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Phytoremediation of Nickel-Contamination Using *Helianthus annuus* L. in Mediterranean Conditions

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Abstract

Nickel contamination poses a serious risk to ecosystems and human health. Phytoremediation provides a sustainable solution. This study evaluates the ability of *Helinathus annuus* L. to tolerate and accumulate nickel under simulated Mediterranean and semi-arid conditions, representing a short-term contamination event with nickel-enriched irrigation. Laboratory experiments assessed growth, tolerance, and Ni distribution within plant tissues. Results showed that Ni uptake increased with concentration, mainly in roots, while translocation to aerial parts remained limited. The bioconcentration factors ranged from 1.32 to 2.55, and the translocation factors from 0.46 to 0.60, indicating efficient uptake but restricted metal mobility. Higher water availability enhanced Ni absorption, suggesting that soil moisture facilitates metal transport and root activity. *Helinathus annuus* L. demonstrated good tolerance at moderate Ni levels but reduced growth and accumulation efficiency at higher concentrations, confirming its potential for phytostabilization in Mediterranean soils affected by metal contamination.

Keywords: nickel contamination; phytoremediation; *H. annuus*; nature-based solutions

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1. Introduction

Environmental contamination has increased over the last 200 years due to industrial activities, fossil fuel combustion, agriculture, and mining [1,2]. Heavy metals, produced through processes such as metal smelting, coal burning, and industrial emissions, are widespread pollutants [3–5]. The soil that is essential for plant growth and ecosystem health, can be negatively affected by contaminants from industrial, agricultural, or improper waste disposal sources, impacting both ecosystems and human health [6,7]. Unlike natural contaminants, heavy metals are not biodegradable and tend to accumulate in soils [8].

Nickel (Ni) is a heavy metal with rust-resistant properties used in many different industries, including making stainless steel, electroplating, and batteries. Ni can be released from human activities emitted as oxides, sulfides, soluble compounds, and to a lesser extent, as metallic Ni, the biggest source of Ni particles in the air is from burning fuels like oil and coal [9]. Ni has the capacity to bioaccumulate within the soil and water

environments, exerting deleterious effects on ecosystem health and biodiversity, for this reason Ni contamination represents a significant hazard to both the environment and human health [10–12]. Its toxicity has been demonstrated to disrupt biological processes in plants and animals, resulting in reduced growth rates, reproductive issues, and even mortality in certain species [13].

Elevated concentrations of Ni have been observed to induce chlorosis and necrosis in plant tissue; this phenomenon can be caused by the disruption of iron uptake and metabolism within the plant organism [14]. The presence of Ni in plants has been demonstrated to have a negative effect on growth, which is evidenced by the inhibition of root growth and the disruption of essential processes such as photosynthesis and nutrient uptake [15]. The alteration of chloroplast structure, the impairment of enzyme activities, and the induction of oxidative stress have been demonstrated to result in a reduction in crop yield [16]. The transportation of water is impaired, thereby exacerbating dehydration and the presence of Ni in soil has a direct impact on plant health, it also changes the chemical and physical composition of the substrate, reducing the nutrients available and affecting soil microflora causing environmental degradation and slowing down the natural regeneration of ecosystems damaging soil fertility [17,18].

The implementation of mitigation strategies, including phytoremediation, soil amendments, biochar addition, and microbial-assisted remediation, is essential to reduce Ni toxicity and safeguard plant health and productivity [13].

Furthermore, the presence of Ni in food chains has the potential to pose a threat to human health through the consumption of contaminated water and crops [17].

Additionally, Ni exposure has been linked to several health concerns in humans, the inhalation of Ni particles has been demonstrated to result in respiratory ailments, including asthma and bronchitis also Ni can be the cause of carcinogenesis, cardiovascular diseases, and neurological disorders [19,20].

Within the regulatory framework, European Directive 2004/107/EC and Italian legislation [21] establish explicit limits for the presence of heavy metals in soils and water. However, despite the existence of regulatory frameworks, the remediation of contaminated sites remains a complex challenge due to the high costs of conventional technologies, thus rendering phytoremediation an attractive alternative.

A variety of restoration technologies are available for the remediation of soils contaminated by heavy metals, phytoremediation has been shown to offer distinct advantages in terms of cost-effectiveness, environmental friendliness, and high public acceptance [22]. Phytoremediation, a technique employing plants to mitigate the adverse effects of heavy metals on the environment [23], encompasses various approaches, including phytoextraction, phytodegradation, phytostabilization, and phytovolatilization [24,25].

Phytoremediation is regarded as a promising eco-friendly method for mitigating pollution in contaminated sites. Although the potential of this approach is demonstrated by full-scale and pilot studies that also reveal certain disadvantages, including variable costs which are dependent on contaminant concentration and soil properties [26,27]. It is important to note that phytoremediation has been shown to be cost-effective, particularly in the context of organic contaminant removal. Additionally, there is a possibility that it may also contribute to the sequestration of CO_2 [28].

In the context of the exigency to mitigate climate change, it is important to contemplate the selection of plant species not solely for their remediation capacities, but also for their prospective contributions to carbon sequestration [29,30]. The strategic selection of species that possess the capacity for high carbon storage capacity can position phytoremediation as a dual-purpose solution, thereby addressing objectives related to both pollution remediation and climate change mitigation [31].

Hyperaccumulators are defined by their capacity for accelerated growth and brief biological cycles, often exhibiting the capability to accumulate metals or metalloids at levels that are hundreds to thousands of times greater than typical concentrations [32,33]. This attribute renders them a subject of considerable interest, as their inherent properties suggest a heightened capacity for efficient metal uptake and enhanced biomass production, thereby facilitating more straightforward harvesting procedures [34,35].

The capacity of a plant to accumulate heavy metals from contaminated soils is predominantly attributable to the behavior of the plant's root system upon exposure to the pollutant, indeed metal ions are known to enter the root symplast and are subsequently chelated by organic ligands, thus mitigating toxicity [36]. Under the alkaline conditions of the cytoplasmic matrix, amino acids are conducive to metal complexation [37]. Carboxylic acids are likely to be the predominant chelating agents for Ni in roots, particularly when elevated concentrations of Ni are retained within root vacuoles following extended cultivation periods [38].

One common metric to measure a plant's ability to clean up pollution is its translocation factor (TF). TF represents the ratio of Ni concentration in the aboveground plant parts to the Ni concentration in the root and rhizome system. It indicates how effectively a plant moves heavy metals from its roots to its leaves, which is crucial for cleaning up contaminated soil using phytoremediation [39]. Even though being a hyperaccumulator is highly advantageous for phytoremediation, a plant with rapid growth and significant biomass production could be suitable even with modest levels of hyperaccumulation.

Helinathus annuus L. is extensively utilized in Italy for its role in agricultural and energy sectors. Its seeds are harvested for agro-food consumption. *H. annuus* is a crucial raw material for biofuel production, contributing to renewable energy sources [40–42]. *H. annuus* shows great promise for phytoremediation due to its rapid biomass production. Studies have demonstrated that sunflowers can effectively remove heavy metals from contaminated soils, making them a valuable tool for environmental cleanup [43–48]. Several studies have assessed the phytoremediation capacity of *H. annuus*, yielding favorable outcomes [49–51]. Phytoremediation abilities of Ni by *H. annuus* vary depending on the conditions. Evaluating these capacities under different conditions makes it possible to select the most suitable plant species for the phytoremediation of polluted soils, to assess the adaptability of plants to climate change, it is essential to consider not only their ability to decontaminate, but also their resilience to changes in environmental conditions [52–54]. The limited number of studies assessing the phytoremediation capacity of *H. annuus* under Mediterranean and semi-arid conditions highlights the need for further investigation.

The aim of this study is to assess the ability of *H. annuus* to accumulate and translocate Ni under Mediterranean and semi-arid water conditions, simulating acute contamination events caused by runoff of contaminated water. The present study aims to contribute to phytoremediation strategies by examining the uptake and distribution of Ni in *H. annuus*.

2. Materials and Methods

An experimental investigation was conducted under laboratory conditions to assess the efficacy of remediation for Ni-contaminated soils in two different hydraulic conditions, simulating a pollutant event due to Ni transport in an aqueous solution.

The plants used in the experiment were derived from seeds purchased from a commercial supplier, to simulate a simple and easily replicable operational approach to phytoremediation. Both growth and tolerance tests were carried out by measuring the entire plant at regular intervals of weeks. The seeds were germinated in a plate containing a thin layer of soil substrate, which was kept consistently moist by daily misting. After approximately three weeks, when the seedlings exhibited a sufficiently developed root system and stem to

allow transplantation, each plant was transferred into an individual pot. The experiment was conducted using a total of 18 pots, with one plant per pot, arranged in three replicates for each experimental condition.

The study began with a growth test, wherein the growth of *H. annuus* was evaluated within mediterranean climatic condition and semi-arid condition, a tolerance test for each hydraulic condition, and an evaluation of phytoremediation capacity for each hydraulic condition. This involved placing eighteen samples of plants in separate pots, each with a diameter of 0.25 m and a height of 0.20 m and 3 samples of control pot. Each pot had a horizontal surface area of 0.049 m^2 and a capacity of 0.01 m^3.

The substrate consisted of a calcareous soil typical of the Bari area (Italy), with a pH of 7.5, a bulk density of approximately 1.4 Mg/m^3, and carbon and nitrogen contents of about 1.5% and 0.5% of the dry weight (dw), respectively.

Tap water was used for irrigation, with bicarbonate levels of 269.0 mg/L, calcium at 30.70 mg/L, potassium at 27.80 mg/L, magnesium at 9.30 mg/L, nitrate (N) at 8.10 mg/L, phosphate (P) at 1.20 mg/L, and fluorides at 1.0 mg/L.

The experiment included two irrigation regimes: one based on a precipitation level of 600 mm/year, typical of Mediterranean conditions, and a second representing semi-arid conditions with 400 mm/year. Accordingly, a total of 18 pots were used, nine for each irrigation treatment, resulting in continuous irrigation rates of 1.644 mm/day (0.0805 L/day) and 1.096 mm/day (0.0536 L/day), respectively.

The tolerance test was conducted by irrigating *H. annuus* plants with Ni-enriched solutions prepared by dissolving $NiSO_4 \cdot 6H_2O$ in tap water. Ni contamination of the soil was induced gradually through daily irrigation over a 12-day period, aiming to reach final concentrations of 120 mg Ni/kg and 80 mg Ni/kg of dry soil.

The samples irrigated under 600 mm/y were named TM120 and TM80, indicating treatments under Mediterranean conditions with target concentrations of 120 mg Ni/kg and 80 mg Ni/kg, respectively, while those irrigated under 400 mm/y were named TD120 and TD80, representing treatments under semi-arid conditions with the same contamination targets. The corresponding control pots were labeled C600 for mediterranean conditions and C400 for dry conditions.

For the Mediterranean regime, the Ni concentrations in the irrigation water were approximately 497 mg/L and 331 mg/L to reach the 120 mg/kg and 80 mg/kg targets, respectively. Under semi-arid conditions, the corresponding concentrations were approximately 747 mg/L and 498 mg/L. These contamination levels were selected to evaluate the phytoremediation potential of *H. annuus* in relation to the Italian regulatory threshold for Ni in soils (120 mg Ni/kg, D) [21]. The contamination simulated a short-term pollution event, in which high Ni concentrations are introduced by runoff following intense rainfall in industrial areas, resulting in temporary exceedance of regulatory limits.

After 12 weeks from the beginning of the Ni contamination treatment, the plants were harvested and divided into roots, stems, and leaves, each part was washed with tap water to remove soil particles and other debris. The tissues were cut into small fragments and dried at 70 °C until constant weight, afterwards, the dried material was triturated to a particle size of approximately 0.2 mm and homogeneously mixed, to obtain a fine and uniform powder that ensures representative subsampling and improves the efficiency of the subsequent chemical digestion. Finally, 0.50 g of the prepared plant material was placed in a desiccator to prevent moisture uptake from the atmosphere and to maintain a stable dry weight prior to analysis.

The soil samples were initially treated with a mixture of concentrated nitric acid (HNO_3) and hydrochloric acid (HCl) in a closed system equipped with a quartz power system, utilizing a 7:1 ratio. The mixture was contained within a sealed fluorocarbon

polymer bottle with a fume extraction system. The resulting liquid was diluted to 50.0 mL in acid-cleaned vials. For the analysis of Ni, dried soil samples (2 g) were digested with aqua regia (a mixture of concentrated HNO_3 and HCl in a 1:4 ratio) in 100.0 mL digestion tubes covered by a funnel.

Digestion occurred at 155 °C until approximately 5 mL remained in the tube. This process was repeated with an additional 25 mL of aqua regia. The resulting solution was filtered using 10 µm membrane filters, and the filtrate was diluted to 25.0 mL with deionized and distilled water for Ni analysis.

Throughout the experiments, environmental conditions such as light, atmospheric humidity, and air temperature were maintained at controlled levels. Nickel concentrations in plant tissues were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES), which was also used for the analysis of soil samples [55,56].

To evaluate the plant's capacity to accumulate nickel from the soil, the Bioconcentration Factor (BCF) was calculated as the average between the Ni concentration of all tissues and the Ni concentration in the soil (dry weight basis). To assess the movement of Ni from roots to aboveground tissues, the Translocation Factor (TF) was calculated as the ratio between the Ni concentration in the whole aerial biomass (stems + leaves) and the Ni concentration in roots [39].

$$\mathrm{BCF} = \frac{[[\mathrm{Ni}]_{\mathrm{stem}} + [\mathrm{Ni}]_{\mathrm{root}} + [\mathrm{Ni}]_{\mathrm{leaf}}]/3}{[\mathrm{Ni}]\mathrm{soil}} \quad \mathrm{TF} = \frac{[\mathrm{Ni}]_{\mathrm{stem}} + [\mathrm{Ni}]_{\mathrm{leaf}}}{[\mathrm{Ni}]_{\mathrm{root}}}$$

This expression provides an integrated measure of metal transfer to aboveground tissues and reflects the plant's overall translocation efficiency under the applied nickel contamination treatments.

To validate the results obtained, statistical analyses were conducted to assess the effects of pollutant concentration and plant organ on Ni accumulation in *H. annuus* under each irrigation regime. Two-way ANOVAs were performed separately for the 600 mm/y and 400 mm/y conditions, both for Ni concentrations and for the total Ni mass accumulated in plant tissues. For bioconcentration (BCF) and translocation (TF) factors, a two-way ANOVA including water regime and treatment was applied. When significant effects were detected, Tukey's post hoc tests were used to identify pairwise differences. All analyses were performed in R (version 2024.12), with statistical significance set at $p < 0.05$.

3. Results and Discussion

The phytoremediation capacity was evaluated by analyzing Ni accumulation in plant tissues. The average dry biomass of plants roots grown under mediterranean conditions was 0.22 kg (SD = 0.02) of TM120 plants, 0.23 kg (SD = 0.02) in TM80 plants, and 0.26 kg (SD = 0.02) in the control C600. The stem biomass reached 0.1 kg (SD = 0.01) in TM120, 0.15 kg (SD = 0.01) in TM80, and 0.18 kg (SD = 0.01) in C600, while the leaf biomass was 0.1 kg (SD = 0.01), 0.14kg (SD = 0.01), and 0.15 kg (SD = 0.01) for TM120, TM80, and C600, respectively. Under semi-arid conditions (400 mm y^{-1}), root dry biomass amounted to 0.18 kg (SD = 0.01) for TD120, 0.18 kg (SD = 0.01) for TD80, and 0.19 kg (SD = 0.01) for the control C400. Stem biomass was 0.1 kg (SD = 0.01) in TD120, 0.13 kg (SD = 0.01) in TD80, and 0.15 kg (SD = 0.01) in C400, whereas leaf biomass reached 0.09 kg (SD = 0.01), 0.1 kg (SD = 0.01), and 0.14 kg (SD = 0.01) for TD120, TD80, and the control, respectively.

The growth test shown in Figure 1, that consisted in evaluating the plants under stable and contamination-free conditions, in order to compare their development under the two different irrigation regimes, showed a total height increase of approximately 26 cm (ds 2.2) for plants irrigated under the 600 mm/y regime and 23 cm (ds 1.8) under the 400 mm/y regime over a 12-week period. These correspond to average growth rates of 3.13 cm/week and 2.66 cm/week, respectively. The linear regression for the 600 mm/y regime followed

the equation y = 0.0972x + 2.4948 (R^2 = 0.6464), while that for the 400 mm/y regime was y = 0.0479x + 2.3457 (R^2 = 0.2541).

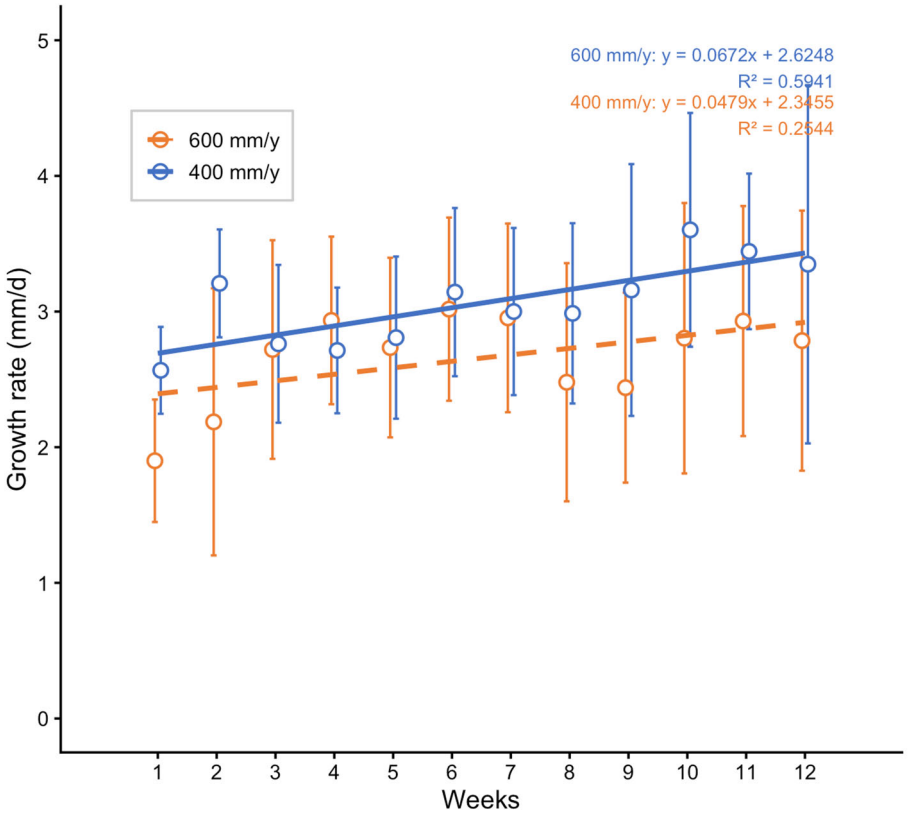

Figure 1. Weekly growth rate of *H. annuus* grown under 400- and 600 mm y^{-1} water regimes. Points represent means ± standard deviation of nine replicates. Linear regression lines illustrate the temporal growth trends for each irrigation level.

To compare the growth rates of the two irrigation regimes, the slope of weekly growth for each plant was calculated, and a *t*-test was applied to the slopes. The comparison yielded no significant differences between treatments ($p > 0.05$). This finding suggests that the increase in water availability did not have a substantial impact on the overall growth rate. However, it is evident from Figure 1 that the growth of the samples cultivated under the 400 mm/y water regime is lower. This result demonstrates the adaptability of *H. annuus* to more severe water conditions, although it was carried out under laboratory conditions that do not consider numerous external variables.

The higher slope and stronger correlation observed under 600 mm/y could indicate a more stable and sustained growth trend, suggesting that greater water availability favored vegetative development, instead the reduced growth rate and lower R^2 value under 400 mm/y reflect a more variable response to limited water supply, consistent with the physiological stress typically induced by semi-arid conditions. Plant growth is still evident, despite the more severe water conditions so this highlight how *H. annuus* can resist even in semi-arid conditions.

Figure 2 illustrates the tolerance response of *H. annuus* to Ni contamination under the two irrigation regimes. Under mediterranean water availability (600 mm/y), growth rates progressively declined with increasing Ni concentration. Control plants (C600) showed the highest and most stable growth throughout the experiment, exhibiting a slightly positive trend (y = 0.0658x + 3.4019; R^2 = 0.777). Plants samples treated with 80 mg Ni/kg (TM80) maintained intermediate values and a nearly stable growth pattern (y = −0.031x + 3.4076; R^2 = 0.314), while those exposed to 120 mg Ni/kg (TM120) showed a marked and continu-

ous decline in growth rate ($y = -0.1672x + 3.3247$; $R^2 = 0.887$). This decline became evident after week 4 and is likely attributable to cumulative Ni toxicity, which is known to impair photosynthetic efficiency and nutrient assimilation in metal-stressed plants [15].

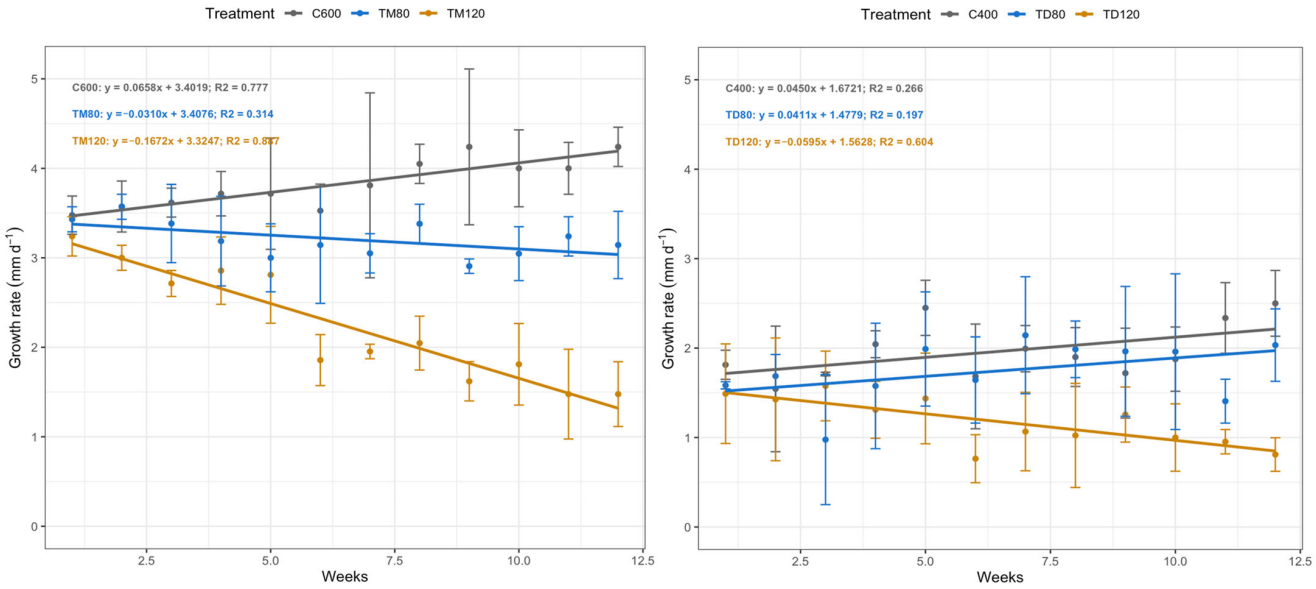

Figure 2. Tolerance test showing growth of *H. annuus* exposed to 0, 80, and 120 mg Ni/kg under 600 and 400 mm/y irrigation. Points show weekly means (n = 3) ± SD, with linear regression lines for each treatment. Statistical analyses on growth slopes are reported in the text.

Under semi-arid conditions (400 mm/y), all treatments displayed lower growth rates compared to the Mediterranean regime, reflecting the additional limitation imposed by reduced water availability. The control samples (C400) still showed the highest growth rate and a weakly positive trend ($y = 0.045x + 1.6721$; $R^2 = 0.266$), whereas TD80 plants exhibited a nearly constant but low growth ($y = 0.0411x + 1.4779$; $R^2 = 0.197$). The TD120 treatment experienced the strongest inhibition ($y = -0.0595x + 1.5628$; $R^2 = 0.604$), suggesting a synergistic effect between Ni stress and water deficit that further reduced the plant's vegetative performance.

To evaluate the combined effect of water availability and Ni concentration on growth dynamics, a two-way ANOVA was performed using the growth slope calculated for each plant over the 12-week interval as the response variable. The analysis showed that both pollutant concentration and water flow rate have a significant influence on growth slope. In particular, the ANOVA revealed a highly significant main effect of concentration ($p < 0.05$), indicating that an increase in concentration leads to a significant reduction in growth rate. Water flow also showed a significant effect ($p < 0.05$), with higher average values under the 600 mm/y treatment compared to 400 mm/y. Post hoc analyses (Tukey's test) conducted confirmed significant differences between concentrations, furthermore, at 400 mm/y, slopes were significantly lower in the TD120 treatment than in both the control ($p < 0.05$) and the TD80 treatment ($p < 0.05$).

The behavior of the two regimes confirms that adequate water availability mitigated the negative impact of Ni contamination, promoting higher growth rates and partially compensating for metal-induced stress.

Factors influencing *H. annuus* tolerance to Ni include genetics, uptake capacity, and detoxification mechanisms; however, it seems that the correspondence between tolerance and Ni pollution suggests higher stress at elevated levels, possibly affecting growth

and metabolism [17]. Further research is needed to understand these mechanisms and their implications.

As shown in Figure 3, Ni concentrations measured in plant tissues after 12 weeks revealed a consistent distribution pattern across both irrigation regimes, with the highest accumulation occurring in roots, followed by stems and leaves. Under Mediterranean conditions (600 mm/y), plants exposed to 120 mg Ni/kg (TM120) accumulated on average 503 mg Ni/kg dw in roots, 194 mg Ni/kg dw in stems, and 109 mg Ni/kg dw in leaves, whereas at the lower contamination level (TM80) the corresponding values were 375 mg Ni/kg dw, 129 mg Ni/kg dw, and 59 mg Ni/kg dw, respectively. Under semi-arid conditions (400 mm/y), the same decreasing trend from roots to aerial parts was observed, with overall lower concentrations. Plants treated with 120 mg Ni/kg (TD120) accumulated 392 mg Ni/kg dw in roots, 155 mg Ni/kg dw in stems, and 61 mg Ni/kg dw in leaves, while those treated with 80 mg Ni/kg (TD80) showed 254 mg Ni/kg dw, 106 mg Ni/kg dw, and 49 mg Ni/kg dw, respectively.

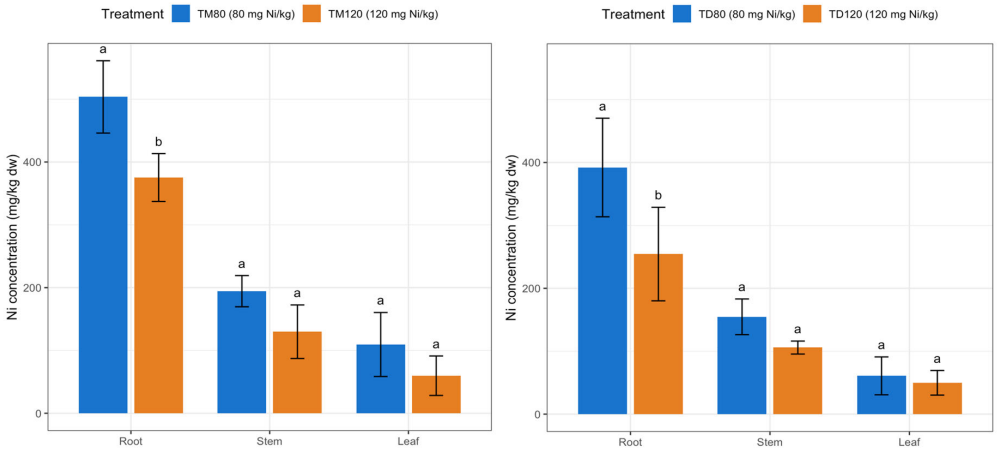

Figure 3. Nickel concentration (mg kg^{-1} dw) in plant tissues (roots, stems, and leaves) after 12 weeks under two irrigation regimes. The (**left**) plot shows plants grown under Mediterranean conditions (600 mm/y), and the (**right**) shows plants grown under dry conditions (400 mm/y). Values represent the mean of three biological replicates ± SD. Letters indicate statistically significant differences among treatments within each tissue ($p < 0.05$).

The two-way ANOVA revealed a highly significant effect of tissue type ($p < 0.001$) and a significant effect of treatment concentration ($p < 0.05$) under both irrigation regimes, whereas the interaction between the two factors was not significant ($p > 0.05$). This indicates that Ni accumulation differs markedly among tissues and increases with the applied Ni concentration, independently of the water regime. Post hoc analyses (Tukey's test) confirmed that roots accumulated significantly more Ni than stems and leaves under all irrigation conditions.

Within the dry regime (400 mm/y), TD120 roots accumulated significantly more Ni than TD80 roots ($p < 0.05$), whereas no differences were detected in stems or leaves ($p > 0.05$). A similar outline was observed under Mediterranean conditions (600 mm/y): TM120 roots showed significantly higher Ni concentrations than TM80 ($p < 0.05$), while stems and leaves did not differ between treatments.

Ni accumulation depended strongly on plant tissue, being highest in roots, intermediate in stems and lowest in leaves, and increasing consistently with the applied contamination level. Across all treatments, Ni remained predominantly restricted to the roots, confirming the limited translocation of the metal to above-ground organs in *H. annuus*.

H. annuus therefore demonstrated effective tolerance to Ni and maintained consistent accumulation in the root under both moisture conditions, supporting its potential application for phytostabilization in Mediterranean environments subject to dry period.

The total amount of Ni accumulated in the plant tissues, as illustrated in Figure 4, confirmed the distribution trends observed in the concentration data. It was evident that the majority of Ni was retained in the root system under both irrigation regimes. In the context of Mediterranean conditions (600 mm/y), plants exposed to elevated levels of contamination (TM120) exhibited a significant accumulation of nickel (Ni) in their roots, reaching approximately 109.59 mg, in contrast to the 69.80 mg observed in plants exposed to TM80. A significantly lower quantity was recorded in the aerial part, with values of 21.15 mg and 19.9 mg being recorded in stems and 10.92 mg and 8.5 mg being recorded in leaves for TM120 and TM80, respectively.

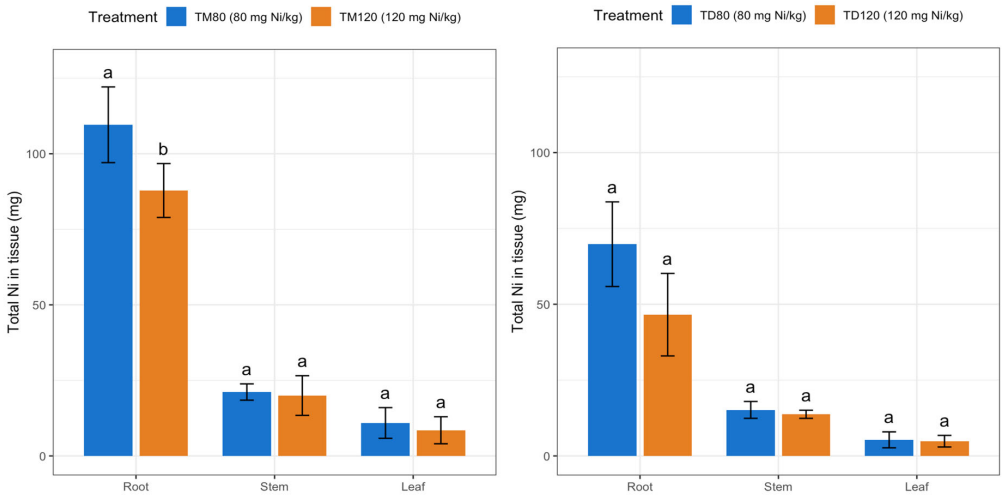

Figure 4. Total amount of Ni accumulated in plant tissues (roots, stems, and leaves) of *H. annuus* after 12 weeks under two irrigation regimes. The (**left**) plot refers to Mediterranean conditions (600 mm/y), while the (**right**) refers to dry conditions (400 mm/y). Values represent the mean of three biological replicates per treatment ± SD. Letters indicate statistically significant differences among treatments within each tissue ($p < 0.05$).

Under semi-arid conditions (400 mm/y), total Ni uptake decreased. Plants from treatment TD120 accumulated around 68.8 mg of Ni in roots and 15.18 mg in stems, while TD80 reached 46.57 mg and 13.73 mg. In the leaves, the total Ni content was limited to 5.3 mg for TD120 and 4.8 mg for TD80.

The analysis of variance showed a highly significant effect of tissue type under both irrigation regimes ($p < 0.001$), confirming that roots retained the largest Ni fraction compared with stems and leaves. The effect of treatment was significant at 600 mm/y ($p < 0.05$) and approached significance at 400 mm/y ($p = 0.050$), while the interaction between tissue and treatment was not significant in either regime ($p > 0.05$), indicating that the pattern of Ni partitioning among tissues remained consistent across treatments.

Tukey's post hoc test confirmed that roots accumulated significantly more Ni than stems and leaves in both regimes ($p < 0.001$). Regarding treatment effects, TM120 plants showed significantly higher total Ni uptake than TM80 in the 600 mm/y regime ($p < 0.05$), while the difference between TD120 and TD80 under 400 mm/y irrigation approached significance but did not exceed the 0.05 threshold ($p = 0.050$). No significant differences between treatments were detected in stems or leaves in either irrigation regime, supporting the observation that Ni translocation from roots to shoots remained limited.

In relation to the preceding findings concerning the data from Figure 3, it can be stated that an increase in environmental Ni concentrations is accompanied by a corresponding increase in Ni uptake and accumulation within *H. annuus* tissues. The roots exhibit the highest Ni concentrations, as they serve as the primary site of uptake from the soil, followed by the stems and then the leaves. This behavior is consistent with that exhibited by sunflowers in general, which are capable of absorbing and storing heavy metals from their surroundings [57–60] The translocation mechanism in plants frequently serves as a detoxification process by chelating heavy metals within inactive sites, the primary storage site for excess Ni is evidently within the plant roots, a trend consistently observed in similar previous studies [61–63].

Figure 5 reports the translocation factors (TF) and bioconcentration factors (BCF) calculated for *H. annuus* under the two irrigation regimes, showing clear differences associated with both water availability and Ni concentration. Under Mediterranean conditions (600 mm y^{-1}), the mean TF values were 0.60 (SD= 0.1) for TM120 and 0.50 (SD= 0.1) for TM80, while the corresponding BCF values were 2.24 (SD= 0.1) and 2.35 (SD= 0.2), respectively. Under semi-arid conditions (400 mm y^{-1}), TF values were 0.55 (SD= 0.1) for TD120 and 0.62 (SD= 0.05) for TD80, and the corresponding BCF values were 1.69 (SD= 0.2) and 1.71 (SD= 0.3), respectively.

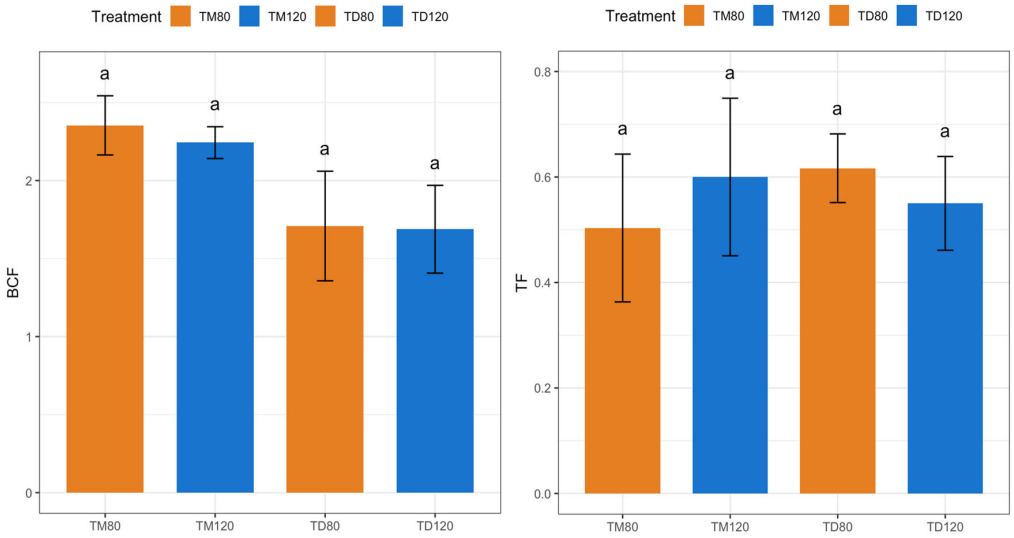

Figure 5. Bioconcentration factor (BCF) and translocation factor (TF) values for each treatment. Values are means ± SD. Different letters would indicate significant differences (Tukey, $p < 0.05$); however, no significant pairwise differences were detected for either BCF or TF, resulting in the same letter across treatments.

The two-way ANOVA performed on BCF revealed a significant effect of water regime, with plants grown under 600 mm y^{-1} showing higher BCF values than those under 400 mm y^{-1}. In contrast there is not significant effect of treatment concentration. Tukey's post hoc test indicated that, despite numerical differences, the four treatments formed statistically overlapping groups, with no treatment pair differing significantly at $p < 0.05$. In contrast, the translocation factor (TF) did not show significant differences between treatments or water regimes. TF values remained relatively stable across all experimental conditions, indicating that the capacity of *H. annuus* to move Ni from roots to aboveground organs is conservative and independent by water availability or Ni concentration in the soil.

Compering with previous literature, the TF values obtained in this study (0.46–0.58) are like the intermediate range. Jadia & Fuleka [42] reported considerably lower TF

values for Ni (0.14–0.24), suggesting reduced movement from roots to shoots, whereas Mohammadzadeh et al. [44] documented much higher TF values (up to 2.6). Similarly, De Bernardi et al. [51] found TF values between 0.84 and 2.04. Such discrepancies highlight that Ni translocation capacity may vary substantially due to differences in plant ecotypes, soil characteristics, or experimental conditions [51,53,64,65].

In this study, the moderately elevated BCF observed under the TM80 treatment indicates a more favorable physiological balance at moderate Ni levels, while TF remained low and stable across treatments.

Overall, *H. annuus* demonstrates a strong capacity to tolerate Ni and to retain it predominantly within the root compartment. Although its low TF values limit its potential for phytoextraction, the combination of high root Ni retention, physiological robustness, and consistent performance across irrigation regimes supports its suitability for phytostabilization in Mediterranean soils contaminated by Ni [51,66,67].

4. Conclusions

The study demonstrates that *H. annuus* shows a consistent growth in Mediterranean irrigation conditions and sufficient development even in semi-arid conditions. Also, *H. annuus* shows a moderate tolerance to nickel Ni contamination, with metal uptake increasing with Ni concentration, especially in the root system. The bioconcentration factors and translocation factor values indicating efficient root absorption but limited metal transfer to aerial parts. These results suggest that Ni accumulation is primarily confined to roots tissues, confirming a phytostabilization rather than phytoextraction behavior.

Water availability influenced both plant growth and metal dynamics, with higher BCF values and greater total Ni accumulation observed under Mediterranean irrigation (600 mm/y) compared to semi-arid conditions (400 mm/y). The reduction in both indicators at higher concentrations highlights a physiological limitation and a possible tolerance threshold between 80 and 120 mg Ni/kg. However, *H. annuus*, with its ability to grow even in unfavorable water conditions, has demonstrated resilience and effectiveness in Ni retention, confirming its potential use in phytostabilisation strategies for contaminated soils in Mediterranean environments and in semi-arid conditions.

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