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

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**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 phytoreductor 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\textsubscript{2}. Nickel impaired the growth activity of both species. Interestingly, *M. crystallinum* was less impacted by NiCl\textsubscript{2} addition. The plant mineral nutrition was differently affected by NiCl\textsubscript{2} exposure depending on the ion, the species and even the organ. In both species, roots were the preferential sites of Ni\textsuperscript{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\textsuperscript{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\textsuperscript{2+}, Pb\textsuperscript{2+} and Ni\textsuperscript{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, phytovolatilization 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\textsuperscript{-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\textsuperscript{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...
depth from Borj-Cedria region (30 km north of Tunis). The following soil properties were determined: pH (in water) 7.6; K⁺ (0.38 mequiv. g⁻¹ soil); Na⁺ (1.31 mequiv. g⁻¹ soil); Ca²⁺ (255.59 mequiv. 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 NiCl₂ in one dose at the beginning of the experiment. After adding Ni²⁺, 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 HNO₃ (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 Ni²⁺ uptake, was depicted by a bioconcentration factor (BCF), provides an index of the ability of the plant to accumulate Ni²⁺ with respect to the concentration of this pollutant in the soil [28]. It is calculated as follows:

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

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 Ni²⁺ 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 weeks later, chlorosis was more severe and necrosis appeared on the oldest leaves with a subsequent falling of these senescing leaves at the highest Ni²⁺ concentrations. In contrast, such toxicity symptoms were not observed on leaves of Ni-treated M. crystallinum plants, even at the highest Ni²⁺ concentration. Both root and shoot biomass decreased significantly in both species with increasing Ni²⁺ concentrations (Figure 2a and 2b). On average, the reductions recorded at 100 µM NiCl₂ 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 NiCl₂. 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 NiCl₂ (~79% and ~37% as compared to the control values respectively).

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 macronutrients, Ca²⁺, Mg²⁺ and K⁺ concentrations decreased significantly with increasing Ni²⁺ external concentration in M. crystallinum shoots. For this species, root Ca²⁺ increased significantly, while Mg²⁺ and K⁺ remain almost constant. In Ni-treated B. juncea plants, shoot and root concentrations of Ca²⁺ and Mg²⁺ were notably higher than those of the control, whereas, K⁺ remained unchanged. For micro-nutrients, Ni treatment led to a significant increase of Fe²⁺ and Mn²⁺ concentrations in B. juncea shoots. However, addition of Ni²⁺ significantly reduced shoot Fe²⁺, Zn²⁺ and Mn²⁺ 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 Mo⁶⁺ and Zn²⁺ concentrations. A similar trend was found for root Fe²⁺ and Mn²⁺ concentrations, only at low treatment (25µM NiCl₂).
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 prerogative 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 Mesembryanthemum 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. crystallinum, the nickel accumulation and translocation were much less deleterious.
Table 1: Macro- (Mg\(^{2+}\), Ca\(^{2+}\), K\(^{+}\)) and micro-nutrient (Fe\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\)) concentrations in shoots and roots of Ni\(^{2+}\)-treated M. crystallinum and B. juncea. Means (\(n = 6\) per treatment ± SE) followed by the same letters are not significantly different at \(P \leq 0.05\).

| NiCl\(_2\) (µM) | Ca\(^{2+}\) µg/g DW | Mg\(^{2+}\) µg/g DW | K\(^{+}\) µg/g DW | Fe\(^{2+}\) µg/g DW | Mn\(^{2+}\) µg/g DW | Zn\(^{2+}\) 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        |                     |                     |

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 \(P \leq 0.05\).

| 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               | 0.222 ± 0.011b  | 0.103 ± 0.017a  | 0.331 ± 0.028a      | 0.078 ± 0.004a|
| 25              | 0.222 ± 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|

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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 (Ca²⁺, Mg²⁺ and K⁺) accumulation in the shoots, and micronutrient (Fe²⁺, Zn²⁺ and Mn²⁺) 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, 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 Ni²⁺ concentration in M. crystallinum (R² = 0.29) as compared to B. juncea (R² = 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 tolerance better Ni in their leaves. According to literature, there are 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 Ni²⁺ 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 Ni²⁺ accumulation, our results indicate that both species were able to absorb Ni²⁺ and to translocate it toward their shoots, but the Ni²⁺ was mainly accumulated in roots (Figure 3). In the shoots, Ni²⁺ 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 Ni²⁺ 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 Ni²⁺ binding capacity (i.e. low organic matter and clay content). Generally, Ni²⁺ becomes more totally available under acidic conditions [45]. Elevated pH concomitant with high organic matter content increases the sorption of Ni²⁺ 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 Ni²⁺ 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 umlfolius [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* (Figure 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 phytorextraction potential, owing to its capability to accumulate a moderate amounts of Ni\(^{2+}\) in the shoots, the halophyte *M. crystallinum* could be therefore more suitable for metal extraction from moderately polluted sites.

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