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NICKEL SPECIATION AND BIOAVAILABILITY IN SERPENTINE SOILS OF THE EASTERN AND SOUTHERN RHODOPES, BULGARIA

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Abstract

Serpentine soils in the Eastern and Southern Rhodopes, Bulgaria, exhibit elevated nickel (Ni) concentrations and distinct mineralogical characteristics, dominated by serpentine minerals (antigorite, lizardite) and the Ni-rich willemseite. This study assesses Ni speciation, mobility, and potential bioavailability by analysing soil physicochemical properties, iron oxide mineralogy, and selective chemical extractions targeting exchangeable, DTPA-extractable, and iron oxide-associated Ni fractions. Results indicate that Ni distribution depends on pedogenetic development: in moderately developed soils, Ni preferentially associates with amorphous and poorly crystalline iron oxides, while in more advanced soils, well-crystallised oxides stabilise Ni and limit its mobility. The Feo/Fed ratio reflects oxide crystallinity and pedogenic stage, serving as a key control on Ni behaviour. Multivariate analyses, including PCA and cluster analysis, reveal a pedogenetic continuum linking mineralogical inheritance, iron oxide transformation, and Ni fractionation. This framework enables the prediction of potential Ni bioavailability and highlights the importance of pedogenesis and secondary mineral phases in regulating Ni dynamics. The findings improve understanding of Ni behaviour in temperate ultramafic soils and provide a basis for ecological risk assessment and sustainable management of serpentine ecosystems.

Key words*: bioavailability, nickel (Ni), Rhodopes Mountains, serpentine soils, soil geochemistry.*

INTRODUCTION

Nickel (Ni) is widely distributed in the Earth's crust and plays an important role in biogeochemical processes in the soil environment. It is essential for plants and microorganisms, as it participates in the structure and activity of the enzyme urease and other key metabolic processes (Kabata-Pendias, 2011). At the same time, elevated nickel concentrations can be toxic, disrupting plant physiological functions, inhibiting nutrient uptake, and reducing soil microbial activity (Alloway, 2013). Therefore, understanding the factors controlling nickel distribution, mobility, and potential bioavailability in soils is crucial.

Nickel content strongly depends on the geological substrate. The highest concentrations are observed in soils formed on ultramafic and ultrabasic rocks such as peridotites, dunites, and serpentinites (Brooks, 1987). These rocks contain high levels of magnesium and heavy metals, including nickel, chromium, and cobalt. Weathering of these rocks leads to the formation of serpentine soils, characterised by high concentrations of potentially toxic elements, low Ca/Mg ratios, and limited content of essential nutrients (Alexander et al., 2007). The specific geochemical conditions of these soils strongly influence the plant and microbial communities typical of serpentine ecosystems.

The geochemical behaviour of nickel depends not only on its total content but also on its chemical forms. It can be adsorbed on clay minerals, associated with iron and manganese oxides, complexed with organic matter, or structurally incorporated into mineral lattices (Kabata-Pendias, 2011; Alloway, 2013). These forms differ in stability and mobility, determining their potential bioavailability to plants and their role in soil geochemical processes.

Quantitative assessment of metal mobility and bioavailability is commonly achieved using selective chemical extractions. Different extractants allow operational definition of metal fractions associated with specific soil components or characterised by varying mobility (McLean & Bledsoe, 1992). For example, the chelating agent diethylenetriaminepentaacetic acid (DTPA) extracts potentially bioavailable trace metal forms (Lindsay & Norvell, 1978), whereas neutral salts such as $Sr(NO_3)_2$ extract

exchangeable and weakly adsorbed forms. These approaches provide a more realistic assessment of the ecological significance of nickel compared to total concentrations.

Iron oxides also play a key role in nickel retention and distribution. They act as effective sorbents, limiting metal mobility and strongly influencing their stability (Alloway, 2013). Amorphous and poorly crystalline iron oxides can retain more labile nickel forms, while well-crystallised oxides stabilise the metal pool. Oxalate extraction targets nickel associated with amorphous and poorly crystalline Fe and Al oxides, while the sodium dithionite-citrate-bicarbonate (DCB) method dissolves free crystalline iron oxides (Mehra & Jackson, 1960; Schwertmann, 1964). Comparison of these fractions and the Fe_o/Fe_d ratio provide valuable information on soil development and nickel stability.

Serpentinites and ultramafic rocks are widespread across the Balkans, including Bulgaria. The Rhodope Massif is a key area with localised bodies of serpentinites and ultrabasic rocks. In the Eastern and Southern Rhodopes, these lithological features form soils with elevated nickel and other trace elements (Angelova & Kovacheva, 2023). However, information on nickel distribution among soil fractions and the influence of iron oxides on its potential bioavailability remains limited.

Investigating nickel speciation and potential bioavailability in the serpentine soils of the Rhodopes is crucial for understanding the processes controlling its behaviour. A combined approach integrating selective chemical extractions, soil physicochemical properties, and mineralogical composition provides a comprehensive, process-oriented interpretation of nickel dynamics.

This study aims to analyse nickel speciation, mobility, and potential bioavailability in serpentine soils of the Eastern and Southern Rhodopes, with a focus on the role of iron oxides, pedogenesis, and mineralogical composition. To achieve this, the study evaluates total nickel content, its spatial variability, distribution among different chemical fractions via selective extractions, the influence of iron oxides and their crystallinity, and the relationship between soil physicochemical and mineralogical properties and nickel's potential bioavailability. Multivariate statistical analyses are applied to identify pedogenetic and functional soil groups that reflect gradients in soil development and nickel bioavailability.

MATERIALS AND METHODS

Study area and sampling

The study was conducted in ultramafic regions of the Central and Eastern Rhodope Mountains, southern Bulgaria. The investigated sites included Kazak, Goljamo Kamenyane, Avren, Chernichevo, Kardzhali, Fotinovo, Dyulitsa, Dobromirtsi, Asenovgrad, Cherven, Parvenets, and Gornoslav. The region is characterized by a transitional Mediterranean-continental climate, contributing to considerable spatial variability in environmental conditions.

Surface soil samples (0-20 cm) were collected from each site. Prior to analysis, the samples were air-dried, homogenized, and sieved to <2 mm.

Physicochemical soil analysis

Soil physicochemical properties were determined using standardized analytical procedures. Soil pH was measured potentiometrically in water according to ISO 10390 (2021). Organic matter content was determined following BS EN 13039 (2011). Cation exchange capacity and exchangeable cations were determined using $BaCl_2$ extraction in accordance with ISO 11260 (1994).

Pseudo-total metal concentrations were determined after acid digestion following ISO 11466 (1995). Potentially available nickel was extracted using diethylenetriaminepentaacetic acid (DTPA) according to ISO 14870 (2001), while exchangeable nickel was obtained using 0.1 M $Sr(NO_3)_2$ extraction (van der Ent et al., 2019).

Iron oxide fractions were differentiated by selective chemical extraction. Amorphous iron oxides (Fe_o) were extracted using ammonium oxalate, whereas free iron oxides (Fe_d) were determined using the citrate-bicarbonate-dithionite method (Sparks, 1996). The crystalline iron oxide fraction (Fe_{cr}) was calculated as the difference between Fed and Feo. The Feo/Fed ratio was used as an indicator of pedogenic development and weathering

intensity. Elemental concentrations were measured using ICP-OES.

Mineralogical characterization

Mineralogical composition was analyzed by X-ray diffraction (XRD). Powder diffraction patterns were recorded over an angular range of 5-80° 2θ using Cu Kα radiation. Phase identification was performed using standard reference databases, and quantitative phase analysis was carried out by Rietveld refinement.

Statistical analysis

Differences among sampling sites were evaluated using one-way analysis of variance (ANOVA). Correlation analysis was applied to assess relationships between soil properties and the distribution of nickel fractions. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSIONS

Soil physicochemical properties and total nickel content

The main physicochemical characteristics of the serpentine soils from the Eastern and Southern Rhodopes are summarised in Table 1. Soil reaction varies within a relatively narrow range, from slightly acidic to slightly alkaline (pH 6.05-7.62), with a mean value of 6.84. Such pH conditions are typical for serpentine-derived soils and reflect the buffering effect of the Mg-rich ultramafic parent material (Brooks, 1987; Kabata-Pendias, 2011).

Organic matter content (LOI) is generally low to moderate (2.27-8.30%), characteristic of ultramafic soils with limited biological productivity and reduced potential for humus accumulation (Alexander et al., 2007). The cation exchange capacity (CEC) shows substantial variability (83.1-434.5 mmolc/kg), indicating differences in soil texture, mineralogical composition and degree of pedogenic development across the studied sites. Total Ni concentrations are high across all samples, ranging from 919.9 to 2397.2 mg/kg, with a mean of 1667.9 mg/kg. These values greatly exceed background concentrations typical for non-serpentine soils and clearly indicate a dominant geogenic control on Ni accumulation derived from the ultramafic parent material (Brooks, 1987; Alloway, 2013). Similar concentrations have been reported for serpentine soils into other ultramafic regions worldwide reflecting the structural incorporation of Ni in primary silicate minerals of the parent rock (Alexander et al., 2007; Bani et al., 2009).

Table 1. Descriptive statistics of physicochemical properties, iron oxides and nickel fractions in serpentine soils of the Eastern and Southern Rhodopes

Variable	Mean±SD	Min.	Median	Max.
pH	6.84±0.50	6.05	6.8	7.62
LOI, %	5.27±1.87	2.27	5.23	8.30
CEC, mmolc/kg	220.61±87.94	83.1	201.6	434.5
Exch. Ca, mmolc/kg	100.60±75.33	26.57	96.60	379.64
Exch. Mg, mmolc/kg	117.25±67.09	25.96	119.14	328.22
Exch. K, mmolc/kg	2.75±1.77	0.47	2.09	6.16
Mg/Ca	1.62±1.14	0.1	1.3	4.7
Fine sand (0.2–0.02 mm), %	30.50±14.43	7.01	29.63	62.65
Silt (0.02–0.002 mm), %	15.52±8.64	5.27	13.06	35.14
Clay (<0.002 mm), %	17.53±5.53	7.70	17.16	28.00
Physical clay (<0.02 mm), %	33.05±12.61	12.97	31.43	54.97
Fe_o (oxalate), mg/kg	4153.9±1543.4	1512.0	4166.3	6552.0
Fe_d (DCB), mg/kg	19392.2±10779.7	3318.8	16453.8	41096.3
Fe_o/Fe_d	0.30±0.27	0.09	0.23	1.27
Ni_o (oxalate), mg/kg	398.3±170.1	103.5	427.5	667.5
Ni_d (DCB), mg/kg	335.84±155.68	61.3	346.9	583.8
Ni_o/Ni_d	1.352±0.569	0.407	1.219	3.135
Ni ($Sr(NO_3)_2$), mg/kg	1.212±1.37	0.012	0.601	4.32
Ni (DTPA), mg/kg	46.96±33.11	6.08	40.16	125.28
Ni (total), mg/kg	1667.9±390.8	919.9	1603.9	2397.2
Fe (total), mg/kg	63298.1±13794.6	36302.4	64110.4	93194.5
Antigorite, %	60.55±20.27	19.0	66.0	86.0
Lizardite, %	1.55±4.99	0.0	0.0	20.0
Clinochlore, %	12.7±11.63	0.0	11.0	48.0
Willemseite, %	7.0±6.82	0.0	6.5	24.0
Quartz, %	9.45±11.77	1.0	8.0	55.0

Distribution of nickel among chemical fractions and indicators of mobility

Selective extraction results reveal a pronounced fractionation of nickel among the investigated soil phases (Figure 1). Boxplots show minimum, first quartile (Q1), median, third quartile (Q3), and maximum values for total Ni, oxalate extractable Ni (Ni–Ox; associated with amorphous Fe oxides). The potentially bioavailable fraction, determined by DTPA extraction, represents only a small proportion of the total Ni pool. Ni-DTPA concentrations vary widely (6.08-125.28 mg/kg), with an average of

46.96 mg/kg, indicating that despite the very high total Ni concentrations, its mobility and immediate bioavailability remain limited.

Nickel associated with amorphous and poorly crystalline iron oxides (Ni-oxalate) ranges from 103.5 to 667.5 mg/kg (mean 398.3 mg/kg). In contrast, nickel bound to free crystalline iron oxides (Ni-DCB) varies between 61.3 and 583.8 mg/kg (mean 335.8 mg/kg). This distribution indicates that a significant portion of Ni has been secondarily incorporated into Fe-oxide phases during pedogenesis.

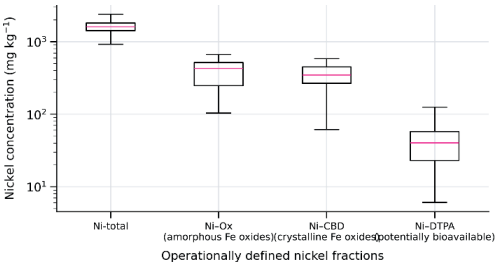


Figure 1. Nickel speciation in serpentine soils from the Eastern and Southern Rhodopes, Bulgaria

The exchangeable fraction extracted with $Sr(NO_3)_2$ is extremely low (0.01-4.32 mg/kg), confirming that easily mobile Ni forms represent only a negligible component of the overall Ni pool in these soils. These results highlight that total Ni content alone is not a reliable indicator of ecological risk or plant availability, as nickel speciation exerts a fundamental control on its environmental behaviour (Kabata-Pendias, 2011; Alloway, 2013).

Iron oxides and the Fe_o/Fe_d pedogenic indicator

Amorphous (Fe_o) and free crystalline (Fe_d) iron oxide contents show substantial variability among the studied serpentine soils. Fe_o concentrations range from 1,512 to 6,552 mg/kg, while Fe_d reaches values up to 41,096 mg/kg. Consequently, the Fe_o/Fe_d ratio - commonly used as an indicator of iron oxide crystallinity and pedogenic development - varies widely between 0.09 and 1.27.

This variability indicates that the investigated soils represent a broad pedogenic spectrum, ranging from weakly developed serpentine regoliths dominated by crystalline Fe oxides to more evolved soils characterised by a greater contribution of amorphous and poorly crystalline Fe phases. Such variability reflects progressive transformations of Fe oxides during pedogenesis and highlights the dynamic role of these phases in regulating Ni stabilisation.

The relationship between Fe_o/Fe_d and potentially bioavailable nickel (Ni-DTPA), illustrated in Figure 2, suggests that higher Ni-DTPA values occur mainly in soils exhibiting intermediate Fe_o/Fe_d ratios. In this figure, boxplots show the minimum, first quartile (Q1), median, third quartile (Q3), and maximum values for oxalate-extractable iron (Fe_o), dithionite-citrate-bicarbonate-extractable iron (Fe_d), and their ratio (Fe_o/Fe_d), used as an indicator of iron oxide crystallinity and degree of pedogenetic development, with the y-axis presented on a logarithmic scale.

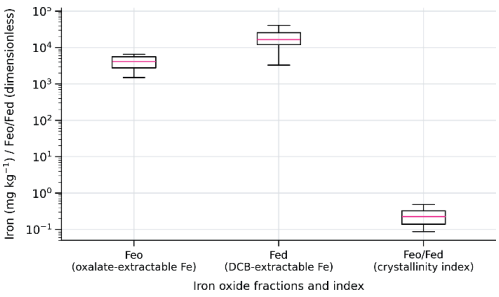


Figure 2. Distribution of iron oxide fractions and Fe_o/Fe_d ratio in the investigated serpentine soils

These systems are characterised by a substantial presence of amorphous and poorly crystalline iron oxides, which act as highly reactive sorbents that temporarily retain nickel in relatively labile forms. This observation is consistent with mechanisms described in the literature, in which amorphous Fe oxides function as transitional reservoirs that control Ni mobility before its stabilisation in more crystalline phases (Chardot et al., 2007; Echevarria, 2018). Overall, iron oxides play a central role in regulating nickel mobility in serpentine soils. The wide variability of the Feo/Fed ratio reflects different stages of oxide transformation and soil ageing (Blume & Schwertmann, 1969; Schwertmann & Taylor, 1989). Amorphous and poorly crystalline Fe oxides provide large reactive surfaces and bind nickel less strongly than well-crystallised oxides, potentially enhancing its availability

(Chardot et al., 2007; Echevarria, 2018). With progressive pedogenic development and increasing crystallinity of Fe oxides, nickel becomes more efficiently stabilised, and its mobility decreases, in agreement with the conceptual model proposed by Echevarria (2018), which emphasises the role of amorphous Fe phases as key Ni buffers in moderately developed ultramafic soils.

Multivariate analysis (PCA and cluster analysis)

Principal component analysis (PCA; Figure 3), presented as a biplot with PC1 and PC2 axes showing both variable loadings and sample scores, integrates soil physicochemical parameters, Ni fractions, iron oxide characteristics, and mineralogical composition, revealing a clear pedogenic gradient that controls Ni speciation and potential bioavailability. The first principal component (PC1) is dominated by variables related to Fe oxides (Fe_o, Fe_d, Fe_o/Fe_d) and associated Ni fractions, highlighting the central role of iron oxide dynamics in Ni retention and redistribution. The second principal component (PC2) primarily reflects variations in pH, texture, and mineralogical composition, indicating the influence of lithogenic control and soil physicochemical conditions.

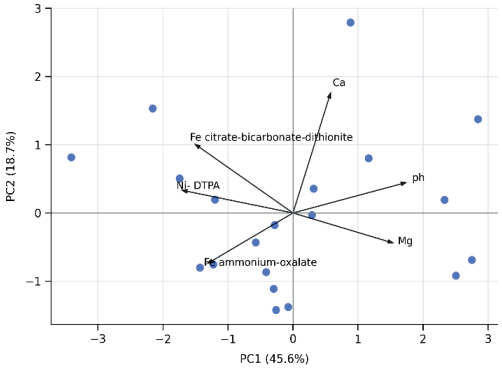


Figure 3. Principal component analysis (PCA) of serpentine soils from the Eastern and Southern Rhodope Mountains.

Cluster analysis (Figure 4), displayed as a hierarchical dendrogram based on standardized physicochemical parameters, Ni fractions, and iron oxide indicators, further supports the PCA results by grouping soils into several functional clusters that reflect differences in pedogenic development, mineralogical inheritance, and Ni mobility. These clusters form a pedogenic sequence ranging from weakly developed soils with relatively higher proportions of labile Ni to more stabilised systems where Ni is increasingly fixed in crystalline Fe oxides and secondary mineral phases. Soils at intermediate developmental stages form distinct clusters characterised by higher Ni-DTPA and Ni-oxalate values. In contrast, both less developed and more stabilised systems show a dominance of structurally and oxide-bound Ni. The consistency between PCA and cluster analysis confirms the existence of a pedogenic continuum controlling Ni stabilisation mechanisms in serpentine soils.

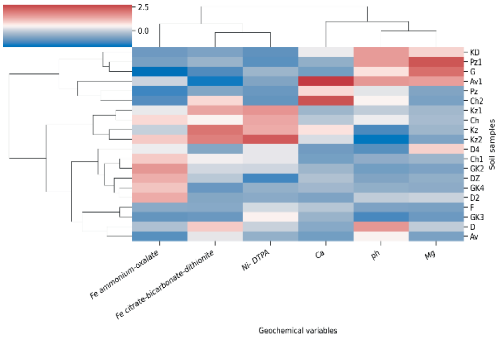


Figure 4. Hierarchical cluster analysis (HCA) of serpentine soils from the Eastern and Southern Rhodope Mountains

Mineralogical composition and its influence on nickel mobility and bioavailability

X-ray diffraction (XRD) analysis indicates that the mineralogical composition of the studied soils is dominated by primary ultramafic minerals typical of serpentinised parent materials. The dominant mineral phase in all soils is antigorite, with contents ranging from 19 to 86% and an average of approximately 60.6%. In several sites (Gornoslav, Dyulitsa, Parvenets) antigorite reaches particularly high proportions, indicating strong lithogenic control and relatively limited chemical weathering.

Lizardite occurs in fewer samples, with contents reaching up to 20%. Its presence, together with antigorite, indicates different serpentinisation pathways and variable stability of serpentine phases during pedogenesis. Both minerals belong to the serpentine group and represent

major structural hosts of Ni through isomorphic substitution of Mg^{2+} by Ni^{2+} within octahedral positions of the crystal lattice (Brooks, 1987; Kabata-Pendias, 2011).

A particularly important mineralogical observation is the identification of willemseite (Ni-rich serpentine) in several soils, with contents reaching up to 24%. Willemseite provides direct evidence for structurally bound nickel incorporated within the primary mineral lattice during rock formation. This mineralogical control explains the combination of high total Ni concentrations and very low exchangeable and DTPA-extractable fractions observed in the studied soils. Similar mineralogical controls on Ni behaviour have been reported in other ultramafic regions (Alexander et al., 2007; Bani et al., 2009).

Among the secondary phases, clinochlore plays an important role, reaching up to 48% in some samples (e.g., Golyamo Kamenyane). Clinochlore forms during secondary transformation of serpentine minerals and may retain Ni through surface sorption and partial structural incorporation. Quartz occurs as a secondary component (up to 55%), reflecting silica enrichment during weathering but contributing little to Ni retention. Montmorillonite is present only in minor amounts (< 9%), indicating limited development of advanced clay mineral phases and a relatively moderate pedogenic intensity.

Mineralogical composition, therefore, exerts a fundamental control on Ni behaviour by determining both the initial geochemical reservoir and its subsequent transformation during pedogenesis. The dominance of serpentine minerals indicates that a large proportion of Ni remains structurally bound within primary ultramafic silicate phases, exhibiting extremely low mobility and minimal bioavailability (Brooks, 1987; Kabata-Pendias, 2011).

With progressive weathering, part of the Ni released from primary minerals becomes incorporated into secondary phases. Clinochlore and Fe oxides act as intermediate geochemical reservoirs, temporarily retaining Ni. Amorphous and poorly crystalline Fe oxides function as reactive sorbents, whereas well-crystallised oxides provide more stable long-term fixation (Schwertmann & Taylor, 1989; Chardot et al., 2007).

This mineralogical-geochemical evolution forms a pedogenic continuum in which structurally bound Ni dominates in early stages of serpentine minerals, intermediate stages involve redistribution toward amorphous Fe oxides and clinochlore, and more advanced stages lead to the stabilisation of Ni in crystalline oxides and secondary mineral phases. Similar pedogenic sequences have been documented in temperate and Mediterranean ultramafic systems and provide a key framework for explaining the observed spatial variability in Ni mobility and plant accumulation (Chardot et al., 2007; Bani et al., 2013; Echevarria, 2018).

Conceptual model of Ni stabilisation in serpentine soils

The results obtained in this study allow the development of a conceptual model describing the mechanisms controlling nickel stabilisation in serpentine soils of the Eastern and Southern Rhodopes (Figure 5). The conceptual diagram illustrates the role of serpentine minerals (antigorite, lizardite, willemseite) as primary Ni reservoirs through structural incorporation, and highlights the contribution of secondary minerals and iron oxides in regulating Ni mobility. The model integrates mineralogical inheritance from ultramafic parent material with pedogenic transformations affecting Ni speciation and mobility.

In the initial stage, nickel is predominantly structurally incorporated into primary serpentine minerals such as antigorite, lizardite, and willemseite, where it substitutes for Mg^{2+} in the octahedral positions of the crystal lattice. Under these conditions, Ni is largely immobile and poorly available for biological uptake.

During progressive weathering and pedogenesis, partial dissolution of serpentine minerals releases Ni into the soil system. A significant portion of this Ni is subsequently associated with amorphous and poorly crystalline Fe oxides, which act as highly reactive sorbents and temporary geochemical reservoirs. This stage is characterised by relatively higher proportions of labile Ni fractions, reflected in increased Ni-DTPA and Ni-oxalate concentrations.

With further pedogenic development, iron oxides progressively transform into more crystalline phases, enhancing the long-term stabilisation of nickel through stronger sorption and co-precipitation mechanisms. Consequently, Ni becomes increasingly immobilised in crystalline Fe oxides and secondary mineral phases, leading to reduced mobility and lower potential bioavailability.

Overall, this conceptual framework highlights the combined influence of lithogenic inheritance and pedogenic transformation processes on the distribution, mobility, and environmental behaviour of nickel in serpentine soils.

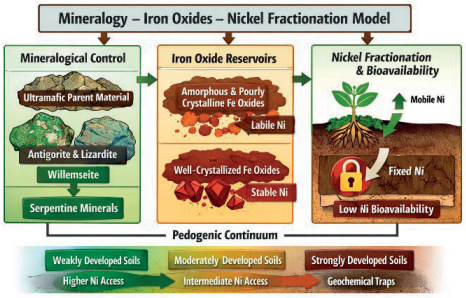


Figure 5. Conceptual model of nickel (Ni) behaviour in serpentine soils of the Eastern and Southern Rhodopes (Generated using Microsoft Copilot)

CONCLUSIONS

This study provides a comprehensive assessment of nickel (Ni) speciation, mobility, and potential bioavailability in serpentine soils of the Eastern and Southern Rhodopes, highlighting the key role of mineralogical inheritance and pedogenetic processes.

The soils are characterised by high total nickel concentrations, confirming the dominant geogenic control exerted by the ultramafic parent material. However, the potentially bioavailable fraction of Ni is limited, indicating that total element content alone does not reflect ecological risk.

Mineralogical analysis shows that serpentine minerals - antigorite and lizardite - serve as the primary reservoirs of nickel through structural incorporation. The detection of willemseite provides direct evidence for stably fixed nickel and explains its low mobility. Secondary mineral phases, primarily clinochlore and iron oxides, act as intermediate geochemical sinks, modifying nickel distribution and regulating its mobility. Amorphous and poorly crystalline Fe oxides retain Ni in more labile pools, while well-crystallised oxides provide long-term stabilisation.

Multivariate analysis identifies a pedogenetic continuum, where intermediately developed soils exhibit higher potential Ni bioavailability, whereas poorly or highly developed soils function as stable geochemical “traps”. This continuum indicates that plant-accessible nickel is spatially heterogeneous and strongly controlled by mineralogy and iron oxide dynamics rather than total Ni content.

The proposed conceptual model, "mineralogy – iron oxides – nickel fractionation", provides a process-oriented framework for predicting Ni behaviour in temperate ultramafic ecosystems. The results are of both fundamental significance for understanding Ni biogeochemistry and practical value for ecological risk assessment and management of serpentine soils.

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