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Contribution of Bentonit to obtain a heavy metals (Cr III and Zn II) tolerant for saltbush plants (*Atriplex halimus* L.)

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Abstract

This present study puts in evidence the incidences of heavy metals (Zn (II) and Cr (III)) and their concentrations on the potentialities of growths of Saltbush plants *Atriplex halimus*. L and its capacities of tolerance and accumulation of these deferent metals cultivated on two substrate: sand and sand+ bentonit. One carried out one first place a characterization of a bentonit of layer by various physicochemical analyses (diffraction of x-rays, spectroscopy will infrared.), which showed that the bentonit sample used is made up primarily of montmorillonites. Then the study was carried out according to an experimental device in blocks, led in hydroponic culture under optimal conditions, we studied the effect of three concentrations (0, 250 and 500mg/l) of two metals chromium and zinc on some parameters of growth and development of this species cultivated on two substrates, one amended out of bentonit and the other on inert sand. The management of metals in plants is a complex phenomenon that depends mainly on the plant species and the metal. Because of the multitude of organic molecules involved in this process, and their roles that is not always clearly established. In our study different behavior both zinc and chromium metals were found, the implication of the physicochemical properties of substrate " Bentonite " strongly influenced on the growth of *Atriplex halimus* and fixation rate increase.

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Introduction

Zinc and chromium are among the most important heavy metals in industrial releases (Khan *et al.,* 1995; Bakhti *et al.,* 2001; Ayari *et al.,* 2005; Veli and Alyuz 2007). Heavy metals are dangerous for the environment because they are not degradable (Fellet *et al.,* 2007; Rehman *et al.*, 2008). In addition, they are enriched during mineral and biological processes, and eventually accumulate in nature (Raskin and *et al.,* 1997). They can also be absorbed directly through the food chain, leading to chronic or acute effects (Auboiroux *et al.,* 1996; Arfaoui *et al.*, 2008).

Current research has focused on the development of low-cost treatment processes such as bioremediation or phytoremediation, indeed it appears that there are tolerant plants that absorb and accumulate heavy metals in their tissues while fixing them under immobile forms, which has opened a new avenue for research into the potential for treating water and soil contaminated by these pollutants (Raskin *et al.,* 1997; Salt *et al.,* 1998; Meagher 2000; Elangoven *et al.,* 2008; Fellet *et al.,* 2007).

Phytoremediation includes a set of techniques that consist in using green plants to extract metals from soils and waters by accumulating them in their tissues (harvestable part), or making them less mobile and less toxic. This technique can be the subject of various strategies such as phytoextraction, phytodegradation, phytostabilization and rhizofiltration (Ben Ghnaya *et al.,* 2007; Hernandez *et al.*, 2007). Atriplex halimus L. (Chenopodiaceae) is a saltbushe specie in arid and semi-arid Mediterranean areas (drought-resistant) (Ben Hassine *et al.,* 2008; Martinez *et al.,* 2005; Alicata *et al.,* 2002).

This species, with its relatively large aboveground and root biomass, is an effective and relatively inexpensive tool in the fight against erosion and desertification and plays an important role in the rehabilitation of degraded lands (Abbad *et al.,* 2004). and improvement of the physical properties of marginal lands (Chisci *et al.,* 2001). It is a species renowned for its great hardiness, ecological range, polymorphism and dominant allogamous reproduction (Haddioui *et al.,* 2001).

It has attracted the attention of many researchers and has been the material of choice for several investigations.

In fact, recent studies have highlighted the promoter character of the species, which showed high absorption capacities of certain metal cations such as cadmium, zinc, selenium and lead (Lutts *et al.,* 2004; Vickerman *et al.*, 2002). It is able to accumulate significant amounts of these elements without presenting growth inhibition or mortality (Belarbi, 2008).

The work undertaken is divided into two parts, the first of which is devoted to an overview of the structure and general properties of bentonite, as well as a description of its purification to remove the impurities it contained in its natural state. . Then a characterization by the different physico-chemical analyzes (measurement of the cation exchange capacity, X-ray diffraction, infrared spectroscopy).

The second part deals with the study of the effects of chromium and zinc on the growth, development and biomass yield of Atriplex halimus as well as the evaluation of its absorption capacities in these two elements., wich was cultivated on two substrate: sand and sand+ bentonit.

In Algeria, the use of native plants as remedial plants is the subject of research, and it is in this context that the work that we present is interesting.

The aim of this study was to investigate the Atriplex halimus capacity to remediate polluted soil by Cr and ZN, in both forms and the effects of chromium and Zinc in its two valence states, Cr(III) and Zn (II) on the metal concentrations in plants and bioavailability of metals in different substrate soils. The experiment was carried out in plastic pots for evaluate the tolerance, metal accumulation and translocation to aerial biomass by Atriplex halimus L. in relation to the soil conditions (Sand and sand + bentonite).

Materials and methods

Area site

All the experiments in this work were carried out on the same lot of clay from the Roussel deposit (Maghnia) located in North West Algeria. (34°53'56.05"N latitude, 1°40'0.80"O longitude).

Characterization of the bentonite used

Materials used

The chemicals used are Merck reagents RP (NaCl: 99.5%, HCl: 37%, $BaCl_2$, $2H_2O$: 99%, $ZnSO_4$, $7H_2O$: 99%, Methylene blue $C_{16}H_{18}ClN_3S$, 2H2O: 82% and Triethanolamine: 98%).

Purification of the clay sample used

The bentonite used, which comes from the natural environment, thus contains associations with minerals such as carbonates and hydroxides, which form coatings covering the surfaces of clay particles (Caillère *et al.*, 1982). These impurities are removed by washing with relatively dilute acid (Bouras, 2003, Bakhti, 2005). Most of the clay in the fraction less than 2.mu.m. 500g of raw bentonit, crushed and sieved to 0.1mm, are first slurried in a six-liter tall vessel with distilled water with stirring for 24 hours to allow the layers of the clay to disperse. After stirring, the suspension is passed through the 50µm sieve and then acidified with N / 20 HCl to a pH of between 1.4 and 2. The acidified suspensions are stirred for 24 hours and then decanted. . The mixture is then washed with distilled water until the liquid overlying the pellet remains opalescent, indicating the onset of deflocculation. The suspensions are agitated followed by decantation, with replacement of the supernatant solution with distilled water. Finally, the bentonit, suspended in water, is then recovered over a height of 15cm.

The preliminary treatment of natural bentonit by sodium homoionization consists not only of removing all the crystalline phases (quartz, feldspar, calcite) (Decarreau, 1990), but also of replacing all the exchangeable cation of natures various by sodium cations. It also allows to have well-defined particle size fractions smaller than 2.mu.m. After washing with hydrochloric acid, the clay is homoionized with a 1N NaCl solution. Then, by means of a series of washing with distilled water, the clay is dispersed by stirring for 4 hours and then the mixture is allowed to stand for 24 hours. Samples are taken from the two-liter high test tubes at depths of 10cm. This operation is repeated twice in succession to allow access to a maximum cation exchange rate. The sodium clay thus prepared is washed with distilled water several times until the disappearance of the chloride ions (negative test with silver nitrate), homogenized by mechanical stirring and then dried at 40°C. The solid obtained is designated subsequently by sodium bentonit and be noted B-Na.

Measurement of cation exchange capacity

The cation exchange capacity of sodium bentonite was determined by the conductemitric method.

Conductivity titration method

0.1g of clay of sodium bentonite is suspended in 25ml of distilled water. Using a magnetic stirrer with standard titration using a solution of Zn SO 4 (0.0125M), after each addition of 0.5ml of the titrant solution, the conductance value after stabilization is noted. To have a better precision in the results and to take into account, as much as possible, the participation of each ion in the measurement of the conductivity. Conductance values corrected to compensate for dilution effects.

Blank test

In this case, the titration curve was established in the absence of the clay (Fig. 01) to determine the variation of the conductivity as a function of the volume of $ZnSO_4$ The equivalent point (the intersection of the two straight halves) is obtained graphically, by regression calculation and equalization of the equations of the two linear branches of the graph. The results, shown in Fig. 02, show that the value of c.e.c. sodium bentonite is: c.e.c. (B-Na)=100meq/100g of clay. The value of the c.e.c. of our sodium clayobtained is similar to that given by the literature for sodium bentonite from Maghnia (Bakhti, *et al.*, 2001).

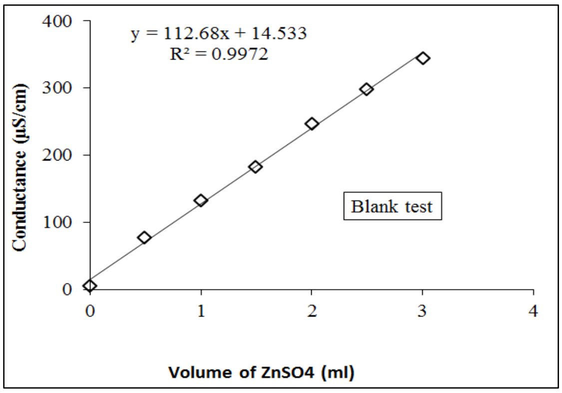


Fig. 1. Variation of the conductance according to the volume of ZnSO4.

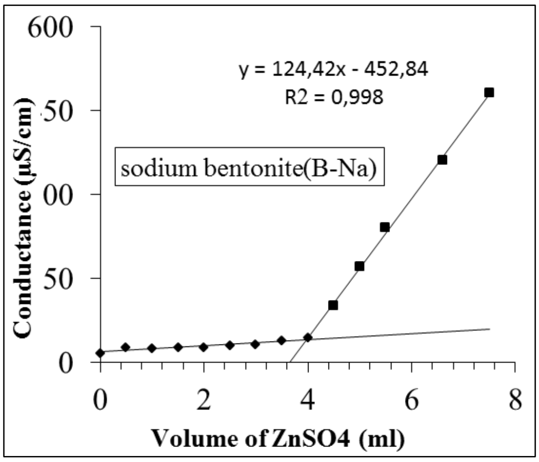


Fig 2. Variation of the conductance according to the volume of ZnSO4.

X-ray diffraction analysis of bentonit

Previous work on the study of the characterization of our natural bentonite Bakhti, A., 2005) showed that the mineral consists mainly of montmorillonite calcium, given the abundance of this element (Ca) in these soils. This clay is characterized by the lines around 15,18, 4,48, 3,25, 2,58 and 1,68 Å, and a small amount of kaolinite, characterized by lines 7,19, 3, 55, and 2.34 Å (Fig. 03). The diffractogram of the raw clay (Fig. 03) also shows the presence of impurities that consist mainly of quartz, calcite and dolomite. On the X-ray diffractogram of our purified bentonite (B-Na), saturated with calcium and dried at 105 ° C. (FIG. 04), it is noted that the purification made it possible to eliminate al most all the impurities present in our bentonite. We also note the characteristic diagram of montmorillonite with notably all the lines mentioned above (lines around 15.67, 4.46, 3.25, 2.55 and 1.69 Å). The X-ray diffractogram also reveals the presence of small amounts of kaolinite (lines around 7.27, 3.58, and 2.35 Å.

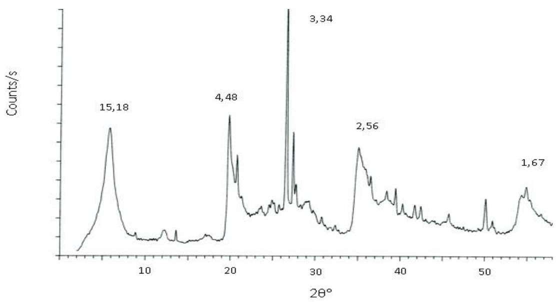


Fig. 3. Diffractogram of natural bentonite (Bakhti, A., 2005).

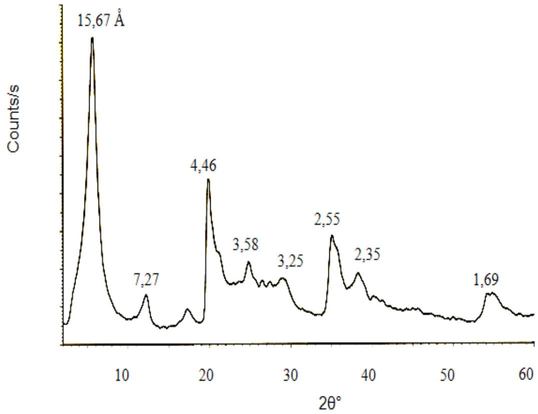


Fig. 4. Diffractogram of purified (B-Na) and saturated calcium bentonite.

Infrared spectroscopy analysis of bentonit

Examination of the infrared spectra of natural and sodium bentonite (Fig 05 and 06) leads to the following findings:

A band at 1638cm^{-1} is attributed to deformation vibrations of water molecules present in the interfoliar space (Bodoardo, *et al.*, 1994).

A shoulder band at approximately 3634 cm^{-1} attributable to the structural hydroxyl elongation vibrations of the octahedral layer of montmorillonite and kaolinite.

A broad band at 3465 cm^{-1} due to the vibrations of structural hydroxyls, but also to water molecules present in large quantities in natural clay. An intense absorption band located at 1033 cm^{-1}, which characterizes the phyllosilicates . (Cailläre, *et al.*, (1982). , corresponds to the valence vibrations of the Si-O bond in the purified clay (B-Na), it is displaced around 1042cm $^{-1}$.

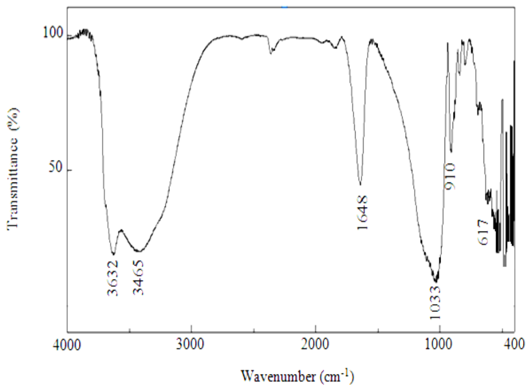


Fig 5. Infrared spectrum of natural bentonite.

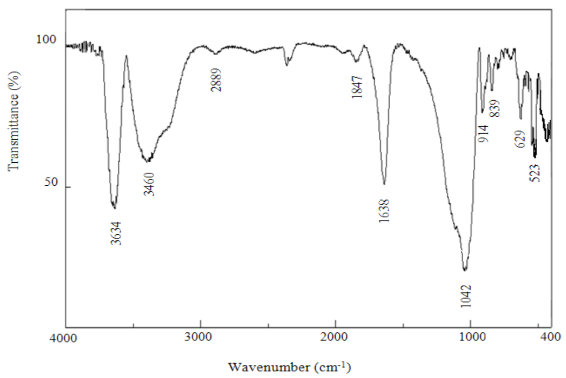


Fig 6. Infrared spectrum of sodium bentonite (B-Na).

The bands at 523, 468 and 405cm-1 are respectively attributed to the Si-O-M bond deformation vibrations, M can be Mg, Al or Fe. (Cailléré, *et al.*, (1982). X-ray diffraction analysis of natural bentonite revealed the presence of mainly calcium montmorillonite and impurities, which consist mainly of quartz, calcite and dolomite, which were eliminated by purification. Examination of the Infrared spectrum of natural and sodium bentonite reveals the characteristic bands of montmorillonite, the OH group of water and the valence of the Si-O bond.

The study undertaken by Bakhti, (2005), presents the adsorptive properties of Maghnia bentonite vis avis the two metal ions Cr (III) and Zn (II), the sodium clay fixes these cations in a relatively short time (50 minutes). Examination of the influence of pH gave a maximum fixation at pH 4 for Cr (III) and 7 for Zn (II), but the saturation threshold of this clay in these cations remains below the theoretical limit of which is 99meq/100g.

The retention capacity of these two cations increases with pH. The maximum absorption is obtained at pH 4 for Cr (III) and at pH 7 for Zn (II); the saturation threshold of sodium bentonite Cr (III) (13.2 mg / g equals 76 meq / 100g) and Zn (II) (23.6mg / g equals 72meq / 100g) is less than the theoretical exchange limit (99meq / 100g). This indicates that for these two cations ion exchange phenomena are mainly involved and the difference between the retained amount of metal on the B-Na solid and its theoretical exchange capacity observed could be attributed in part to the competitive effect of H_3O^+ ions present in the solution. (Bakhti, 2005).

Plant material, growth conditions and Application of chrom and Zinc

The experiment was conducted in a greenhouse conditions. which is located to the Northwest of Algeria (0°5'7.97"E longitudes, 35°53'32.29"N latitudes). Sand was used as a culture substrate, taken from the sea (Mostaganem beach is located to the Northwest of Algeria) which is demineralized and characterized by a retention capacity of 19% and an electrical conductivity of 1.15mS/m. The bentonite used was taken from the site called Roussel from the Maghnia deposit located in the wilaya of Tlemcen (Algeria). The bentonite is extracted from the deposit in the form of clumps of 5 to 15cm, the mottess are grinded with the mortar to reduce them in grains smaller than 0.2mm, the capacity of retention of the mixture sand + 10% bentonite is of 25% and an electrical conductivity from 1.75mS/m. The seeds used in this experiment were harvested from their mother plants in November 2014, at the khadra nursery (Mostaganem), located 500 m from the sea.

Sowing took place at the rate of 10 seeds per pot, at the stage of 02 leaves this number was reduced to 3. The irrigation solution is provided by a nutrient solution of KNOP "Heller" diluted (1/10). The sterilized grains were sown in perforated plastic pots with a volume of 900g, 18 pots filled with demineralized sand and 18 pots filled with mixture (sand + 10% bentonite). In the first two weeks irrigation is done only by distilled water to induce germination, by the third week, we proceeded to irrigation by the medium KNOP. Diluted (1/10) The irrigation frequency is three (03) times a week.

The irrigation solution is placed in plastic plates or pots are arranged, ensuring the feeding of plants by the upward flow (by capillarity) of the nutrient solution. Throughout the preparation period of the plant material, the nutrient solution is prepared at a pH of 5.-5.6, was renewed every 02 to 03 days to ensure the stability of the pH. To test the effect of the three (03) concentrations (0, 250 and 500mg / l) of the two metals in the form of (Zn (NO_3) $6H_2o$) and ($CrCl_3.6H_2O$) on both substrates: sand and (sand + 10% bentonite).

After approximately 25 weeks from the sowing date, the metal solutions were applied to the plants at predefined concentrations according to the device, and for a volume equal to the retention capacity. The metal solution of Zn (NO_3) 6 H_2O is always prepared between (5-5.6) against the solution ($CrCl_3$ 6H_2O) is prepared at pH = 4.5, and this to avoid the precipitation of chromium which theoretically starts at pH = 5.

Determination of heavy metals in plants and metal fractionation in soils

Saltbush plants Atriplex halimus *L.* were harvested four weeks after metals application and separated into three parts: roots, stems and leaves. The plants were thoroughly washed in tap water, rinsed three times with distilled water and oven-dried at 105°C to a constant weight. Dry weights were recorded and plant material was prepared for acid digestion, samples (1g aerial parts 'leaves and stems' and 0.3g for the roots) were then digested in 10cm3 concentrated nitric acid (HNO_3) in a warm (60°C) sand bentonit an bath for 2 h, filtered through a Whatman 540 filter paper, made up to volume and then analyzed. Metal concentrations in the soil and plant extracts were determined by atomic absorption spectrophotometry (AAS) at 540nm.

Studies of biometric and physiological parameters

the growth parameter in number of leaves was determined by counting the number of leaves before and after the treatment on each plant,for the stem length parameter, the difference in stem length noted before and after the treatment was determined. Root lengths were measured at the end of the experiment, at the end of the experiment, it is proceeded to the detachment by jet of distilled water to avoid any risk of contamination, the plants are carefully wiped with filter paper is split into their aerial and underground parts. For each plant and each part we have determined: fresh weight, the dry weight, the latter was determined by stoving at 70°C for 48 hours (Nadjmi, *et al.*, Daoud, 2009, Kadukova *et al.*, 2004), trace element contents of (Zn and Cr).

Statistics

All data presented in this study are the mean values of three replicates. Statistical analysis was performed using STATBOX v6.4 statistical software. The experiment was performed on a completely randomized design with three factors (metal , dose and substrat) and three treatments (Cr^{3+} and Zn^{2+} 02 concentrations, and the control). The chromium and zinc contents in roots, stems and leaves for Zn and Cr concentrations in soil results were subjected to analysis of variance (ANOVA); means of the several studied parameters were compared by the NEWMAN- KEULS test ($p < 0.05$).

Results and discussion

On the biometric parameters the toxicity of Cr is more striking for the length of the roots and the growth in numbers of the leaves for the plants cultivated on sand, on the other hand it is noted that there is a very readable increase for the three parameters studied at the plants grown on the mixture sand and bentonite. The number of leaves for plants of Atriplex grown on sand + bentonite and quite important (table 01,02), it is three times more than that presented on sand substrate . There are significant differences in the dose factor for plants grown on sand + bentonite substrate, however there is a reduction for plants grown on sand for Cr at 250 and 500mg /l. The average data presented in Table 03 show that the fresh weights obtained on the roots of the sand + bentonite treatment largely exceed those presented by the sand treatment table 04, it is also noted that the effect of the Cr metal on the fresh weight is marked above all in the plants grown on sand, plants grown on sand + bentonite produce higher root fresh weight.

Table 1. effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand substrate.

Treatment	Substrat (Sand)		
	Lengths of Root	Lenght of Stem	Number of Leaves
Control	$7.087^{C} \pm 0.051$	$8.167^{A} \pm 1.589$	$45.333^{B} \pm 2.309$
Zn II /250	$7.330^{C} \pm 0.130$	$5.033^{A} \pm 0.737$	$49.667^{B} \pm 3.055$
Zn II/500	$6.043^{E} \pm 0.361$	$1.767^{B} \pm 0.929$	$31.333^{B} \pm 3.215$
control	$6.597^{D} \pm 0.206$	$4.133^{A} \pm 1.380$	$38.667^{B} \pm 11.59$
Cr III /250	$5.567^{F} \pm 0.153$	$4.667^{A} \pm 3.499$	$15.667^{B} \pm 2.082$
Cr III/500	$5.063^{G} \pm 0.142$	$0.933^{B} \pm 0.611$	$7.333^{B} \pm 1.528$

Table 2. effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand + 10 % Bentonite.

Treatment	Substrat (Sand + 10% Bentonit)		
	Lengths of Root	Lenght of Stem	Number of Leaves
Control	$8.483^{B} \pm 0.068$	$5.167^{A} \pm 0.404$	$146.667^{A} \pm 76.173$
Zn II /250	$9.063^{A} \pm 0.523$	$5.933^{A} \pm 5.877$	$161.667^{A} \pm 60.252$
Zn II/500	$8.813^{AB} \pm 0.076$	$5.067^{A} \pm 3.323$	$149.000^{A} \pm 36.387$
control	$8.443^{B} \pm 0.051$	$5.732^{A} \pm 0.690$	$142.998^{A} \pm 5.235$
Cr III /250	$8.687^{AB} \pm 0.051$	$5.855^{A} \pm 1.015$	$143.420^{A} \pm 7.535$
Cr III/500	$8.673^{AB} \pm 0.14$	$6.187^{A} \pm 1.489$	$141.222^{A} \pm 19.280$

Table 3. effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand substrate.(fresh weight (g)).

Treatment	Substrat (Sand)		
	Fresh weight (g) of roots	Fresh weight of the stem	Fresh weight of Leaves (g).
Control	$0.706^{C} \pm 0.041$	$1.912^{D} \pm 0.052$	$4.304^{B} \pm 0.291$
Zn II /250	$0.751^{C} \pm 0.055$	$1.775^{DE} \pm 0.108$	$3.825^{B} \pm 0.530$
Zn II/500	$0.643^{D} \pm 0.013$	$1.356^{DEF} \pm 0.383$	$3.590^{B} \pm 0.345$
Control	$0.733^{C} \pm 0.83$	$1.513^{DEF} \pm 0.170$	$4.383^{B} \pm 0.548$
Cr III /250	$0.579^{C} \pm 0.052$	$1.035^{EF} \pm 0.108$	$3.391^{B} \pm 0.257$
Cr III/500	$0.430^{D} \pm 0.026$	$0.797^{F} \pm 0.080$	$2.307^{B} \pm 0.204$

Table 4. effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand + 10 % bentonite substrate. (fresh weight (g)).

Treatment	Substrat (Sand+ bentonite)		
	Fresh weight of roots (g)	Fresh weight of the Stem	Fresh weight of Leaves (g).
Control	$1.455^{B} \pm 0.047$	$2.738^{C} \pm 0.539$	$9.576^{A} \pm 0.798$
Zn II /250	$1.770^{A} \pm 0.080$	$3.072^{BC} \pm 0.299$	$10.793^{A} \pm 1.822$
Zn II/500	$1.368^{B} \pm 0.042$	$4.016^{A} \pm 0.340$	$12.598^{A} \pm 1.999$
Control	$1.210^{B} \pm 0.085$	$2.637^{C} \pm 0.279$	$8.937^{A} \pm 0.876$
Cr III /250	$1.610^{A} \pm 0.154$	$3.587^{AB} \pm 0.497$	$10.814^{A} \pm 0.977$
Cr III/500	$1.198^{B} \pm 0.051$	$2.643^{C} \pm 0.703$	$8.408^{A} \pm 1.822$

Average fresh weights of steams, obtained on sand + bentonite, are higher than those grown on sand only. On the other hand, it is noted that the fresh weight for plants cultivated on sand decreases for zinc and Cr at a concentration of 500mg /l, the lowest weight is obtained on the Cr treatment.

It is also noted that the fresh weight yield of the leaves increases with the increase of the metal doses used on plants grown on sand + bentonite.

The study of the fresh weight variations of the three parts of the plant (roots, stems, leaves) for the sand substrate amended in bentonite reacts with a higher yield by weight, unlike the others cultivated on sand, which seems to support zinc more than Cr.

According to table 05-06 The investigation of resistance to ZnII and CrIII metals from a dry weight yield point of view reveals that the roots, stems and leaves exhibit different behaviors in the two substrates. It is noted that there is a reduction for the plants grown on sand which is more prominent for the Cr, on the other hand there increase in dry weight for all the organs on the substrates amended in bentonite in the whole of the treatments compared to the witnesses.

These results show that the different parts of the A. halimus plant support more ZnII than CrIII, and the role of bentonite in the fixation of one part and the decrease of the toxic effect of the two metals on the other hand.

Only in the increase of NPK uptake rate by plants in the sand-bentonite mixture. Concerning the determination of the two metal zinc and chromium in the soil, the two metals are strongly influenced by the substrate, metal and dose.(tab 07-08).

Table 5. Effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand substrate.(Dry weight (g)).

Treatment	Substrat (Sand)		
	Dry weight of Roots (g)	Dry weight of the Stem	Dry weight of Leaves (g).
Control	$0.146^{C} \pm 0.009$	$0.332^{C} \pm 0.017$	$0.473^{C} \pm 0.021$
Zn II /250	$0.139^{D} \pm 0.004$	$0.313\ ^{C} \pm 0.006$	$0.433^{C} \pm 0.051$
Zn II/500	$0.107^{E} \pm 0.006$	$0.193^{C} \pm 0.050$	$0.374^{C} \pm 0.033$
Control	$0.157^{C} \pm 0.025$	$0.253^{C} \pm 0.045$	$0.501^{C} \pm 0.061$
Cr III /250	$0.106^{D} \pm 0.015$	$0.154^{C} \pm 0.021$	$0.375^{C} \pm 0.034$
Cr III/500	$0.070^{E} \pm 0.008$	$0.120^{C} \pm 0.016$	$0.188^{C} \pm 0.002$

Table 6. effect of concentration of chromium and zinc in different parts of *A. halimus* after 30 days of irrigation treatments cultuved on sand substrate +10 % bentonite. (Dry weight (g)).

Treatment	Substrat (Sand+ bentonite)		
	Dry weight of Roots(g)	Dry weight of the Stem	Dry weight of Leaves (g).
Control	$0.427\ ^{B} \pm 0.011$	$0.834\ ^{B} \pm 0.138$	$1.273^{B} \pm 0.11$
Zn II /250	$0.600^{A} \pm 0.025$	$1.117^{A} \pm 0.125$	$1.549^{A} \pm 0.253$
Zn II/500	$0.418^{B} \pm 0.011$	$1.155\ ^{A} \pm 0.127$	$1.670^{AB} \pm 0.317$
Control	$0.349^{B} \pm 0.033$	$0.735^{B} \pm 0.107$	$1.271^{B} \pm 0.127$
Cr III /250	$0.482^{A} \pm 0.024$	$0.978\ ^{A} \pm 0.185$	$1.527\ ^{A} \pm 0.084$
Cr III/500	$0.358^{B} \pm 0.024$	$0.718^{A} \pm 0.179$	$1.181^{AB} \pm 0.308$

Table 7. average ZnII and CrIII contents in diffrents part of Ariplex halimus plants (in µg / g DM) grown on sand substrate treated with increasing doses of zinc and chromium.

Treatment	Substrat (Sand)			Sand
	Average levels of Zn and Cr in the roots	Average levels of Zn and Cr in the stems	Average levels of Zn and Cr in the leaves .	
Control	$0.0001^{E} \pm 0.000$	$0.0001^{E} \pm 0.000$	0.0001 ± 0.000	$0.000^{E} \pm 0.000$
Zn II /250	$374.686^{B} \pm 19.99$	$146.065^{D} \pm 25.133$	159.373 ± 22.901	$0.519\ ^{E} \pm 0.057$
Zn II/500	$593.104^{A} \pm 11.635$	$376.388^{A} \pm 20.852$	343.831 ± 38.814	$0.501^{E} \pm 0.069$
Control	$0.0001^{E} \pm 0.000$	$0.0001^{E} \pm 0.000$	0.0001 ± 0.000	$0.000^{E} \pm 0.000$
Cr III /250	$24.074^{E} \pm 1.538$	$5.047^{E} \pm 1.510$	3.123 ± 0.883	$0.583^{E} \pm 0.119$
Cr III/500	$38.002^{E} \pm 1.814$	$11.312^{E} \pm 1.391$	8.303 ± 1.331	$0.651^{E} \pm 0.08$

Table 8. Average ZnII and CrIII contents in diffrents part of Ariplex halimus plants (in µg / g of Dry Matters) grown on sand and sand + bentonite substrates treated with increasing doses of zinc and chromium.

Treatment	Substrat (Sand + bentonote)		
	Average levels of Zn and Cr in the roots	Average levels of Zn and Cr in the stems	Average levels of Zn bentonit substart and Cr in the leaves
Control	$0.0002^{E} \pm 0.000$	$0.0001^{E} \pm 0.000$	0.0001 ± 0.000 $0.000^{E} \pm 0.000$
Zn II /250	$153.790^{D} \pm 22.284$	$170.309^{C} \pm 29.272$	172.784 ± 4.91 $29.176^{D} \pm 4.709$
Zn II/500	$302.702^{C} \pm 48.677$	$317.811^{B} \pm 9.284$	208.902 ± 5.683 $69.097^{B} \pm 5.347$
Control	$0.0001^{E} \pm 0.000$	$0.0001^{E} \pm 0.000$	0.0001 ± 0.000 0.000 ± 0.000
Cr III /250	$8.096^{E} \pm 1.573$	$6.477^{E} \pm 1.203$	3.359 ± 0.14 $50.035^{C} \pm 5.942$
Cr III/500	$14.944^{E} \pm 2.455$	$8.972^{E} \pm 0.482$	7.237 ± 0.969 $107.759^{A} \pm 8.859$

Work done by Ben Ghnaya *et al.*, (2007), shows that the dry weight of the aerial and root parts of rapeseed (*Brassica napus* L) plants decreases with the applied concentrations of ZnSO4, (0-100-250-500µM), which have a negative correlation. The photosynthesis activity is truncated from the concentrations of 500µM $ZnSO_4$. It is noted that the fixing for the sand is very low is limited for the two metals Zn and Cr even at high concentration, which is 0.501µg/g for 500mg/l of zinc, is 0.651µg/g for 500mg/l of chromium. For the mixture sand + 10% bentonite fixation is very important and varies depending on the concentration and the metal, for Zinc at a dose of 250mg/l, the mixture sets a quantity of 29.176 (µg/g), which increases proportionally with the dose, which reaches 69.09 (µg / g) for a concentration of 500mg/l. The contents recorded in the sand + bentonite substrate for chromium are higher, (50.035µg/g) for 250mg/l and 107.75 (µg/g) for 500mg/l. (Fig. 16).

For the determination of zinc and chromium in tissues of Atriplex halimus. Chromium is mainly sequestered in the roots and the low translocation of chromium from the underground to the aerial parts is a problem for phytoremediation plant choices (Pulford *et al.*, 2001, Shanker *et al*, 2005, Kabata-Pendias, A. 2004). For our study, the two metals were used to determine the amounts of the ions (Zn^{+2} and Cr^{+3}) in different parts of Atriplex halimus, by comparing the values obtained with the critical concentrations above which the effects of toxicity are possible (150-300mg.kg^{-1}) for zinc and between 20-100mg.kg^{-1} of chromium (III) (Benghnaya *et al.*, 2007, Talidi 2006 and Shanker *et al*, 2005,).

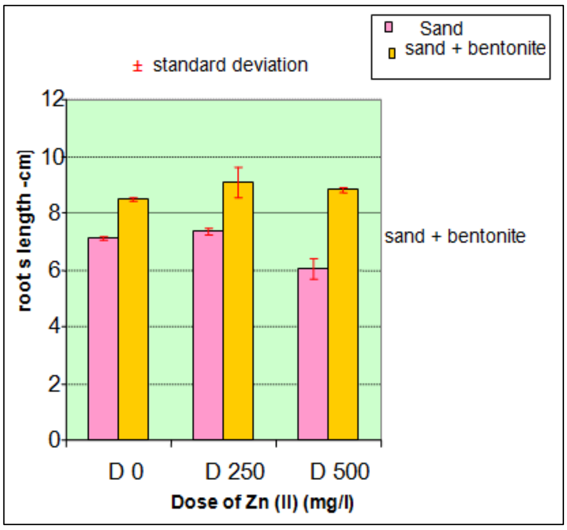


Fig. 7. Root lengths

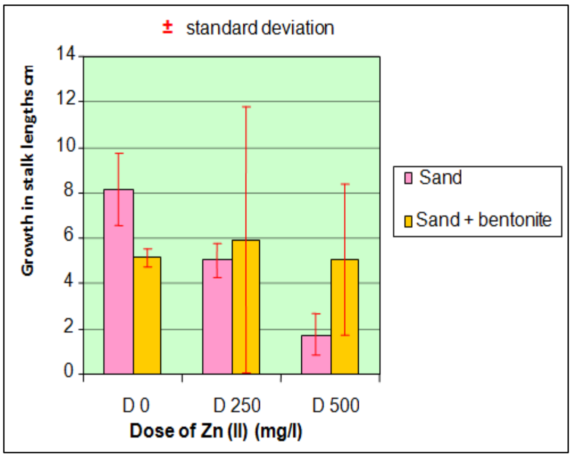
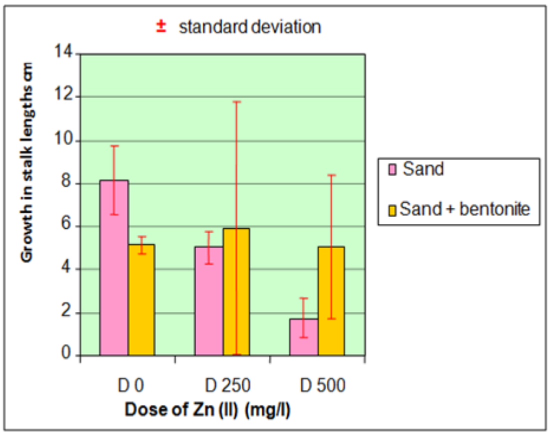


Fig. 8. Growth in stalk lengths.

We find that the obtained zinc content is above this range in the sand substrate, which is clearly noted for the roots (374.68-593.10mg.kg^{-1} dry matter) of (146.06 -376.38mg. kg^{-1} DM) in the stems and (159.37-343.83 mg.kg-1 DM) in the leaves. On the other hand, for the A. halimus plants cultivated on the bentonite-amended sand, the recorded contents are less for the roots (153.79-302.7 of DM) but encouraging for the aerial part, (170.30-327.8mg.kg^{-1} DM) in the stems and (172.78-208.1mg.kg^{-1} DM) in the leaves. (Fig. 18-19).



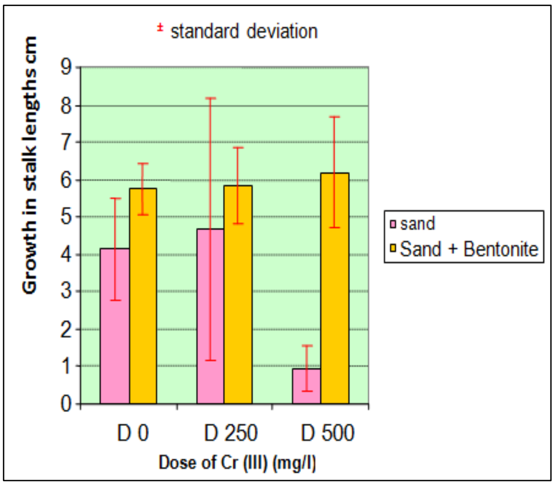
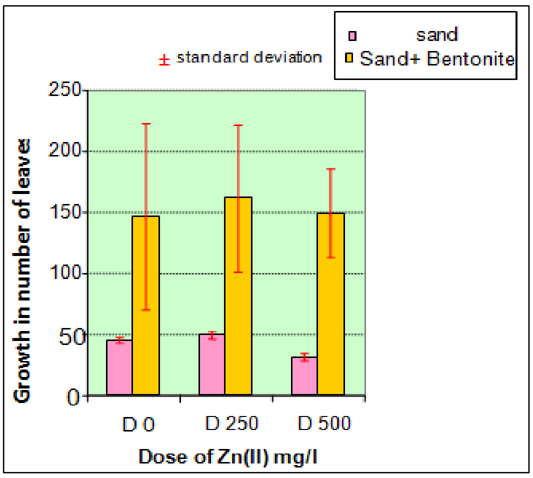


Fig. 9. Growth in number of leaves.



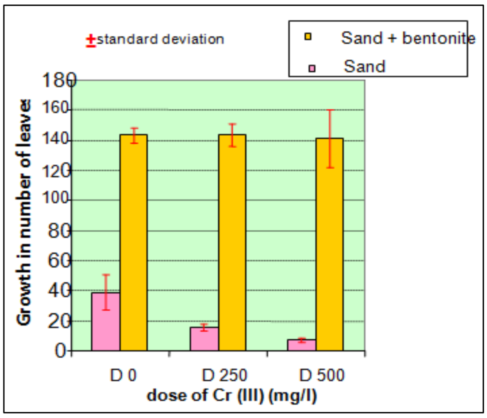
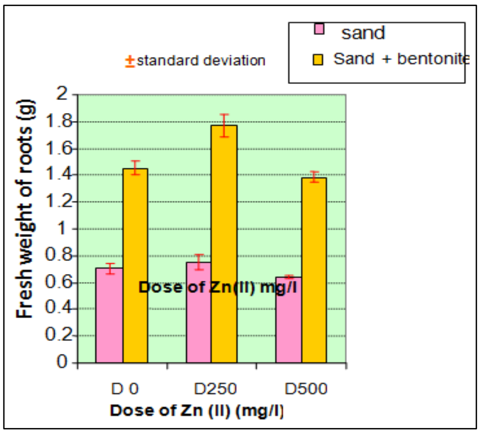


Fig. 10. Fresh weight of roots.



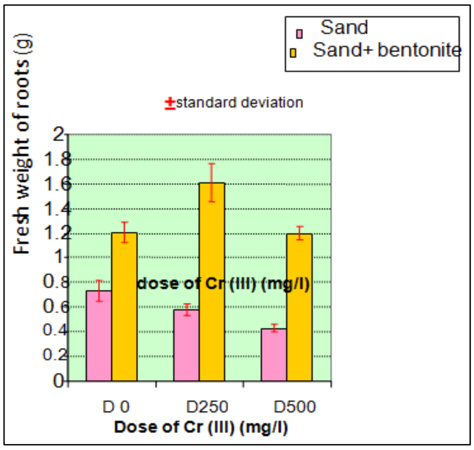
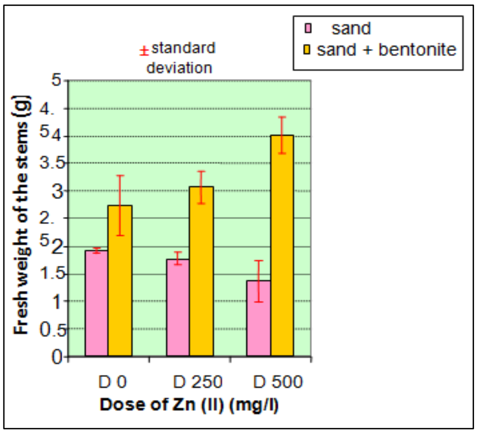


Fig. 11. Fresh weight of the stems.



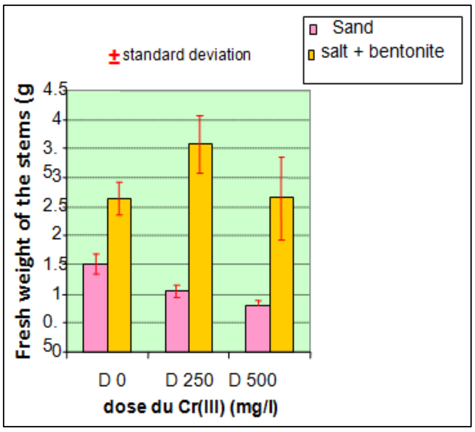
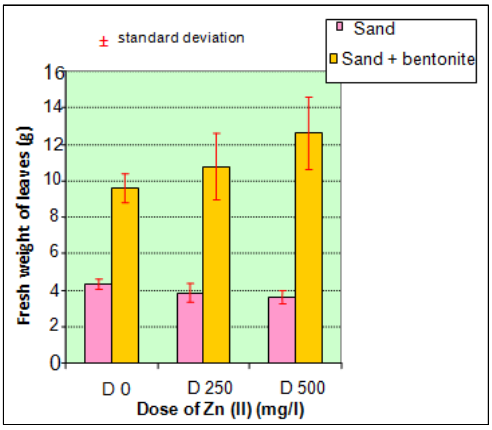


Fig. 12. Fresh weight of leaves.



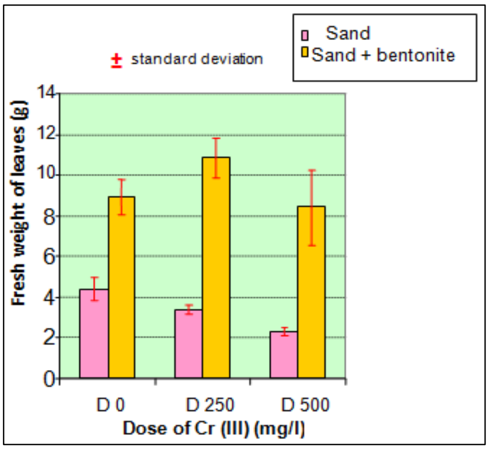
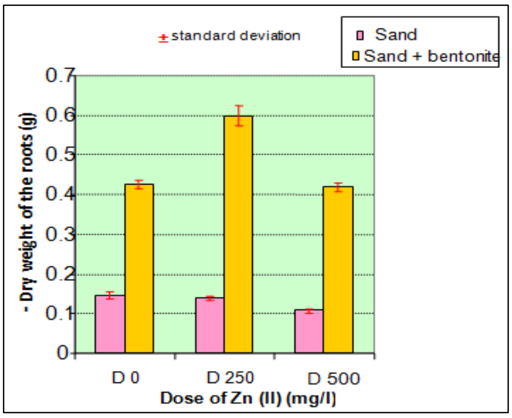


Fig. 13. Dry weight of the roots.



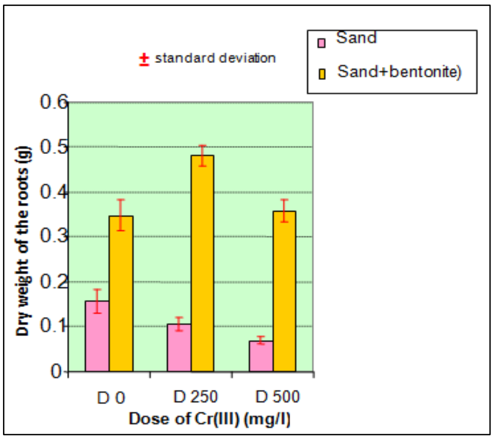


Fig. 14. Dry weight of the stems.

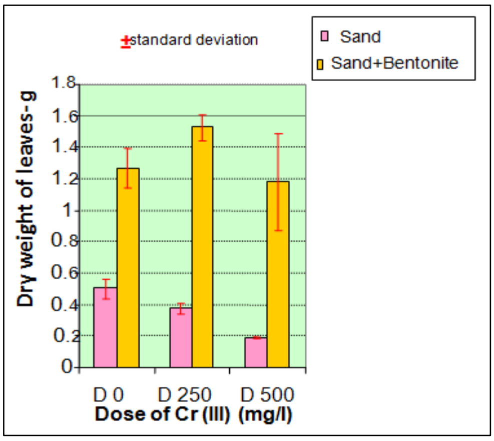
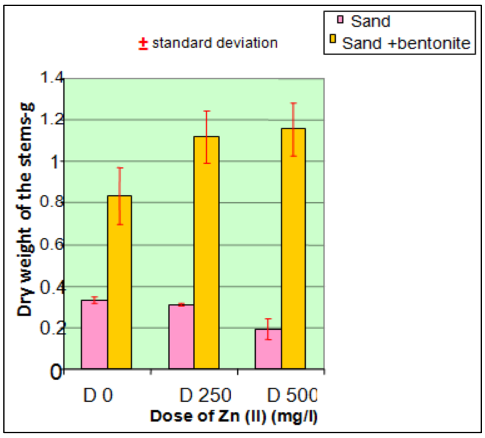
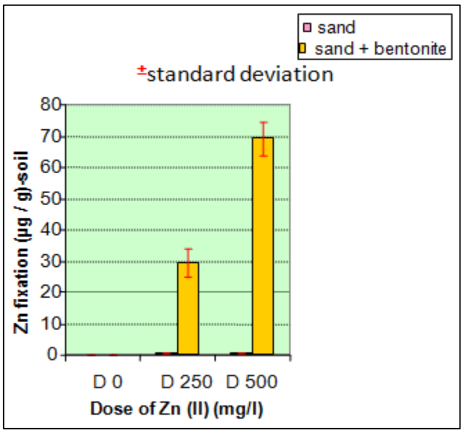
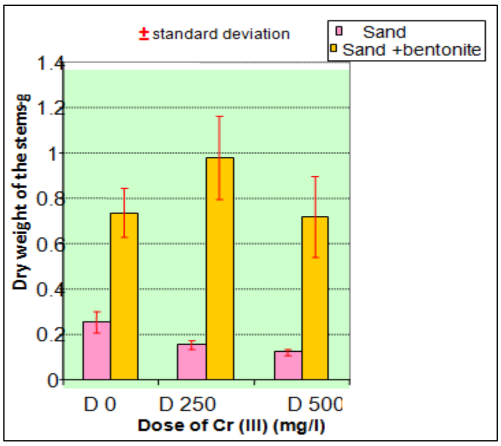


Fig. 16. Dosage of the two metals zinc in the soil.







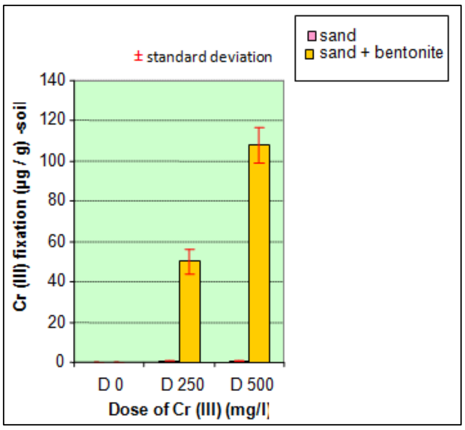
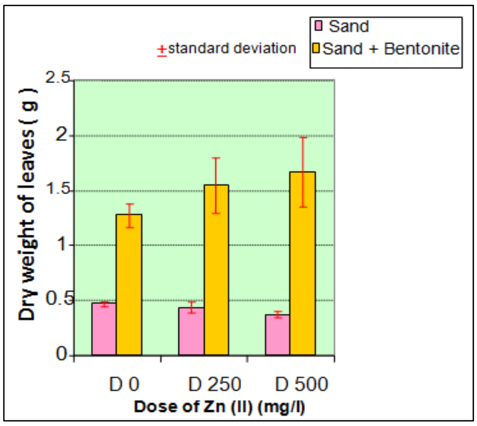
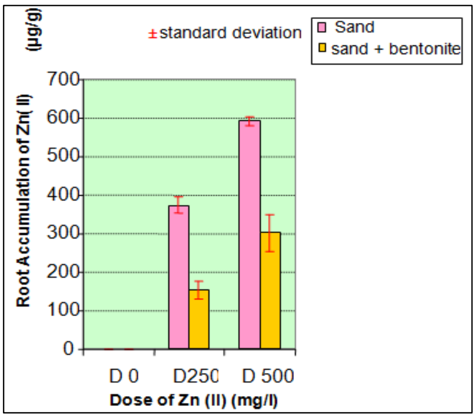


Fig. 15. Dry weight of leaves.

Fig. 17. Dosage in the roots.





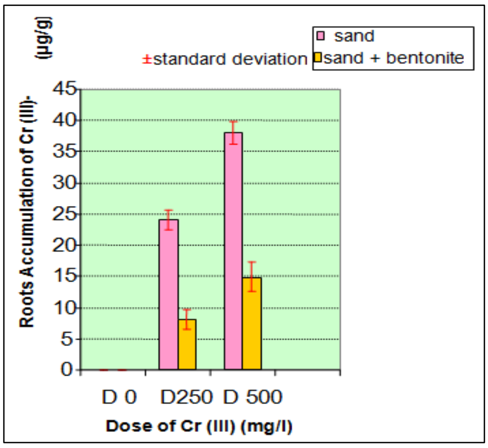
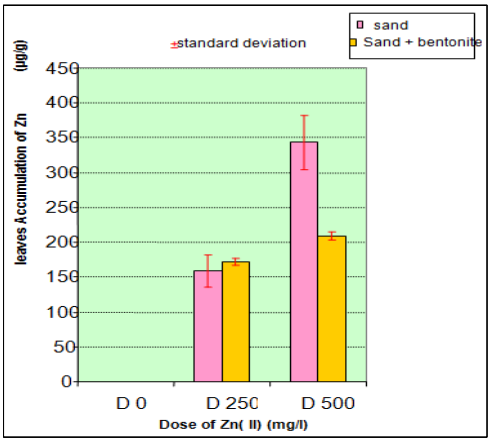


Fig. 18. Dosage in the stems.



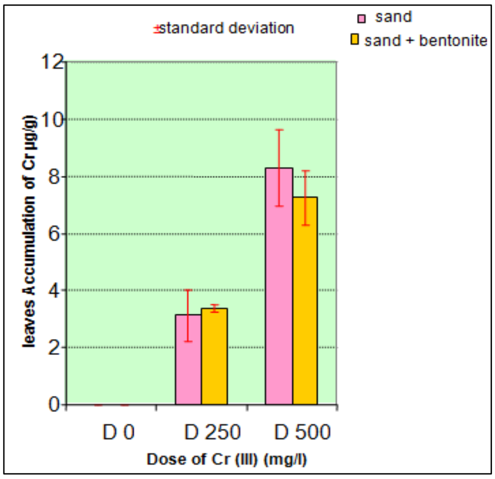


Fig. 19. Dosage of leaves.

Chromium was detected in different parts of the plant but with lower levels than zinc. The highest amounts of chromium are recorded for roots of A.halimus grown on sand that vary between (24.07-38mg.kg^{-1} DM). But the translocation to the aerial part is very weak, we note a content (5.04-11.31mg.kg^{-1}) of dry matter for the stems and of (3.12-8.34) mg.kg^{-1} of dry matter in the leaves, these contents are thus low for plants grown on bentonite-amended sand with levels similar to that of the stems and leaves of plants grown on sand.

The results of the spectrophotometric analysis of the zinc and chromium ions in the two substrates show that the level fixed by the sand is very low for the two metals (ZnII and CrIII), the contents vary from (0.501-0.651µg.g^{-1} dry matter). This is due to a very low cation exchange capacity of sand (Wahla *et al.*, 2008). On the other hand, the mixture of bentonite and sand has remarkable fixation contents for the two metals, the contents for zinc vary from (29.17-69.09 µg.g^{-1} in DM) and the contents of chromium vary from (50.03-107.75µ. gg^{-1} in DM) based on concentrations of 250 and 500mg / l. On the other hand, the examination of these results shows that the two cations Cr^{+3} and Zn^{+2} are not adsorbed equivalently, the adsorption of chromium appears to be greater than that of zinc. According to (Mbarki *et al.*, 2008) the clay-sand mixture promotes a better absorption of the NPK mineral element for the plant and increases the water retention capacity and increases the ion exchanges.

The results obtained from the study of the different correlations in plant biomass, evaluated in fresh weight and dry weight between the aerial and underground part vis avis the two metals, present strong link this suppose that the aerial and root vegetation respond in the same way, from the point of view of growth in the presence of metals, and respond by the same metabolic processes, we find the heavy metals (Cr and Zn).

On the other hand, the correlation between the dry weights and the metal contents (Zn and Cr) is negative for the aerial and root parts, this result confirms the toxic effect of the two metals on the metabolic process of the plant, moreover it seems that the aerial part is more affected than the root part, according to (Shanker *et al* 2005, Davies *et al.*, 2002, Moral *et al.*, 1995), chromium affects the photosynthesis activity and the enzymatic activity at the leaf level and causes a decrease in nutrient uptake by the roots including N, P, K, Fe, Mg, Mn, and according to Lopez *et al.*, (2008), negative correlations between dry weights and soil levels.

Cr (III) were recorded in the roots and aerial parts of wheat. Cervantes *et al.*, (2001), show that Cr (III) at 100µM causes growth inhibitions for 40% of young barley plants.

Although pH is indirectly the main factor that significantly controls the chemical behavior of metals (Merrington *et al.*, 2003), increasing their mobility in acidic conditions (Alloway 1995). Mobility of heavy metals in soil depends greatly on soil pH. Fuller (1977) considered that in acidic soils (pH 4.2–6.6) Cr is moderately mobile, and in neutral to alkaline soils (pH 6.7–7.8), Cr is highly mobile. Furthermore, soil pH constitute important factors affecting metal bioavailability in the soil since the transfer of metals between the readily available and less-available phases of the soil is significantly influenced by the competition for surface exchange sites by other cations (especially H^+) and by the presence of binding surfaces such as the organic matter (Naidu *et al.,* 2003; Fitzgerald *et al.,* 2003; Rieuwerts *et al.*, 2006).

Conclusion

The results obtained from the study concerning the cultivation of Atriplex halimus in a hydroponic medium for five months on two substrates, one amended in bentonite and the other on inert sand, show that the growth of Atriplex halimus in the amended medium in bentonite significantly higher than that of plants grown on sand, this difference is due to the role of bentonite in the formation of a good colloid with sand, is in the increase of the retention capacity of water and mineral elements.

The toxic action of heavy metals (ZnII and CrIII) appears to be attenuated on medium amended in bentonite than on pure sand. This is certainly related to the dynamics of metal ions and the exchange capacities of colloidal bentonite. The growth of the plants measured on the various parts of the plant (root-stems-leaves) correlation analysis, shows that the different organs respond in a coordinated manner to different stresses of metals.

Zinc seems to be more supported by Atriplex halimus plants than chromium, and the effect generated varies with the concentrations provided and the nature of the substrate. Vegetable biomass, evaluated in fresh weight and dry weight, obtained on medium amended to bentonite is much larger than that obtained on sand. While remarkable reductions in this biomass have been recorded, especially in plants grown on sand in the presence of chromium. Cr appears to be more toxic than Zn. The involvement of the physicochemical properties of bentonite in the substrate has been important for the trapping of the two chromium and zinc metals and, depending on the metal concentration, the adsorption of chromium appears to be greater than that of zinc. The amendment in bentonite showed an increase in the biomass rate of the plant, especially the aerial part where the accumulation capacities of the metals increased automatically, especially for zinc.

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