposed to aqueous soil extract. The control indicates *V. faba* roots irrigated with distilled water (Data are the means of three replicas. Bars indicate SD. ** $P < 0.01$).

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Table 1. Growth parameters of *J. curcas* cultivated in the Kalaât Landelous plot (November 2008–July 2011)

Plantation	Attributes	November 2008	July 2011
Block	Height (cm)	35.66 ±13.57a	135.00 ±21.70a*
	Diameter (cm)	0.97 ±0.31a	3.05 ±0.035a*
Surface	Height (cm)	36.00 ±11.26a	126.33 ±44.99a*
	Diameter (cm)	1.00 ±0.33a	3.77 ±1.11a*

Notes: Values are mean ±SD based on 22 measures; For each parameter, columns with same letters are not significantly different at $P < 0.05$, and rows with (*) are significantly different at $P < 0.05$ according to Tukey's test

Plant behavior: One of the strategies used for the rehabilitation of salt-affected soils is the introduction of species with an economic value adapted to these environmental constraints. Two of these promising plants are *J. curcas* and *A. vera* which were planted from November 2008 to May 2012 in the Kalaât Landelous plot. At the end of the experiment, *J. curcas* did not survive under the conditions of the plot. Most of the plants exhibited a slight increase in their growth parameters with only 135 cm of length and 4 cm of diameter after three years of cultivation (Table 1). The nutritional status was determined in the stems and roots (Fig 2). Both organs had high concentrations of Cl and Na ions in their tissues. No significant difference was found between the plants cultivated on the blocks and those taken on from the surface. Mineral elements were more concentrated in the stems. In addition, plants showed drying of their upper tips and defoliation. The defoliation seems a result of many factors such as saline stress, weather conditions (cold and sea winds), drought stress during the period without irrigation and waterlogging during the rainy season. The response of *J. curcas* to salinity and drought was already studied (Díaz-López *et al.*, 2012). In Kalaât Landelous, it seems that plants were sensitive to lower temperature and waterlogging during the cold and rainy season, as confirmed by earlier reports (Luo *et al.*, 2006; Wan *et al.*, 2006).

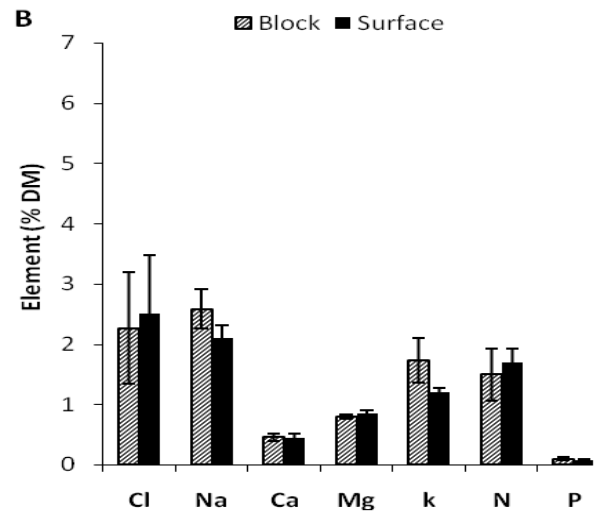
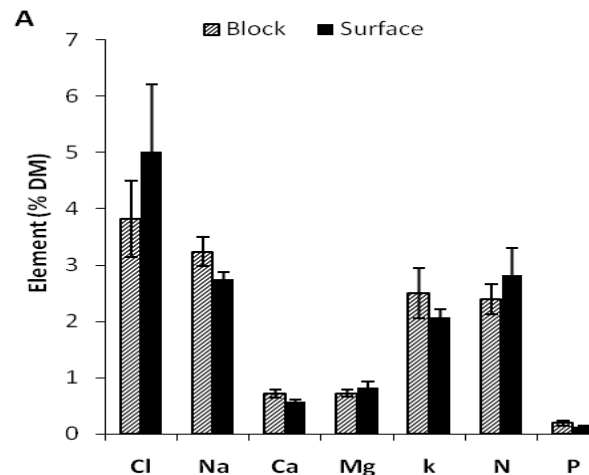


Fig 2. Element levels in the stems (A) and the roots (B) of *J. curcas* planted in Kalaât Landelous and irrigated with TWW. Plants were cultivated on the blocks and on the surface of the soil. Values are mean ±SD based on 12 measures. For each pair of bars, means with same letters are not significantly different at $P < 0.05$ according to Tukey's test. DM: dry matter

Aloevera is a medicinal plant which was also tested in Kalaât Landelous. It is a xerophyte with a strong drought resistance and a certain tolerance to salt stress (Souguir *et al.*, 2015). Sixty samples of *A. vera* were planted only on the blocks, 53 survived at a slow growth rate (Table 2). Despite the high economic value of the leaves, *A. vera* did not produce more than 12 leaves per plant with 24 cm of length.

Table 2. *Aloe* plants mortality and leaf number and growth (March 2010–May 2012)

Attributes	March 2010	May 2012
Mortality (%)	0.00	11.66
Leaf number	8.75 ±3.00a	10.31 ±3.94a
Leaf length (cm)	18.32 ±4.260a	23.59 ±6.93a

Notes: Values are mean ±SD based on 53 measures; For each row, means with same letters are not significantly different at $P < 0.05$ according to Tukey's test

Table 3. Mineral contents in roots and leaves of *A. vera* planted in Kalaât Landelous and irrigated with TWW

Attributes	Mineral contents											
	%				mg/kg							
	Na	K	Ca	Mg	Co	Cu	Fe	Mn	Ni	Pb	Cd	Zn
Leaves	4.24±	1.71±	1.48±	0.58±	0.04±	2.52±	142.30	5.36±	1.16±	0.29±	0.32±	13.30
	0.02	0.10	0.06	0.04	0.01	0.73	±3.81	1.67	0.60	0.13	0.03	±3.7
Roots	2.03±	1.15±	1.56±	0.70±	0.10±	2.25±	203.00	7.88±	2.39±	0.52±	0.26	9.20±
	0.12*	0.03	0.08	0.13	0.00*	0.51	±4.24*	0.79	0.40	0.16	±0.02	1.97
Ranges and permissible limits	-	-	-	-	0.02	3.0-	50-	20-	0.2-	0.1-	0.05-	20-
					-0.5 ^a	12 ^a	200 ^a	400 ^a	2 ^a	0.5 ^a	0.5 ^a	200 ^a
						50 ^b	28 ^b	200 ^b	1.5 ^b	10 ^b	0.3 ^b	50 ^b

Notes: Data represent the means ±SD. * $P < 0.05$ compared with leaves; ^a, Ranges set by Adriano *et al.* (2001) and Kabata-Pendias (2000) in the vegetative aboveground plant organs; ^b, Permissible limits in medicinal plants set by the World Health Organization (Shah *et al.*, 2013; Khan *et al.*, 2008)

Table 4. Mortality and growth in height and diameter of the forest plants grown in Kalaât Landelous and irrigated with TWW

Plants	Mortality (%)	Height (cm)			Diameter (cm)		
		July 2012	June 2014	Increase (%)	July 2012	June 2014	Increase (%)
<i>Pinus halepensis</i>	100±0.00	-	-	-	-	-	-
<i>Eucalyptus gomphocephala</i>	66.75±9.80	45.75±13.03	182.13±26.97	74.88	0.73±0.02	4.42±0.90	83.48
<i>Cupressus sempervirens</i>	51.93±22.53	61.67±9.30	68.71±19.53	10.25	0.67±0.05	0.92±0.27	27.17
<i>Casuarina glauca</i>	42.45±11.63	52.74±8.50	125.91±18.26	58.11	0.52±0.09	2.41±0.50	78.42
<i>Acacia cyanophylla</i>	35.15±25.47	71.25±9.10	186.19±31.04	61.73	0.67±0.02	5.43±0.86	87.66
<i>Atriplex nummularia</i>	8.09±7.33	56.00±7.20	134.35±8.46	58.32	0.64±0.03	4.53±0.26	85.87

Note: Data represent the means of the living plants ±SD

The concentrations of ions (Table 3) did not show significant changes among organs except for Na, which was significantly higher ($P < 0.05$) in leaves than in roots. Heavy metal concentrations did not exhibit significant differences between leaves and roots except for Co and Fe. These levels were lower than the values reported by Adriano *et al.* (2001) and Pendias (2000) who stated that there were no differences between the highest and the lowest concentrations of each metal in the vegetative above ground plant organs. Compared to levels recommended by WHO, our results showed that Co, Cu, Mn, Pb, and Zn concentrations were below the permissible limits in medicinal plants, while Fe (142 - 203 mg/kg), Ni (1.16 - 2.39 mg/kg) and Cd (0.26 - 0.32 mg/kg) concentrations were near or above the permissible limits. Because of its slow growth, low production and capacity to accumulate toxic contaminants, *A. vera* could not be considered as an alternative plant since it could not adapt to the conditions of Kalaât Landelous.

In July 2012, the plot was planted with some forest plants. After approximately 2 years, *A. nummularia* appears to be the most resistant plant to the climatic and soil conditions of the plot with the lowest mortality (8%)

(Table 4). The increases in height and diameter of plants were 58% and 85%, respectively (Table 4). *A. nummularia* is also considered an excellent livestock fodder thanks to its high protein content. Unfortunately, its density was more and its number reduced because of overgrazing and the lack of a sustainable management strategy. The xerohalophyte *Atriplex* is tolerant to water and salt stress (Le Houérou, 1980), and it accumulates large amounts of salt in its tissue, especially in the trichomes located on its leaf surface (Mozafar and Goodin, 1970). These properties make it suitable for the development programs for the rehabilitation of degraded lands.

Another forest plant cultivated in the plot of Kalaât Landelous was *A. cyanophylla*. This plant was introduced in Tunisia the first time in 1930 for rangeland rehabilitation and the protection of soil from erosion, particularly in arid zones. It is used for the production of wood and forage as well, for human nutrition and in pharmacy (Le Houérou, 1980). In this study, we found a low mortality (only 35%) and an increase in growth (Table 4). Several other studies also showed a high tolerance of the different species of *Acacia* to abiotic stress such as salinity (Bui *et al.*, 2014).

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Table 5. Heavy metal contents (mg/kg) in the leaves of plants grown in Kalaât Landelous

Plants	Co	Cu	Fe	Mn	Ni	Pb	Cd	Cr
<i>Eucalyptus gomphocephala</i>	0.06± 0.01	0.06± 0.02	1.16± 0.14	0.19± 0.01	0.00± 0.00	0.08± 0.02	0.02± 0.01	0.04± 0.03
<i>Cupressus sempervirens</i>	0.04± 0.01	0.03± 0.01	5.33± 0.72	0.52± 0.08	0.00± 0.00	0.10± 0.03	0.02± 0.00	0.12± 0.03
<i>Casuarina glauca</i>	0.05± 0.02	0.06± 0.01	3.57± 0.96	1.03± 0.10	0.01± 0.00	0.12± 0.04	0.01± 0.00	0.05± 0.03
<i>Acacia cyanophylla</i>	0.05± 0.02	0.06± 0.02	1.94± 0.51	0.44± 0.01	0.01± 0.00	0.13± 0.02	0.02± 0.00	0.03± 0.02
<i>Atriplex nummularia</i>	0.09± 0.01	0.05± 0.01	2.34± 0.27	0.15± 0.02	0.09± 0.02	0.14± 0.02	0.03± 0.00	0.01± 0.00
Ranges and maximum levels	0.02- 0.5 ^a -	3.0- 12 ^a 15 ^c	50- 200 ^a -	20- 400 ^a -	0.2- 2 ^a -	0.1- 0.5 ^a 0.3 ^d	0.05 -0.5 ^a 0.05 ^d	0.1- 0.5 ^a -
						30 ^b	1 ^b	

Notes: Data represent the means ±SD; ^a, Ranges set by Adriano *et al.* (2001) and Pendias (2000); Maximum levels fixed by the European Commission, 2002^b, 2003^c and 2008^d

Eucalyptus has been cultivated in Tunisia since the French occupation (Poupon, 1972). Different species are commonly used to manage salt-affected soils and shallow saline water tables. However, the sensitivity to salinity varies from one species to another (Feikema and Baker, 2011; Doronila and Forster, 2015). In our study, the species *E. gomphocephala* had shown an excellent growth (75% to 83%) despite a high mortality rate which reached 66% (Table 4). The salt tolerance of some *Eucalyptus* species was reported to be linked to the accumulation of quercitol, an organic osmolyte found in appreciable concentrations in tissues of *Quercus* species (Adams *et al.*, 2005). Studying the effect of long-term irrigation with wastewater on growth, biomass production and water use, Minhas *et al.* (2015) concluded that *Eucalyptus* plantations can act as potential sites for sewage disposal.

Cupressus Sempervirens was able to survive in the plot of Kalaât Landelous. However, plant growth was slow (10% to 27%) and mortality was high (52%) (Table 4). The responses of *Cupressus* to salinity, drought and long-term irrigation with TWW were previously studied (Farahat and Linderholm, 2015; Yigit *et al.*, 2016). *P. halepensis* was found to be the most sensitive plant because no plants survived in the plot (Table 4). Several researchers noted a slowdown of the growth under abiotic stress, especially salinity and drought (Klein *et al.*, 2014; Garah *et al.*, 2016). In the short term, irrigating plants by TWW with low heavy metals concentrations showed no harmful effects on forest species. In fact, the heavy metal (Co, Cu, Fe, Mn, Ni, Pb, Cd and Cr) contents in the plant leaves varied from one species to another, but remained

below the permissible limits in plants that can be used as a fodder (Table 5).

Conclusion

Our experiment was conducted in the region of Kalaât Landelous on a highly saline soil with a salinity >16 dS m⁻¹ and a shallow and saline water table. Plantings of salt-tolerant species are commonly used to manage this type of salt-affected soils as well as to reuse TWW released from the treatment plant of the region. Among the studied species, *J. curcas* and *A. vera* could not be suggested as alternative plants for cultivation. *Jatropha* and *Aloe* plants showed low growth and an ability to accumulate toxic elements in their above ground parts. More attention should be directed towards forest species such as the genus *Atriplex*, *Casuarina*, *Acacia* and *Eucalyptus*. Despite high mortality of some species, they were able to grow under constraints on the plot. They could increase the use of the TWW in irrigation and provide a solution for the rehabilitation of salt affected soils.

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