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# Metal tolerance and phytoremoval ability in *Amaranthus paniculatus* L. grown in nickel-spiked nutrient solution

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**Abstract.** To evaluate a possible utilization in the phytoremediation of metal contaminated substrates, *Amaranthus paniculatus* L. plants were grown for one week in Ni-spiked growth solutions at 0, 25, 50, 100, 150 μM $NiCl_2$ in hydroponics under controlled climate conditions. Results showed a high tolerance to Ni in plants exposed to low Ni concentrations. Tolerance decreased as Ni concentration in the growth solutions enhanced. Ni concentrations in plant organs (root, stem and leaves) revealed a trend to increase in parallel with the enhancement of Ni content in the growth solution. The ability to accumulate Ni in plants was also evaluated by calculating the bioconcentration factor (BCF). An inverse relation between BCF and Ni concentrations in the growth solution was evidenced. Ni phytoremoval ability of *A. paniculatus* plants was particularly appreciable at 25 μM $NiCl_2$, where more than 65% of the initial Ni amount was taken up by plants in one week of treatment. The capability of plants to translocate Ni from roots to shoots (stem+leaves) was evaluated by the translocation factor (T*f*). Results revealed a low T*f* in plants exposed to low Ni concentration, suggesting a tolerance mechanism to protect physiological processes occurring in leaves. Overall, *A. paniculatus* plants showed a valuable capability to phytodecontaminate Ni-polluted waters, particularly at low Ni concentrations.

**Key words:** Heavy metals, hydroponics, phytoremediation, metal bioconcentration

## Introduction

The enhanced levels of HM found in air, soil and water in these last years, mainly due to anthropogenic causes, are considered a relevant threat for food chains and ecosystem survival. Among technologies useful to clean up contaminated substrates from HM, the phytoremediation, *i.e.* the use of plants to remove or render less harmful HM in soils and waters, has emerged as a valuable choice (Salt et al., 1998). Ecological sustainability, economical feasibility, public acceptance, low level of technological demand and low level of energetic input are some of the most important characteristics of phytoremediation. On the contrary, a limit for applying phytoremediation is the metal concentrations that plants can tolerate. Plant species showed an extreme natural variability for this trait. Some plants species, for instance, can withstand and accumulate HM, such as Ni, in concentrations higher than 0,1% of D.W. and are termed hyperaccumulators (Brooks et al., 1977). Other plant species are very sensitive to the exposure to HM, evidencing toxicity symptoms also at low HM concentrations. Adverse effects of HM on photosynthesis, growth, mineral nutrition, hormonal status and water balance, mainly linked to the production of reactive oxygen species within cells, have been extensively described in plants (Clemens, 2006). The assessment of the thresholds of HM toxicity and of the HM bioaccumulation capability represents a basic step to candidate a plant for phytoremediation, even through early screening tests at laboratory scale (Shevyakova et al., 2011; Iori et al., 2011). In this work, results from a laboratory investigation aimed at evaluating the ability of *Amaranthus paniculatus* L. to tolerate, remove and bioconcentrate nickel in a short-term experiment were reported. Nickel is delivered in the environment by industrial and agricultural activities, with possible harmful effects on living organisms. It is an essential micronutrient for plants but at high concentrations it becomes very toxic, inducing several metabolic disorders (Seregin and Kozhevnikova, 2006).

## Materials and Methods

Seeds of *Amaranthus paniculatus* L. were soaked and germinated in darkness on wet filter paper in a growth chamber at 26°C. Young plantlets were transferred to plastic pots filled with three liters of one-sixth-strength Hoagland solution in a controlled climate chamber at photon flux density of 300 µmol $m^{-2}$ $s^{-1}$ for 14 h/day at 25°C/20°C day/night and a relative humidity of 70-80%. Air pumping avoided oxygen deprival. After three weeks, plants were transferred to single pots (six plant per pot) and subjected to 0 (control), 25, 50, 100, 150 µM $NiCl_2$*$6H_20$ in one-sixth-strength Hoagland solution for one week. Solutions were sampled every day to evaluate Ni removal by plants. At the end of the experiment, plants were harvested, carefully washed, separated in their organs, dried in a oven at 80°C and finally weighed. Samples were processed for metal determination (Zacchini et al., 2009). Tolerance index (T*i*) was calculated as: dry weight of plants grown in nickel-spiked solution/dry weight of plants grown in control solution. Bioconcentration factor (BCF) as: nickel concentration in the harvested plant material (mg $Kg^{-1}$)/nickel concentration in the nickel-spiked solution (mg $Kg^{-1}$). Translocation factor (T*f*) as: nickel concentration in the shoots (mg $Kg^{-1}$)/nickel concentration in the roots (mg $Kg^{-1}$)*100. Data were subjected to ANOVA and Duncan's test was used to separate the means.

## Results and Discussion

*A. paniculatus* plants showed a good tolerance to Ni, evaluated by the T*i* (Fig.1). T*i* was higher than 0,8 at 25 µM $NiCl_2$, decreasing at 50 and 100 µM $NiCl_2$. A marked reduction of T*i* was observed in plants exposed at 150 µM Ni-spiked growth solution, with a value lower than 0.4.

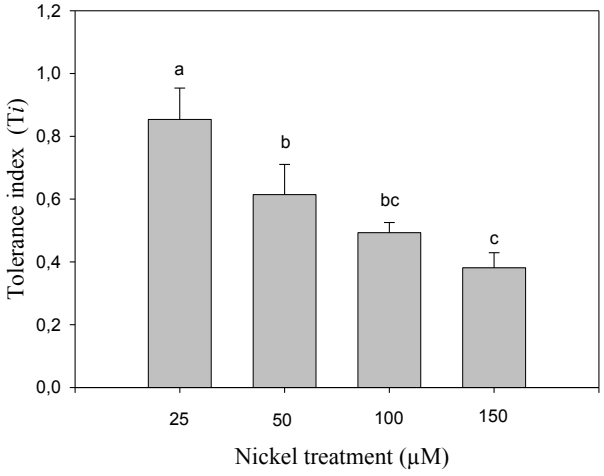


**Fig. 1.** Tolerance index of *A. paniculatus* plants grown in hydroponics for one week in Hoagland solution spiked with $NiCl_2$ at different concentrations (±S.E., n=6). Bars with the same letter are not significantly different (P≤ 0.01, Duncan's test).

These results are in line with the Ni concentration detected in plants. In fact, an increase of metal concentration was observed in the plant organs as Ni concentration in the growth solution enhanced (Tab.1).

| $NiCl_2$ (µM) | Ni concentration | | |
|---|---|---|---|
| | Root | Stem | Leaf |
| 0 | 14 (±3)$^{d}$ | 13 (±3)$^{d}$ | 11 (±3,8)$^{b}$ |
| 25 | 614 (±41)$^{c}$ | 54 (±3)$^{cd}$ | 49 (±3,5)$^{b}$ |
| 50 | 1036 (±158)$^{b}$ | 99 (±7,5)$^{c}$ | 34 (±9,1)$^{b}$ |
| 100 | 1672 (±191)$^{a}$ | 287 (±86)$^{b}$ | 38 (±10)$^{b}$ |
| 150 | 1928 (±95)$^{a}$ | 675 (±41)$^{a}$ | 120 (±21)$^{a}$ |

**Table 1.** Nickel concentration (mg $Kg^{-1}$ D.W.) in the organs of *A. paniculatus* plants grown in hydroponics for one week in Hoagland solution spiked with $NiCl_2$ at different concentrations (±S.E., n=3). Within columns, values with the same letter are not significantly different (P≤0.01, Duncan's test).

In particular, the highest Ni concentrations were detected in the roots of plants exposed to 100 and 150 µM $NiCl_2$, but a remarkable Ni concentration was also measured at 50 µM $NiCl_2$. In stems, Ni concentration was particularly high in plants treated with 150 µM $NiCl_2$. Lower Ni concentrations were found in 50 and 100 µM Ni-treated plants. In leaves, a higher Ni concentration was found in plants exposed to 150 µM $NiCl_2$ in comparison with plants treated with the other Ni concentrations. To evaluate the ability of plants to concentrate Ni from the external solutions in their tissues, the bioconcentration factor (BCF) was calculated. As shown in Fig. 2, a decreasing trend of BCF related to total plant was evidenced as Ni concentrations in the external solutions increased. This is in accordance with data reported by Galardi et al. (2006) in *Alyssum bertolonii*, a Ni-hyperaccumulating plant, exposed under hydroponics to similar Ni concentrations. An ability to concentrate Ni more than 30 fold from the nutrient solution was observed in *A. paniculatus* plants exposed to 25 and 50 µM $NiCl_2$. However, this value is on average near ten fold lower than that reported in Ni-hyperaccumulating plants (Galardi et al., 2007).

The capability of *A. paniculatus* plants to remove Ni from the Ni-spiked growth solution was followed along the experimental time interval. A different Ni phytoremoval trend was exhibited by *A. paniculatus* plants depending on the Ni concentration in the growth solution (Fig. 3). In accordance with the tolerance responses, plants exposed 25 µM $NiCl_2$ exhibited a higher and more constant Ni removal ability along time compared to plants exposed to 50, 100 and 150 µM $NiCl_2$, succeeding in removing more than 65% of the initial Ni content of the growth solution. Lower removal

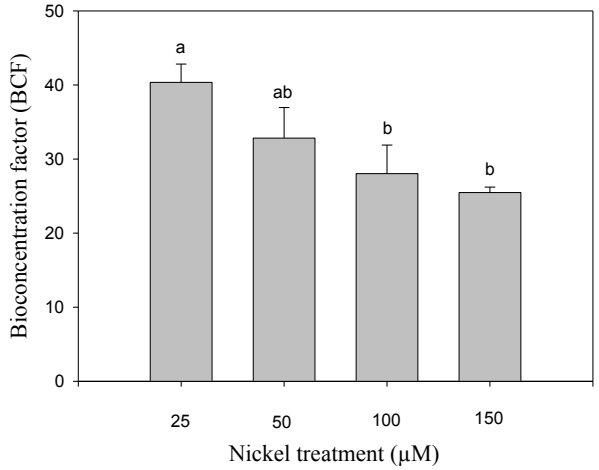


**Fig. 2.** Bioconcentration factor (BCF) in *A. paniculatus* plants grown in hydroponics for one week in Hoagland solution spiked with $NiCl_2$ at different concentrations (±S.E., n=6). Bars with the same letter are not significantly different (P≤0.01, Duncan's test).

abilities were found in plants treated with 50 and 100 μM $NiCl_2$.

Plants exposed to 150 μM $NiCl_2$ showed the lowest Ni removal ability, being the Ni content at the end of the treatment period near 70% of the initial one. Moreover, these plants exhibited a strong reduction of Ni removal ability just after two days from the start of the Ni treatments, evidencing metabolic disturbances exerted by Ni to root uptake processes.

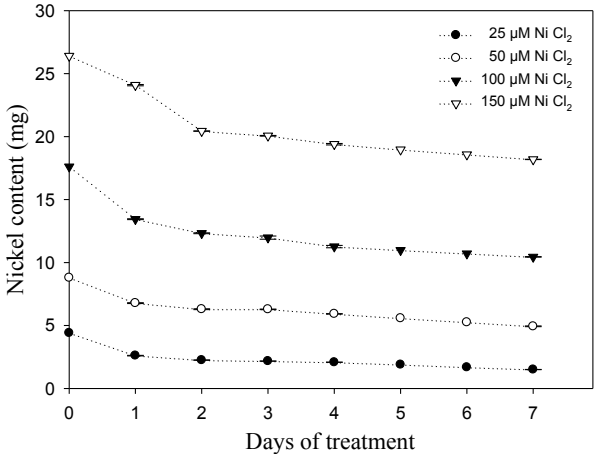


**Fig. 3.** Ni removal ability of *A. paniculatus* plants grown in hydroponics for one week in Hoagland solution spiked with $NiCl_2$ at different concentrations (±S.E., n=6).

The absorbed metal can be translocated from roots to the aerial plant organs trough the xylem fluid, commonly bound to metal chelating compounds. To measure the capability of plants to transfer the absorbed metals to the shoots, the translocation factor (T*f*) was applied.

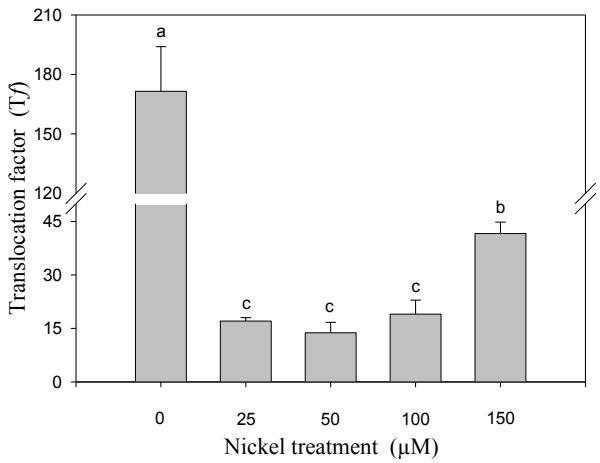


**Fig. 4.** Translocation factor (T*f*) in *A. paniculatus* plants grown in hydroponics for one week in Hoagland solution spiked with $NiCl_2$ at different concentrations (±S.E., n=6). Bars with the same letter are not significantly different (P ≤ 0.01, Duncan's test).

Data in Fig. 4 showed a remarkable higher T*f* in control plants, where Ni concentration in roots depended only on the seed supply, compared to that of plants treated with different Ni concentrations. This result was consistent with Galardi et al. (2007), even if in that work the ratio between T*f* of control plants and that of Ni-treated plants was far lower than that found in our experiment. Among Ni-treated plants, a higher T*f* was calculated in plants exposed to 150 μM $NiCl_2$. The T*f* values observed for *A. paniculatus* in this experiment are notable lower than those calculated from data reported by Galardi et al. (2007) in different ecotypes of *Alyssum bertolonii*, a Ni-hyperaccumulator plant, exposed to Ni in similar experimental conditions. In control plants, the highest T*f* can be ascribed to the physiological Ni demand of shoots to sustain leaf metabolic functions, such as enzyme activities and photosynthetic reactions. On the contrary, in 150 μM Ni-treated plants, a higher T*f* can be associated to an impairment of the metabolic processes regulating metal transport, due to the extremely high Ni concentration in the roots. The lower T*f* observed in plants exposed to 25, 50 and 100 μM $NiCl_2$, unrelated to the metal concentration in the growth solution, can be attributed to a tolerance response aiming at reducing metal presence in leaf cells, preserving physiological functions (Seregin and Kozhevnikova, 2006).

## Conclusion

*A. paniculatus* plants showed a different capability to tolerate, remove and accumulate Ni, depending on the metal concentrations of the growth solution. Results evidenced that, up to a concentration of 50 μM $NiCl_2$ in the growth solution (a Ni concentration near 150 fold higher than that allowed in waters by Italian law), plants can maintain adequate physiological functions, allowing to accumulate remarkable amounts of Ni in their tissues

while tolerating them. Although the Ni bioconcentration potential expressed by this plant species was far lower than that reported for Ni-hyperaccumulation plants, the good ability to remove Ni from contaminated solutions, especially at low Ni concentrations, represents a valuable characteristic to exploit for the utilisation of this plant species for the decontamination of Ni-polluted waters.

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