RESEARCH ARTICLE **(Open Access)**

Time-Dependent Nickel Immobilization by Nano-Montmorillonite Clay in Serpentine Soils

TEUTA BUSHI [1*], AIDA BANI[2], FRANZ OTTNER[3]

[1] Department of Physics, Agricultural University of Tirana, Albania

[2] Department of Environment and Natural Resources. Agricultural University of Tirana, Tirana, Albania

[3]Institute of Applied Geology, Department of Civil Engineering and Natural Hazards, Austria

*Corresponding author: Teuta Bushi; teuta.halilaj@ubt.edu.al

Abstract

Serpentine soils in Albania are naturally enriched in nickel (Ni), which limits agricultural productivity due to Ni toxicity. This study aimed to evaluate the effectiveness of nano-montmorillonite clay in remediating Ni-rich soils using the nickel hyperaccumulator *Odontarrhena Chalcidica* as a test plant. The experiment was conducted in a greenhouse under a completely randomized design with three replications. Each experimental unit consisted of a 2 kg plastic pot. Four doses of nano-montmorillonite were tested: 0.0, 21.4, 43.4, and 64.0 g kg^{-1} (0%, 1.07%, 2.17%, and 3.2%), corresponding to field application rates of 0, 30, 60, and 90 t ha^{-1}, respectively. The effects of the treatments were evaluated at two time intervals: 60 days and 6 months. The main innovation of this study lies in the application of nano-clay technology for Ni immobilization and the assessment of its long-term efficiency. Results demonstrated a strong remediation effect of nano-montmorillonite. After 6 months, the application of 43.4 g kg^{-1} clay reduced water-extractable Ni by 77.6% compared to the control (from 8.50 mg kg^{-1} to 1.89 mg kg^{-1}). The highest dose (64.0 g kg^{-1}) further decreased the concentration to 1.64 mg kg^{-1}. In addition, the amendment significantly lowered Ni accumulation in plants and increased biomass production, confirming a substantial reduction in phytotoxicity.

Overall, nano-montmorillonite proved to be an effective and sustainable strategy for improving soil safety and agricultural productivity in Ni-contaminated serpentine soils, aligning with EU environmental standards

Keywords: clay, nano-montmorillonite, hyperaccumulator, *Odontarrhena chalcidica*, serpentine soil

1. Introduction

Globally, regions underlain by ultramafic bedrock give rise to serpentine soils, which are naturally enriched with high concentrations of nickel (Ni) [2]. These Ni-rich soils present a major challenge for agriculture due to the phytotoxic effects of excess Ni, which can inhibit plant growth, reduce biomass, and cause nutrient imbalances [13]. In many regions, including parts of the Balkans, these geogenic conditions restrict land use and agricultural productivity.

Clay minerals—particularly swelling types such as bentonite and montmorillonite—are recognized for their high cation exchange capacity (CEC) and large specific surface area, which enable strong adsorption and fixation of metal cations like Ni [7]. Supporting this, Kumararaja et al. (2016) demonstrated that the application of bentonite effectively reduced the uptake of heavy metals, including Ni, in edible crops, thereby mitigating potential health risks [19].

Recent advances in nanomaterials have opened new possibilities for improving the performance of such soil amendments. Nano-structured clays, such as nano-montmorillonite, exhibit enhanced reactivity and metal-binding capacity owing to their increased surface area and abundance of active sites [17, 18]. However, the long-term stability and field effectiveness of these nano-amendments, particularly their sustained capacity to reduce metal bioavailability—remain insufficiently explored.

This study aimed to evaluate the time-dependent immobilization of Ni in serpentine soils using locally

*Corresponding author: Teuta Bushi; teuta.halilaj@ubt.edu.al

(Accepted for publication 28.11.2025)

***ISSN**: 2218-2020,*

sourced nano-montmorillonite clay. The experiment investigated changes in Ni bioavailability and its accumulation in the nickel hyperaccumulator *Odontarrhena chalcidica* over two distinct periods: short-term (60 days) and long-term (6 months). The primary goal was to assess the durability and efficiency of nano-montmorillonite for potential long-term soil remediation.

2. Materials and Methods

2.1 Experimental Setup

A greenhouse experiment was conducted using a completely randomized design with three replications [16]. Each experimental unit consisted of a 2 kg plastic pot filled with serpentine soil collected from the Tropoja region (0–20 cm depth). The soil was characterized by a high total Ni content (1,739 mg kg^{-1}) and a neutral pH (7.0) [5, 15].

2.2 Treatments

Four doses of nano-montmorillonite clay (0.0, 21.4, 43.4, and 64.0 g kg^{-1}), corresponding to field application rates of 0, 30, 60, and 90 t ha^{-1}, were thoroughly mixed with the soil. The clay was sourced from the Domosdova field in Prrenjas, Albania, and identified as smectite-dominant nano-montmorillonite (90%) [7, 17].

2.3 Plant Cultivation and Sampling

Seeds of *Odontarrhena Chalcidica* were sown on March 1, 2024. After germination, plants were thinned to two per pot and irrigated regularly to maintain 60–70% field capacity. Fertilization was applied at a rate of 3 g per pot using a balanced NPK fertilizer (20:20:20). Soil and plant samples were collected at two time intervals: 60 days and 6 months after sowing [6, 16].

2.4 Analytical Methods

Soil Analysis

Water-extractable Ni was determined using a water-based extraction method. In addition, soil pH, organic matter content, cation exchange capacity (CEC), and water-extractable metals were analyzed following Ure et al 1993 [12].

Plant Analysis

Shoots and roots were separated, thoroughly washed, dried at 80°C to constant weight, and weighed to determine biomass. Nickel concentrations were measured using X-ray fluorescence (XRF).

Nickel uptake and translocation were assessed using the following indices [3, 6]:

Bioaccumulation Factor in Plant (BFP): Ratio of Ni concentration in the aerial parts to that in the soil.

Bioaccumulation Factor in Root (BFR): Ratio of Ni concentration in the roots to that in the soil.

2.5 Statistical Analysis

Regression analysis was performed to evaluate the relationship between clay dose and Ni concentration in both soil and plant tissues. Pearson correlation coefficients (r) were calculated to assess linear relationships among all measured variables [16].

Nickel Immobilization over Time

The application of nano-montmorillonite significantly reduced the concentration of water-extractable Ni in a dose-dependent manner at both sampling intervals (Figure 1). The immobilization effect increased over time, with all treatments showing lower Ni availability after 6 months compared to 60 days [17, 19].

The highest clay dose (64.0 g kg^{-1}) reduced water-extractable Ni by 78.9% after 60 days and by 84.7% after 6 months relative to the control [7, 10]. This progressive immobilization indicates not only immediate Ni adsorption but also stronger, long-term binding mechanisms—possibly due to aging effects and the gradual incorporation of Ni into the clay mineral structure.

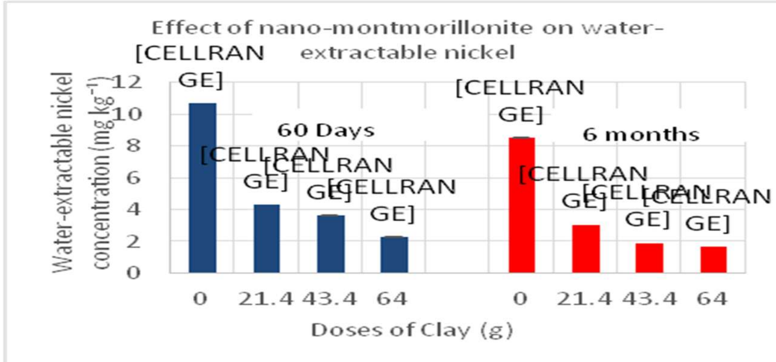

Figure 1. Effect of nano-montmorillonite amendment on water-extractable nickel concentration in serpentine soil after 60 days and 6 months. Values represent mean ± SD (n = 3). Different lowercase letters indicate significant differences between treatments within each sampling time ($p < 0.05$).

Plant Growth Response

The immobilization of nickel in the soil led to a marked improvement in plant growth and a reduction in metal-induced stress. The dry biomass of *Odontarrhena Chalcidica* shoots showed a significant positive correlation with increasing doses of nano-montmorillonite (Figure 2). After 60 days, shoot dry biomass increased from 3.1 g pot^{-1} in the control to 5.4 g pot^{-1} at the highest clay application rate (64.0 g kg^{-1}), representing a 74% enhancement. This positive growth response highlights the effectiveness of nano-montmorillonite in mitigating nickel phytotoxicity by decreasing its bioavailability in the soil solution.

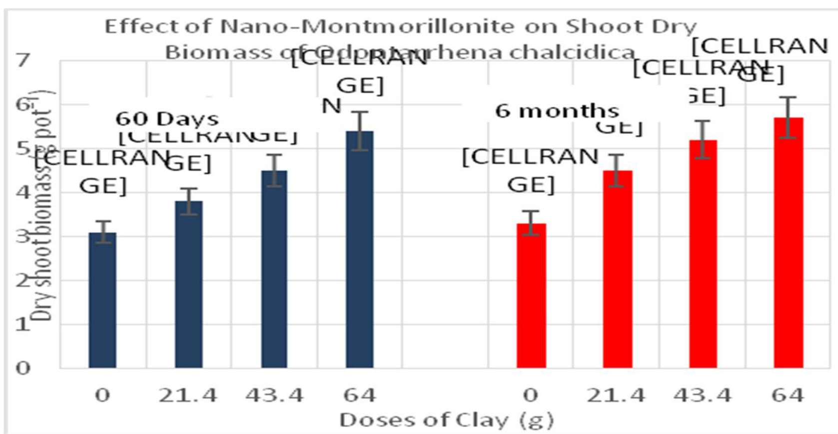

Figure 2. Dry shoot biomass of *Odontarrhena chalcidica* as influenced by different doses of nano-montmorillonite after 60 days and 6 months. Vertical bars represent ± SD (n=3). Different letters indicate significant differences among treatments ($p < 0.05$).

Bioaccumulation and Translocation Factors

To further evaluate the impact of the clay amendment on nickel phytoavailability, the Bioaccumulation Factor in Plant (BFP) and Bioaccumulation Factor in Root (BFR) were calculated (Table 1). Both BFP and BFR decreased significantly with increasing nano-montmorillonite application. For instance, at the 64.0 g kg^{-1} dose, BFP decreased by 31% and BFR by 25% compared to the control, indicating a strong reduction in nickel uptake efficiency. This supports the hypothesis that nickel was progressively immobilized in the soil matrix and rendered less accessible for plant absorption.

The Translocation Factor (TF)—the ratio of nickel in shoots to roots—also declined with higher clay doses, albeit remaining high overall. This indicates that while *O. Chalcidica* retains its inherent ability to translocate nickel to the shoots, the process is moderated in the presence of the clay, likely due to reduced nickel mobility in the rhizosphere.

*Corresponding author: Teuta Bushi; teuta.halilaj@ubt.edu.al
(Accepted for publication 28.11.2025)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

Table 1. Bioaccumulation Factor in Plant (BFP), Bioaccumulation Factor in Root (BFR), and Translocation Factor (TF) of nickel in last harvest of *Odontarrhena chalcidica* as affected by nano-montmorillonite application.

Clay Dose (g kg^{-1})	BFP	BFR	TF
0.0	9.00	1.52	7.10
21.4	7.85	1.38	6.45
43.4	6.95	1.25	6.02
64.0	6.17	1.14	5.89

Correlation Analysis

The relationships between all measured parameters were quantified using Pearson correlation analysis (figure 3). The clay application rate was the dominant factor, showing a very strong negative correlation with Ni concentration in shoots (r = -1.00), BFR (r = -0.999), and BFP (r = -0.997).

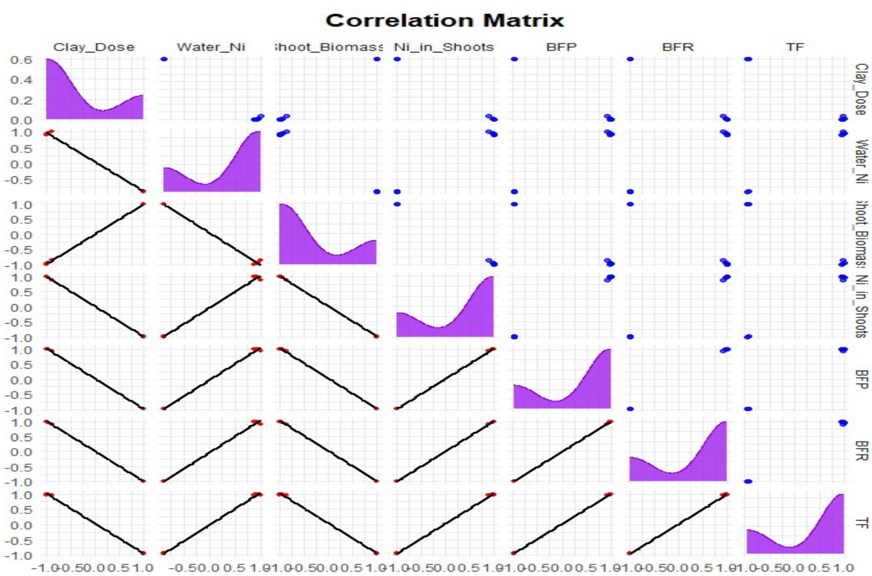

Figure 3. Pearson correlation matrix showing the relationships between clay dose, soil nickel availability, plant growth, and nickel uptake parameters.

This indicates that increasing the clay dose was strongly correlated with a decrease in nickel uptake and bioaccumulation. Soil pH showed a moderate positive correlation with clay dose (r = 0.89), suggesting that the amendment may also slightly influence soil pH over time. Plant biomass was positively correlated with clay application (r = 0.95) and negatively correlated with water-extractable Ni (r = -0.94), reinforcing that reduced nickel bioavailability directly improved plant growth.

3. Discussion

The results of this study clearly demonstrate that nano-montmorillonite clay is highly effective in reducing nickel bioavailability in serpentine soils, with its immobilization effect strengthening over the 6-month period. The pronounced decline in water-extractable Ni can be attributed to the high specific surface area and cation exchange capacity (CEC) of nano-montmorillonite, which enable strong chemisorption of Ni^{2+} ions [11, 8]. The additional decrease in Ni availability between 60 days and 6 months suggests the occurrence of aging processes, during which nickel becomes increasingly incorporated into the clay interlayers or forms inner-sphere complexes, thereby reducing its mobility and reversibility.

These findings are consistent with previous research on bentonite and other clay-based amendments [1, 3], but they underscore the superior performance of nano-structured montmorillonite. The simultaneous increase in plant biomass and reduction in tissue nickel concentrations indicate that the amendment effectively mitigated Ni phytotoxicity while maintaining sufficient metal availability to support the physiological functions of the hyperaccumulator. This balance is particularly important for applications in phytoremediation and the safe cultivation of plants in metal-enriched soils [4, 9].

From a practical standpoint, the use of locally sourced nano-montmorillonite represents a sustainable and economically viable strategy for managing nickel-rich serpentine soils in Albania and comparable regions [14]. Similar pedogenetic processes have been reported in Mediterranean serpentine soils, such as those in northwestern Italy [9], supporting the geogenic origin of nickel enrichment observed in Albanian soils. The demonstrated long-term stability of nano-montmorillonite in this study reinforces its potential for field-scale application, contributing to improved soil health, environmental safety, and agricultural productivity.

5. Conclusion

The application of nano-montmorillonite clay significantly and sustainably reduced nickel bioavailability in serpentine soil, with immobilization efficiency increasing over time. This resulted in markedly lower nickel uptake by *Odontarrhena chalcidica* and a corresponding increase in plant biomass, confirming the alleviation of nickel-induced phytotoxicity. Overall, nano-montmorillonite presents a promising and durable soil amendment for enhancing the safety and productivity of Ni-enriched agricultural soils.

6. References

1. Adriano DC: **Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals**. Springer, New York; 2001.
2. Alloway BJ: **Sources of heavy metals and metalloids in soils**. In: Alloway B (ed): **Heavy metals in soils**. Environmental Pollution 22. Springer, Dordrecht; 2013.
3. Baker AJM, Reeves RD, Hajar ASM: **Heavy metal accumulation and tolerance in British populations of the metallophyte Thlaspi caerulescens J. & C. Presl (Brassicaceae)**. New Phytologist 1994, **127**(1):61-68.
4. Bani A, Echevarria G: **Can organic amendments replace chemical fertilizers in nickel agromining cropping systems in Albania?** International Journal of Phytoremediation 2019, **21**(1):43-51.
5. Bani A, Echevarria G, Montargès-Pelletier E, Gjoka F, Sulçe S, Morel JL: **Pedogenesis and nickel biogeochemistry in a typical Albanian ultramafic toposequence**. Environmental Monitoring and Assessment 2014, **186**(7):4431-4442.
6. Bani A, Echevarria G, Mullaj A, Reeves R, Morel JL, Sulçe S: **Nickel hyperaccumulation by Brassicaceae in serpentine soils of Albania and northwestern Greece**. Northeastern Naturalist 2009, **16**(sp5):385-404.
7. Bhattacharyya KG, Gupta SS: **Adsorption of a few heavy metals on natural and modified kaolinite and montmorillonite: A review**. Advances in Colloid and Interface Science 2008, **140**(2):114-131.
8. Bonifacio E, Barberis E: **Phosphorus dynamics during pedogenesis on serpentinite**. Soil Science 1999, **164**(12):960-968.
9. Bonifacio E, Zanini E, Boero V, Franchini-Angela M: **Pedogenesis in a soil catena on serpentinite in north-western Italy**. Geoderma 1997, **75**(1-2):33-51.
10. Bradl HB: **Adsorption of heavy metal ions on soils and soils constituents**. Journal of Colloid and Interface Science 2004, **277**(1):1-18.
11. Chardot V, Echevarria G, Gury M, Massoura S, Morel JL: **Nickel bioavailability in an ultramafic toposequence in the Vosges Mountains (France)**. Plant and Soil 2007, **293**(1):7-21.
12. Ure AM, Quevauviller P, Muntau H, Griepink B: **Speciation of heavy metals in soils and sediments. An account of the**

improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. International Journal of Environmental Analytical Chemistry 1993, **51**:135-151.

13. Kazakou E, Adamidis GC, Baker AJM, Reeves RD, Godino M, Dimitrakopoulos PG: **Species adaptation in serpentine soils in Lesbos Island (Greece): Metal hyperaccumulation and tolerance**. Plant and Soil 2010, **332**(1-2):369-385.
14. Kidd PS, Bani A, Benizri E, Gonnelli C, Hazotte C, Kisser J, Echevarria G: **Developing sustainable agromining systems in agricultural ultramafic soils for nickel recovery**. Frontiers in Environmental Science 2018, **6**:44.
15. Shallari S, Schwartz C, Hasko A, Morel JL: **Heavy metals in soils and plants of serpentine and industrial sites of Albania**. Science of the Total Environment 1998, **209**(2-3):133-142.
16. Sokal RR, Rohlf FJ: **Biometry: The Principles and Practice of Statistics in Biological Research**. 4th edition. W.H. Freeman and Co., New York; 2012.
17. Uddin MK: **A review on the adsorption of heavy metals by clay minerals, with special focus on the past decade**. Chemical Engineering Journal 2017, **308**:438-462.
18. Wang L, Wang Y, Ma F, Tankpa V, Bai S, Guo X, Wang X: **Mechanisms and reutilization of modified biochar used for removal of heavy metals from wastewater: A review**. Science of the Total Environment 2019, **668**:1298-1309.
19. Yi X, Liang X, Yingming X, Xu Q, Huang Q, Lin W, Sun Y: **Remediation of heavy metal-polluted agricultural soils using clay minerals: A review**. Pedosphere 2017, **27**(2):193-204.