Journal of
Bioremediation & Biodegradation

Amari et al., J Bioremediat Biodegrad 2016, 7:2
DOI: 10.4172/2155-6199.1000336

Research Article Open Access

Evaluation of the Ni^{2+} Phytoextraction Potential in *Mesembryanthemum crystallinum* (Halophyte) and *Brassica juncea*

Amari T[1]*, Ghnaya T[1], Sghaier S[1], Porrini M[2], Lucchini G[2], Attilio Sacchi G[2] and Abdelly C[2]

[1]*Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisia*
[2]*Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, 20133 Milan, Italy*

Abstract

Among heavy metal stressors, nickel (Ni) pollution is one threatening risk to the environment. In this view, the growing concerns about environmental pollution have stimulated the efforts to promote the individuation of phytoextractor plants that are able to tolerate and accumulate toxic metals, including Ni, in the aerial parts. More recently, it has been suggested that halophytes, i.e. native salt-tolerant species, could be more suitable for metal extraction, from saline soils than glycophytes, most frequently used so far. In the framework of this approach, we evaluated here the Ni-phytoextraction ability of the halophyte *Mesembryanthemum crystallinum* comparatively to the model species *Brassica juncea*. Plants were maintained for 3 months on a soil containing 0, 25, 50, and 100 ppm $NiCl_2$. Nickel impaired the growth activity of both species. Interestingly, *M. crystallinum* was less impacted by $NiCl_2$ addition. The plant mineral nutrition was differently affected by $NiCl_2$ exposure depending on the ion, the species and even the organ. In both species, roots were the preferential sites of Ni^{2+} accumulation, but the fraction translocated to shoots was higher in *M. crystallinum* than in *B. juncea*. The relatively good tolerance of *M. crystallinum* to Ni suggests that this halophyte is more efficient to extract Ni^{2+} than *B. juncea*.

Keywords: Halophyte; Nickel; Phytoextraction; *M. crystallinum*; Tolerance

Introduction

Environmental pollution by heavy metals represents a major threat to human, animal and plant health [1,2]. Nowadays, land contamination with heavy metals has become a serious problem in the world. In Tunisia, saline depressions with low population levels, often represent a sink of industrials and urban waste and many of them are contaminated by Cd^{2+}, $Pb^{2+}$and Ni^{2+} [3]. Heavy metals are released into environment by natural and anthropogenic sources. The most significant anthropogenic sources are Human activities, particularly industry, urbanism and agricultural practices [4]. Among heavy metals, Nickel (Ni) is recognized as a dangerous environmental pollutant [5]. It has adverse effects on human health such as Allergic dermatitis, cancer of the lungs, nose and sinuses [6,7]. Cancers of the throat and stomach have also been attributed to its inhalation [8]. In plants, Ni toxicity affects various physiological processes such as water relationship and photosynthesis activity [9,10] nitrogen metabolism and nutrient uptake [11]. In addition, there is increasing evidence that Ni toxicity is associated with oxidative stress [12,13] as reflected by the increase in the concentration of free radicals, which can overwhelm cell's intrinsic antioxidant defenses and can lead to cell damage or death [7,14]. The growing concerns about environmental pollution have stimulated the efforts to propose new approaches on the remediation of environment. In this way, several physicochemical techniques were used to clean up metal-contaminated soils. Yet, these metal removing processes are quite expensive and can severely inhibit soil fertility with subsequent negative impacts on the ecosystem [15]. Hence, biological treatment, especially phytoremediation, has emerged as a promising technology contributing to reduce the concentrations of Ni in contaminated soils to acceptable levels within a reasonable time frame. This approach based on the capability of selected plants to grow and accumulate metals is an environmental-friendly and relatively cheap technique comparatively to physicochemical methods [16,17]. Phytoremediation includes phytoextraction, phytostabilization, phytovolatization and rhizofiltration [18]. As far as heavy metals are concerned, phytoextraction is especially suitable since those pollutants could not be degraded. Among the Ni-accumulating plants, there is a discrete group of the hyper accumulators that accumulate metal in the shoots to the level of over 1000 µg/g dry weight [19]. All of these plants are typical glycophytes lacking salt-tolerance mechanisms and can therefore not be used to extract metals from salt-affected soils. Recently, it has been suggested that halophytes species, i.e. native salt tolerant species could be more suitable for heavy metal phytoremediation than glycophytes, most frequently used so far [20,21]. Interestingly, literature indicates that halophytes may be useful for phytoremediation [22,23] increasing the interest for halophytic plant utilization to extract several toxic metals [24,25]. Information regarding Ni-phytoextraction using halophytes is scarce. *M. crystallinum* is a dicotyledonous halophyte from the Aizoaceae family (order: Caryophyllales), commonly known as ice plant, and naturally present in environments characterized by an excess of toxic ions. It has been established as an extremely stress-tolerant model system [26]. This halophyte can yield 20 and 30 t ha^{-1} biomass and has been shown to accumulate up to 40% NaCl on a dry weight basis. In order to better characterize the Ni-phytoextraction capacity of this halophyte, the plant behavior upon exposure to nickel was compared to *B. juncea*, a typical glycophyte species commonly used for this purpose [27]. We paid a particular attention to plant growth parameters, and to Ni^{2+} distribution between roots and shoots.

Materials and methods

Soil characteristics and treatments

The soil used in this study was collected from the horizon 0–20 cm

***Corresponding author:** Amari T, Laboratoire des Plantes Extrêmophiles, Centre de Biotechnologie de Borj-Cédria, BP 901, 2050 Hammam-lif, Tunisia, Tel: +21679325848; Fax: +21679325848; E-mail: taoufik.amari@gmail.com

Received January 02, 2016; **Accepted** February 24, 2016; **Published** February 29, 2016

Citation: Amari T, Ghnaya T, Sghaier S, Porrini M, Lucchini G, et al. (2016) Evaluation of the Ni^{2+} Phytoextraction Potential in *Mesembryanthemum crystallinum* (Halophyte) and *Brassica juncea*. J Bioremediat Biodegrad 7: 336. doi: 10.4172/2155-6199.1000336

depth from Borj-Cedria region (30 km north of Tunis). The following soil properties were determined: pH (in water) 7.6; K^+ (0.38 µequiv. g^{-1}soil); Na^+ (1.31 µequiv. g^{-1}soil); Ca^{2+} (255.59 µequiv. g^{-1}soil); electric conductivity EC (86.66 µs cm^{-1}); organic matter content (0.47%). The sandy-loam soil was distributed into 24 large plastic pots, each containing 5 kg of air-dried soil. For Ni treatments, the soil was artificially contaminated with 25, 50 and 100 µg Ni g^{-1}soil. Ni was added as aqueous solution of $NiCl_2$ in one dose at the beginning of the experiment. After adding Ni^{2+}, the soil was equilibrated for 21 days during three cycles of saturation with tap water and was thereafter air dried.

Culture condition

Seeds of *Mesembryanthemum crystallinum* and *Brassica juncea* were sown directly in soil, in order to obtain uniform seedlings. Four weeks-old seedlings were selected and transplanted into each pot (3 plants per pot). The experiment was conducted for a period of three-months and it carried out in an open-air area under natural light and ambient temperature, in order as to keep all plants under conditions as similar as possible to those in the field.

Plant growth

At harvest, shoots were harvested and successively rinsed three times with cold water and blotted between two layers of filter paper. Roots were carefully removed from the substrate and dipped in a cold solution of HCl (0.01 M) during 5 min to eliminate heavy metals adsorbed at the root surface, and then washed three times with cold distilled water and blotted dry with filter paper. The fresh weight was immediately estimated, and the dry weight was measured after 48 h of desiccation in an oven at 60°C.

Nutrient concentrations and nickel accumulation

Dried samples (*c.a.* 300 mg) were ground to a fine powder using a stain-less mill and digested by concentrated HNO_3 (10 ml) in a microwave digester (ETHOS D, milestone, Italy) at 100°C. Thereafter, Ni and nutrients concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer, Sciex-Elan 5000).

Bioconcentration factor: The Ni^{2+} uptake, was depicted by a bioconcentration factor (BCF), provides an index of the ability of the plant to accumulate Ni^{2+} with respect to the concentration of this pollutant in the soil [28]. It is calculated as follows:

$$BCF = \frac{Ni^{2+} \text{concentration in dry shoots at the harvest}}{\text{Initial concentration of } Ni^{2+} \text{in soil}}$$

Pigment content

Pigments were extracted by placing 50 mg of fresh leaf in 2 mL of 100% acetone. The samples were incubated in darkness until complete chlorophyll extraction. Chlorophyll and carotenoids contents in supernatants were analyzed spectrophotometrically at 644.8, 661.6 and 470 nm [29].

Statistical analysis

Analyses of variance (ANOVA) with orthogonal contrasts and mean comparison procedures were used to detect differences between treatments. Mean separation procedures were conducted using the multiple range tests with Fisher's least significant difference (LSD) ($P < 0.05$).

Results

Plant morphology and growth

Results related to the effect of Ni^{2+} on plant morphology are presented in Figure 1. Ni-exposure of *B. juncea* plants induced early morphophytotoxicity symptoms as young leaf chlorosis, which was visible 10 days after starting the treatment. Two week later, chlorosis was more severe and necrosis appeared on the oldest leaves with a subsequent falling of these senescing leaves at the highest Ni^{2+} concentrations. In contrast, such toxicity symptoms were not observed on leaves of Ni-treated *M. crystallinum* plants, even at the highest Ni^{2+} concentration. Both root and shoot biomass decreased significantly in both species with increasing Ni^{2+} concentrations (Figure 2a and 2b). On average, the reductions recorded at 100 µM $NiCl_2$ in shoot biomass was 65% and 30% respectively, for *B. juncea* and *M. crystallinum*. For both species, the reduction percentage observed in root biomass reached *ca*. 60 % as compared to the control at 100 µM $NiCl_2$. As a result, the whole plant biomass production of both species was adversely affected by Ni addition (Figure 2c), with *B. juncea* more impacted than *M. crystallinum* at 100 µM $NiCl_2$ (−79% and −37% as compared to the control values respectively).

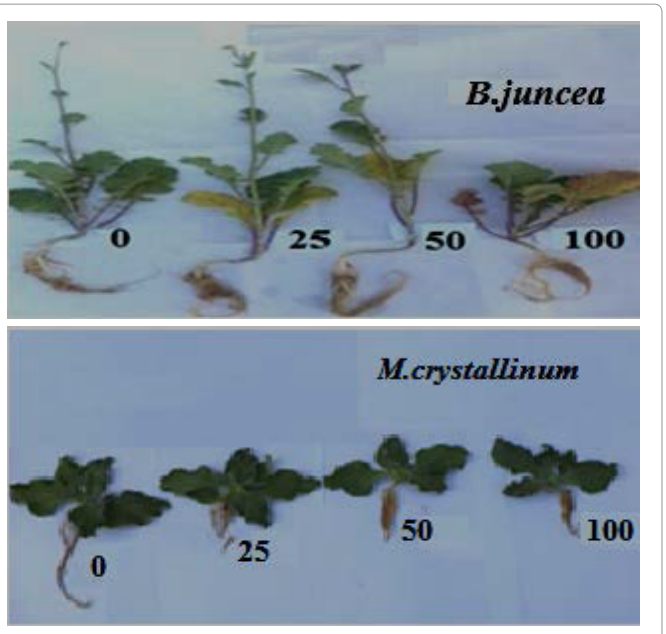

Figure 1: Morphological aspect of *Brassica juncea* and *Mesembryanthemum crystallinum* plants subjected in soil to different Ni concentrations during 3 months.

Plant mineral status

Significant differences were found in the nutrient uptake and accumulation pattern of Ni-treated plants depending on the element, the species investigated and even the organ (Table 1). With respect to macro-nutrients, Ca^{2+}, Mg^{2+} and K^+ concentrations decreased significantly with increasing Ni^{2+} external concentration in *M. crystallinum* shoots. For this species, root Ca^{2+} increased significantly, while Mg^{2+} and K^+ remain almost constant. In Ni-treated *B. juncea* plants, shoot and root concentrations of Ca^{2+} and Mg^{2+} were notably higher than those of the control, whereas, K^+ remained unchanged. For micro-nutrients, Ni treatment led to a significant increase of Fe^{2+} and Mn^{2+} concentrations in *B. juncea* shoots. However, addition of Ni^{2+} significantly reduced shoot Fe^{2+}, Zn^{2+} and Mn^{2+} concentration in *M. crystallinum.* In *B. juncea* roots, a slight increase of micro-nutrients concentrations was noted. In contrast, for *M. crystallinum,* nickel treatment resulted in a significant decrease of Mo^{2+} and Zn^{2+} concentrations. A similar trend was found for root Fe^{2+} and Mn^{2+} concentrations, only at low treatment (25µM $NiCl_2$).

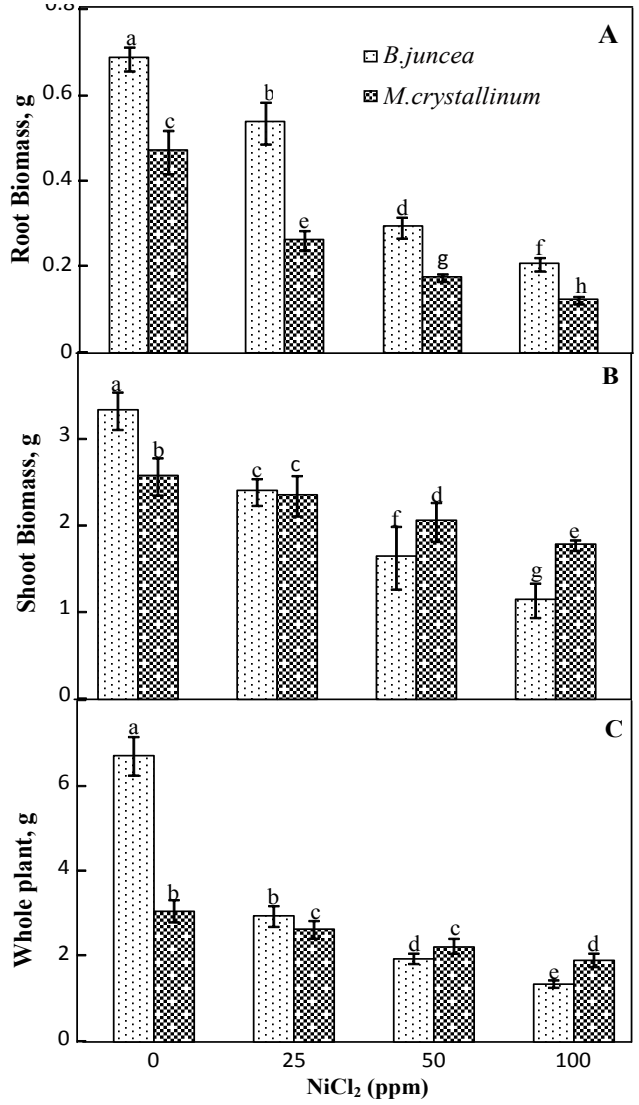

Figure 2: Effect of different $NiCl_2$ external concentrations on root biomass (**A**), shoot biomass (**B**) and the whole plant dry weight (**C**) of *M. crystallinum* and *B. juncea* after 3 months of treatment. Means (*n* = 6 per treatment ± SE) with at least one same letter are not significantly different at P ≤ 0.05.

Ni^{2+} effect on chlorophyll and carotenoid contents

The photosynthetic pigments of Ni-treated *B. juncea* plants was adversely impacted as reflected by the significant decrease of Chl a, Chl b, and total Chl concentrations (Table 2). For instance, compared to the control, the reductions recorded at 100 µM $NiCl_2$ in Chl a, Chl b and total Chl were 39%, 55%, 44%, respectively. In contrast, for *M. crystallinum* plants, Ni^{2+} led to a slight decrease of Chl a, Chl b and total Chl concentrations, excepting in the 100 µM $NiCl_2$ dose, Ni-treated *M. crystallinum* plants showed a significantly higher Chl concentration as compared to the control (Table 2). For both species, the carotenoid concentration was generally constant following Ni exposure, whereas it decreased significantly in *B. juncea* at the highest $NiCl_2$ concentration (Table 2).

Ni^{2+} accumulation and translocation

In treated plants, Ni^{2+} concentrations increased markedly in both under- and above-ground organs following Ni exposure (Table 3). It is noteworthy that roots of both *B. juncea* and *M. crystallinum* accumulated much more Ni^{2+} than did shoots. *M. crystallinum* shoot Ni^{2+} concentrations were significantly higher than *B. juncea* (for instance 78 µg g^{-1} DW and 57 µg g^{-1} DW at 100 µM $NiCl_2$ respectively), the same trend was also observed in roots (for instance 371 µg g^{-1} DW and 152 µg g^{-1} DW at 100 µM $NiCl_2$ respectively). The phytoextraction potential of a given species depends not only on metal shoot concentration but also on shoot biomass production. In terms of shoot Ni^{2+} content (calculated as the product of the shoot metal concentration by its biomass), *M. crystallinum* translocated more Ni^{2+} toward shoots as compared to *B. juncea* irrespective of $NiCl_2$ concentration (Figure 3). For instance, at 100 µM $NiCl_2$, shoot Ni^{2+} contents were 141 µg $plant^{-1}$ and 66 µg $plant^{-1}$ in *M. crystallinum* and *B. juncea*, respectively. The higher phytoextraction capacity of *M. crystallinum* is better highlighted by using the nickel absorption efficiency, which showed higher values for *M. crystallinum* as compared to *B. juncea* plants (Table 3).

Discussion

Phytoextraction, the establishment of plants to extract heavy metals from contaminated sites is particularly challenging due to the high toxicity of these pollutants which commonly hamper plant growth. The identification of Ni-accumulator plant species represents the major prerequest for further rehabilitation of Ni contaminated soils. Recently, it has been reported that halophytes species would be candidate for this purpose compared to glycophytes [25,30,31]. For example, [32] have shown that *Messembryanthemum crystallinum* is more tolerant to Cu stress than *Arabidopsis thaliana*. Similarly, [12] clearly showed that *Chenopodium botrys* an annual halophyte may remove up to 180 g Cd ha^{-1}, which is 6 times more than Cd removal by the hyperaccumulator *Noccaea caerulescens*.

In the present study, the two tested species showed a different pattern in response to the addition of Ni^{2+} in the soil. Results showed that the halophyte species *M. crystallinum* was more tolerant to Ni^{2+} than the glycophyte *B. juncea* (Figure 1). Indeed, nickel-induced chlorosis and foliar necrosis were visible only in *B. juncea* plants, whereas for *M.*

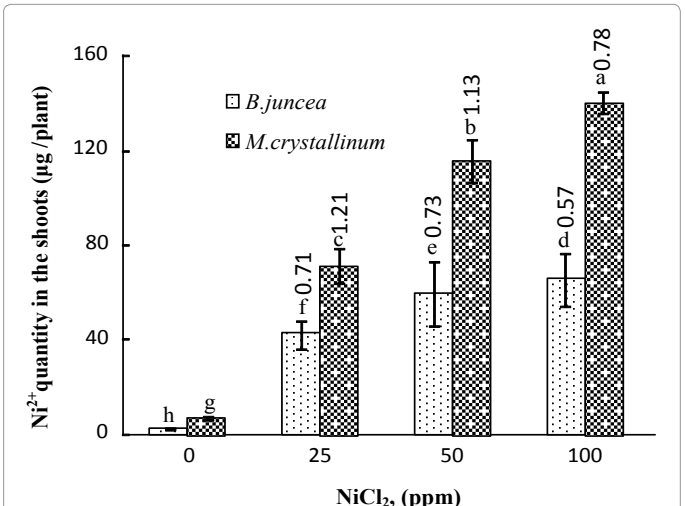

Figure 3: Nickel amount (µg $plant^{-1}$) in shoots of *M. crystallinum* and *B. juncea*. The values above histograms correspond to the bioconcentration factor (BCF) for Ni-treated plants. Plants were exposed for 3 months to increasing $NiCl_2$ concentrations in the soil. Means (n = 6 per treatment ± SE) with at least one same letter are not significantly different at P ≤ 0.05.

$NiCl_2$ (µM)		0	25	50	100
Ca^{2+} µg/g DW					
M. crystallinum	Shoots	15.34 ± 1.03a	16.22 ± 1.02b	20.10 ± 1.76c	20.31 ± 1.05d
	Roots	34.51 ± 0.38a	5.35 ± 0.15c	6.93 ± 0.21c	16.55 ± 0.42b
B. juncea	Shoots	12.62 ± 0.80c	14.52 ± 0.31b	16.73 ± 0.22b	24.61 ± 0.54a
	Roots	5.88 ± 0.16b	5.40 ± 0.14b	6.29 ± 0.06b	8.00 ± 0.20a
Mg^{2+} µg/g DW					
M. crystallinum	Shoots	5.41 ± 0.27a	4.95 ± 0.01a	5.28 ± 0.12a	5.87 ± 0.16a
	Roots	3.26 ± 0.01b	1.79 ± 0.08c	2.53 ± 0.46b	5.22 ± 0.69a
B. juncea	Partie aérienne	2.73 ± 0.17b	2.75 ± 0.13b	3.50 ± 0,03b	5.17 ± 0.03a
	Roots	1.76 ± 0.06b	1.80 ± 0.05b	2.11 ± 0.02b	3.37 ± 0.10a
K^+ µg/g DW					
M. crystallinum	Shoots	32.29 ± 1.55a	23.80 ± 0.33b	26.68 ± 0.67ab	28.01 ± 0.23a
	Roots	30.90 ± 0,29a	11.91 ± 0,13b	10.03 ± 0.26b	11.74 ± 0.63b
B. juncea	Shoots	21.11 ± 1.31a	21.46 ± 0.39a	17.05 ± 0.23b	22.69 ± 0.38a
	Roots	10.01 ± 0.26a	11.09 ± 0.31a	9.45 ± 0.14ab	12.63 ± 0.42a
Fe^{2+} µg/g DW					
M. crystallinum	Shoots	0.48 ± 0.02a	0.40 ± 0.01a	0.32 ± 0.01b	0.28 ± 0.03b
	Roots	3.09 ± 0.02b	1.72 ± 0.05c	2.81 ± 0.53b	5.36 ± 0.79a
B. juncea	Shoots	0.17 ± 0.01b	0.34 ± 0.02a	0.21 ± 0.01b	0.35 ± 0.02a
	Roots	1.89 ± 0.08b	2.28 ± 0.05b	2.49 ± 0.06b	4.33 ± 0.12a
Mn^{2+} µg/g DW					
M. crystallinum	Shoots	32.71 ± 1.90a	20.05 ± 0.11b	30.58 ± 0.51a	30.42 ± 0.75a
	Roots	24.32 ± 0.07a	14.13 ± 0.37b	20.64 ± 1.01a	29.95 ± 6.34a
B. juncea	Shoots	20.53 ± 1.64c	34.40 ± 2.22b	29.63 ± 0.01b	42.65 ± 0.27a
	Roots	15.93 ± 0.44b	15.11 ± 0.89b	19.64 ± 0.58a	22.88 ± 0.47a
Mo^{2+} µg/g DW					
M. crystallinum	Shoots	3.57 ± 0.40a	3.33 ± 0.11b	3.00 ± 0.07b	2.94 ± 0.12b
	Roots	25.70 ± 2.88a	9.59 ± 0.31ab	4.08 ± 0.54b	3.73 ± 0.26b
B. juncea	Shoots	3.49 ± 0.23b	3.27 ± 0.13b	3.68 ± 0.03b	4.45 ± 0.10a
	Roots	4.39 ± 0.10a	3.65 ± 0.17b	3.63 ± 0.01b	4.49 ± 0.04a
Zn^{2+} µg/g DW					
M. crystallinum	Shoots	41.45 ± 2.35a	19,50 ± 0.38b	19.87 ± 0.16b	13.13 ± 0.32b
	Roots	52.48 ± 1.57a	16.08 ± 0.13b	17.63 ± 0.41b	27.97 ± 0.46b
B. juncea	Shoots	19.07 ± 1.34a	19.31 ± 1.36a	20.90 ± 0.27a	17.02 ± 0.08b
	Roots	16.84 ± 0.61b	16.69 ± 0.44b	17.80 ± 0.62b	20.02 ± 0.33a

Table 1: Macro- (Mg^{2+}, Ca^{2+}, K^+) and micro-nutrient (Fe^{2+}, Mn^{2+}, Mo^{2+}, Zn^{2+}concentrationsin shoots and roots of $NiCl_2$-treated *M. crystallinum* and *B. juncea*. Means (n = 6 per treatment ± SE) followed by the same letters are not significantly different at P ≤ 0.05.

$NiCl_2$ (ppm)	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl Total (mg/g FW)	Car (mg/g FW)
M. crystallinum				
0	0.089 ± 0.014a	0.048 ± 0.006a	0.137 ± 0.010a	0.044 ± 0.006a
25	0.075 ± 0.001b	0.038 ± 0.002b	0.113 ± 0.001b	0.038 ± 0.004b
50	0.083 ± 0.010b	0.050 ± 0.003a	0.132 ± 0.007a	0.043 ± 0.007a
100	0.107 ± 0.003c	0.052 ± 0.002a	0.159 ± 0.004c	0.048 ± 0.008a
B. juncea				
0	0.228 ± 0.011a	0.103 ± 0.017a	0.331 ± 0.028a	0.078 ± 0.004a
25	0.192 ± 0.011b	0.072 ± 0.006b	0.264 ± 0.012b	0.084 ± 0.004b
50	0.183 ± 0.016b	0.068 ± 0.006c	0.251 ± 0.017b	0.080 ± 0.006ab
100	0.139 ± 0.009c	0.046 ± 0.003d	0.186 ± 0.012c	0.058 ± 0.005c

Table 2 : Effect of $NiCl_2$ on leaf chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl), and carotenoid (Car) concentration of *M. crystallinum* and *B. juncea*. Means (n =6 per treatment ± SE) followed by the same letters are not significantly different at P ≤ 0.05.

crystallinum seedlings, such toxicity symptoms were not observed even at a shoot tissue concentration higher than 78 µg g^{-1} dry mass. In both species, Ni negatively affected the plant growth (Figure 1). Biomass productivity of shoots and roots were significantly reduced in response to Ni stress, with root being more impacted than shoot (Figure 2a and 2b). The analysis of total chlorophyll concentrations in apical leaves (Table 2) confirmed that *B. juncea* was more sensitive to nickel than *M. crystallinum*. The severe Ni-induced leaf chlorosis observed in *B. juncea* was associated with lower pigment concentrations in leaves as previously reported in Ni-treated *Hordeum vulgare* and *Triticum aestivum* seedlings [33,34]. The abovementioned Ni-related impact on the plant phenotype and/or biomass production may result from direct (toxicity of Ni^{2+} accumulated in tissues) and/or indirect factors, including the alteration of mineral nutrition, the impairment of the photosynthetic

$NiCl_2$ (ppm)	Roots	Shoots
	µg Ni^{2+} g/DW	µg Ni^{2+} g/DW
M. crystallinum		
0	4.25 ± 0.49a	2.89 ± 0.16a
25	40.46 ± 0.80b	30.36 ± 0.62b
50	103.39 ± 1.05c	56.35 ± 1.70c
100	370.98 ± 7.73d	78.33 ± 1.45d
B. juncea		
0	3.45 ± 0.14a	0.84 ± 0.06a
25	42.79 ± 1.48b	17.75 ± 1.32b
50	96.46 ± 1.81c	36.33 ± 0.36c
100	152.63 ± 3.56d	57.11 ± 0.43d

Table 3: Nickel concentration in roots and shoots of *M. crystallinum* and *B. juncea*, after 3 months of treatment. Means (n = 6 per treatment ± SE) with at least one same letter are not significantly different at P ≤ 0.05.

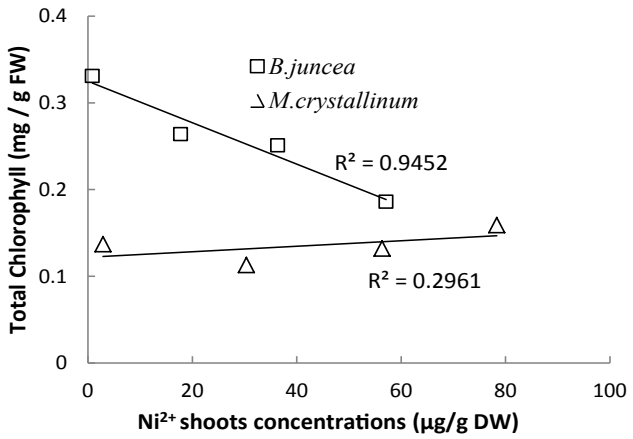

Figure 4: Relationship between shoot Ni^{2+} concentration and total chlorophyll concentration in *M. crystallinum* and *B. juncea* exposed for 3 months to increasing $NiCl_2$ concentrations in the nutrient solution. Means (n = 6 per treatment ± SE) followed by the same letters are not significantly different at P ≤ 0.05. Bars marked with same letter are not significantly different at p= 0.05.

process and the disturbance or imbalance of the plant water status. One of the likely mechanisms that can explain the superiority of the halophyte to maintain its growth potential and to tolerate heavy metals could be, at least partly, linked to the maintenance of an adequate nutrients uptake [31]. Yet, this is not the case in *M. crystallinum*. In our experiment, Ni-stress adversely affected macro-nutrient (Ca^{2+}, Mg^{2+} and K^{+}) accumulation in the shoots, and micronutrient (Fe^{2+}, Zn^{2+} and Mn^{2+}) in the roots of this species. It is known that, heavy metals, including nickel, may compete with essentials nutrients absorption and translocation, which adversely affects the plant mineral status, and even lead to nutrient deficiencies [35,36]. Generally, the effect of toxic heavy metals on nutrients uptake depends at least on two major mechanisms that play a pivotal role in generating metal toxicity. First, the competition for the common binding sites due to the comparable ion radii [37]. For example, Ni has a similar character to Mg, Ca, Fe, and Zn [9]. Second, the decline in nutrient uptake may also result from the metal-induced metabolic impairment that affects the constitution and enzyme activities of cell membranes [38]. Photosynthetic pigments may be used as indicators of metal stress damage [39] and may predict subsequent events at the organism level [40]. In *M. crystallinum*, the total chlorophyll and carotenoid concentrations were slightly affected in the 0–50 ppm $NiCl_2$ range before significantly increasing at the highest dose likely as a result of rudimentary effect of Ni on Chl and Car biosynthesis concomitant to important reduction of leaf area under 100 ppm Ni leading to the increase of Chl concentration in leaves of these plants (Table 2). The absence of a strong correlation between the shoot chlorophyll concentration and the shoot Ni^{2+} concentration in *M. crystallinum* (R^2= 0.29) as compared to *B. juncea* (R^2= 0.94) (Figure 4) suggests that nickel is very likely efficiently sequestered in the aboveground organs of the former halophyte species, thus providing a powerful protection of the photosynthetic machinery and hence tolerate better Ni in their leaves. According to literature, there are at several mechanisms that could govern metal tolerance in halophytes when compared to glycophytes. Thus, efficient sequestration was considered an important process allowing the resistance to heavy metals in tissues by its complexation with ligands and/or by their exclusion from metabolically active cytoplasm by moving them into inactive compartments, mostly vacuoles and cell walls [30,41]. It is also worth mentioning that Ni^{2+} tolerance is also strongly coupled with an effective protection against oxidative stress by the induction dynamic ROS-scavenging system [42,43]. Some of these processes might be involved in the rudimentary toxicity signs of Ni expressed by the halophyte *M. crystallinum*.

Regarding Ni^{2+} accumulation, our results indicate that both species were able to absorb Ni^{2+} and to translocate it toward their shoots, but the Ni^{2+} was mainly accumulated in roots (Figure 3). In the shoots, Ni^{2+} concentrations were significantly lower as compared to those measured in previous work, when the two tested species are cultivated in a Ni enriched nutrient solution [10]. This suggests that Ni^{2+} is less bioavailable in soil than in hydroponic medium. Heavy metals in soils are intimately associated with different soil components and their mobility and availability in rooting medium is determined mainly by the way metals are bound to these soil components. Numerous edaphic factors such as, pH, soil texture, and organic matter content can affect the heavy metals bioavailability in soils and then the metal accumulation by plants [44]. The soil used in this study is a sandy-loam soil with a limited Ni^{2+} binding capacity (i.e. low organic matter and clay content). Generally, Ni^{2+} becomes more totally available under acidic conditions [45]. Elevated pH concomitant with high organic matter content increases the sorption of Ni^{2+} by soils and thus reduces its bioavailability [46]. Bioavailability of nickel was not assessed after artificial contamination in our substrate. As a consequence, despite a low pH and a low amount of organic matter, it cannot be excluded that a portion of the added Ni became unavailable for a direct absorption by the plant.

When cultivated on Ni-contaminated soils, most of Ni^{2+} taken up was accumulated in roots. Several species adopt this strategy and accumulate toxic metals in the roots such as *Thlaspi arvense*, *Zea mays* [47], *Hordeum vulgare* [48] and *Rubus ulmifolius* [49]. In plants, it was suggested that nickel could be transported in association with citrate and malate [50,51] as a nickel-peptide complex or as a nickel–histidine complex [52]. Furthermore, nickel may also be sequestered in the cation exchange sites of the walls of xylem parenchyma cells and immobilization in the vacuoles of roots [50]. However, this behavior is not suitable in plants used for phytoextraction of metals.

Beside concentrations, a total amount of metals accumulated in the shoots is considered as the most important parameter to evaluate the potential of phytoextraction in plants. *M. crystallinum* accumulated much more nickel in the shoots as compared with the glycophyte *B. juncea* (Figure 3). In addition, examining the Bioconcentration Factor (BCF), which is a common index used to estimate plant ability to pump heavy metals from the substrate and to compare species for

phytoextraction potentials, revealed that in soil. *M. crystallinum* showed a higher aptitude to bioaccumulate Ni^{2+} than *B. juncea* (Figue 3). For example, BCF values were 0.78 and 0.57 respectively in *M. crystallinum* and *B. juncea* exposed to 100 ppm $NiCl_2$.

Conclusion

Our finding indicated that, on Ni^{2+}-polluted soils, the halophyte species *M. crystallinum* is more tolerant to nickel than *B. juncea* and that such a tolerance is associated with a high potential of Ni^{2+} accumulation in aboveground materials. BCF and the amounts of extracted Ni^{2+} values indicated that *M. crystallinum* is more efficient to extract nickel from contaminated soil than *B. juncea*. Regarding the phytoextraction potential, owing to its capability to accumulate a moderate amounts Ni^{2+} in the shoots, the halophyte *M. crystallinum* could be therefore more suitable for metal extraction from moderately polluted sites.

References

1. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48: 909-930.
2. Anjum NA, Ahmad I, Mohmood I, Pacheco M, Duarte AC, et al. (2012) Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids-a review. Environ Exp Bot 75: 307-324.
3. Ghnaya T, Nouairi I, Slama I, Messedi D, Grignon C, et al. (2005) Cadmium effects on growth and mineral nutrition of two halophytes: Sesuvium portulacastrum and Mesembryanthemum crystallinum. J Plant Physiol 162: 1133-1140.
4. Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. ISRN Ecology : 1-20.
5. Mishra S, Dwivedi SP, Singh RB (2010) A review on epigenetic effect of heavy metal carcinogenesis on human health. Open Nutraceut J 3: 188-193.
6. Khan MA, Ahmad I, Rahman I (2007) Effect of environmental pollution on heavy metals content of Withania somnifera. J Chin Chem Soc 54: 339-343.
7. Das KK, Das SN, Dhundasi SA (2008) Nickel, its adverse health effects & oxidative stress.Indian J Med Res 128: 412-425.
8. Duda-Chodak A, Baszczyk U (2008) The impact of nickel on human health. J Elementol 13: 685-696.
9. Chen C, Huang D, Liu J (2009) Functions and toxicity of nickel in plants: 695 recent advances and future prospects. Clean 37: 304-313.
10. Amari T, Ghnaya T, Debez A, Taamali M, Youssef NB, et al. (2014) Comparative Ni tolerance and accumulation potentials between Mesembryanthemum crystallinum (halophyte) and Brassica juncea: metal accumulation, nutrient status and photosynthetic activity. J Plant Physiol 171: 1634-1644.
11. Gajewska E, Wielanek M, Bergier K, Sklodowska M (2009) Nickel induced depression of nitrogen assimilation in wheat roots. Acta Physiol Plant 31: 1291-1300.
12. Hao F, Wang X, Chen J (2006) Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci 170: 151-158.
13. Gajewska E, Odowska M (2007) Effect of nickel on ROS content and antioxidative enzyme activities in wheat leaves. Biometals 20: 27-36.
14. Krystofova O, Shestivska V, Galiova M, Novotny K, Kaiser J, et al. (2009) Sunflower Plants as Bioindicators of Environmental Pollution with Lead (II) Ions. Sensors (Basel) 9: 5040-5058.
15. Gardea-Torresdey JL, Peralta-Videab JR, Rosaa G, Parsons JG (2005) Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. Coord. Chemosphere 249: 1797-1810.
16. Petra K, Juan B, Pilar Bernal M, Flavia N, Charlotte P, et al. (2009) Trace element behaviour at the root–soil interface: Implications in phytoremediation. Environ Exp Bot 67: 243-259.
17. Greipsson S (2011) Phytoremediation. Nat Educ Knowl 2: 7.
18. Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56: 15-39.
19. Baker AJM, McGrath SP, Sidoli CMD, Reeves RD (1994) The possibility of in situ heavy metal decontamination of polluted soils using crops of metal-accumulating plants. Resour Conserv Recy 11: 41-49.
20. Mazharia M, Homaeed M (2012) Annual halophyte Chenopidium botrys can phytoextract cadmium from contaminated soils. J B App Sci Re 2: 1415-1422.
21. Zaier H, Ghnaya T, Ghabriche R, Chmingui W, Lakhdar A, et al. (2014) EDTA-enhanced phytoremediation of lead-contaminated soil by the halophyte Sesuvium portulacastrum. Environ Sci Pollut Res Int 21: 7607-7615.
22. 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.
23. Eisa SS, Eid MA (2011) Cadmium accumulation in Atriplex halimus subsp. schweinfurthii and its influence on growth, proline, root hydraulic conductivity and nutrient uptake. Aust J Basic Appl Sci 5: 88-95.
24. Lefèvre I, Marchal G, Ghanem ME, Correal E, Lutts S (2010) Cadmium has contrasting effects on polyethylene glycol–sensitive and resistant cell lines in the mediterranean halophyte species Atriplex halimus L. J Plant Physiol 167: 365-374.
25. Milic D, Lukovic J, Ninkov J, Zeremski Skoric T, Zoric L, et al. (2012) Heavy metal content in halophytic plants from inland and maritime saline areas. Cent Eur J Biol 7: 307-317.
26. Ibdah M, Krins A, Seidlitz H, Heller W, Strack WD, et al. (2002) Spectral dependence of flavonol and betacyanin accumulation in Mesembryanthemum crystallinum under enhanced UV radiation. Plant Cell Environ 25: 1145-1154.
27. Sainger M, Sharmab A, Bauddhc K, Ahlawat Saingerd P, et al. (2014) Remediation of nickel-contaminated soil by Brassica juncea L. cv. T-59 and effect of the metal on some metabolic aspects of the plant. Bioremediation J 8: 100-110.
28. Zayed A, Gowthaman S, Terry N (1998) Phytoaccumulation of trace elements bywetland plants: I. Duckweed. J Environ Qual 27: 715-721.
29. Lichtenthaler HK, Welburn A (1983) Determination of total carotenoids and chlorophyllsa and b of leaf extract in different solvents. Biochem Soc Trans 603: 591-592.
30. Ghnaya T, Zaier H, Baioui R, Sghaier S, Lucchini G, et al. (2013) Implication of organic acids in the long-distance transport and the accumulation of lead in Sesuvium portulacastrum and Brassica juncea. Chemosphere 90: 1449-1454.
31. Zaier H, Ghnaya T, Lakhdar A, Baioui R, Ghabriche R, et al. (2010) Comparative study of Pb-phytoextraction potential in Sesuvium portulacastrum and Brassica juncea: tolerance and accumulation. J Hazard Mater 183: 609-615.
32. Thomas JC, Malick FK, Endreszl C, Davies EC, Murray KS (1998) Distinct response to copper stress in the halophyte Mesembryanthemum crystallinum. Physiol Plant 102: 310-317.
33. Shalygo NV, Kolensikova NV, Voronetskaya VV, Averina NG (1999) Effects of Mn2+, Fe2+, Co2+ and Ni2+ on chlorophyll accumulation and early stages of chlorophyll formation of greening barley seedling. Russ J Plant Physiol 46: 496-501.
34. Yusuf M, Fariduddin Q, Hayat S, Hasan SA, Ahmad A (2011) Protective response of 28-homobrassinolide in cultivars of Triticum aestivum with different levels of nickel. Arch Environ Contam Toxicol 60: 68-76.
35. Kidd P, Barcelo J, Bernal MP, Navari-Izzo F, Poschenrieder C, et al. (2009) Trace element behaviour at the root–soil interface: implications in phytoremediation. Environ Exp Bot 67: 243-59.
36. Saito A, Saito M, Ichikawa Y, Yoshiba M, Tadano T, et al. (2010) Difference in the distribution and speciation of cellular nickel between nickel-tolerant and non-tolerant Nicotiana tabacum L. cv. BY-2 cells. Plant Cell Environ 33: 174-187.
37. Nishida S, Aisu A, Mizuno T (2012) Induction of IRT1 by the nickel-induced iron-deficient response in Arabidopsis. Plant Signal Behav 7: 329-331.
38. Ros R, Morales A, Segura J, Picazo I (1992) In vivo and in vitro effects of nickel and cadmium on the plasma ATPase from rice (Oryza sativa L.) shoots and roots. Plant Sci 83: 1-6.
39. Alam MM, Hayat S, Ali B, Ahmad A (2007) Effect of 28-homobrassinolide treatment on nickel toxicity in Brassica juncea. Photosynthetica 45: 139-142.
40. Van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13, 195-206.

41. Reboreda R, Caçador I (2007) Halophyte vegetation influences in salt marsh retention capacity for heavy metals. Environ Pollut 146: 147-154.

42. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9: 490-498.

43. Saiyood S, Vangnai AS, Inthorn D, Thiravetyan P (2012) Treatment of total dissolve solids from plastic industrial effluent by halophyte plants. Water Air Soil Pollut 223: 4865-4873.

44. Jean L, Bordas F, Gautier-Moussard C, Vernay P, Hitmi A, et al. (2008) Effect of citric acid and EDTA on chromium and nickel uptake and translocation by Datura innoxia. Environ Pollut 153: 555-563.

45. Panda GC, Das SK, Bandopadhyay TS, Guha AK (2007) Adsorption of nickel on husk of Lathyrus sativus: behavior and binding mechanism. Colloids Surf B Biointerfaces 57: 135-142.

46. Burke DJ, Weis JS, Weis P (2000) Release of metals by the leaves of the salt marsh grasses Spartina alterniflora and Phragmites australis. Estuar Coast Shelf Sci 51:153-159.

47. Seregin IV, Kozhevnikova AD, Kazyumina EM, Ivanov VB (2003) Nickel toxicity and distribution in maize roots. J Plant Physiol 50: 711-718.

48. Andreeva IV, Govorina VV, Vinogradova SB, Yagodin BA (2001) Nickel in plants. Agrokhimiya 20: 82-94.

49. Marques AP, Moreira H, Rangel AO, Castro PM (2009) Arsenic, lead and nickel accumulation in Rubus ulmifolius growing in contaminated soil in Portugal. J Hazard Mater 165: 174-179.

50. Seregin IV, Kozhevnikova AD (2008) Roles of root and shoot tissues in transport and accu-mulation of cadmium, lead, nickel, and strontium. J Plant Physiol 55: 1-22.

51. Amari T, Lutts S, Taamali M, Lucchini G, Sacchi GA, et al. (2016) Implication of citrate, malate and histidine in the accumulation and transport of nickel in Mesembryanthemum crystallinum and Brassica juncea. Ecotoxicol Environ Saf 126: 122-128.

52. Kerkeb L, Kramer U (2003) The role of free histidine in xylem loading of nickel in Alyssum lesbiacum and Brassica juncea. Plant Physiol 131: 716-724.