Temporal patterns of nickel transfer from soil to rice in terraced paddy fields affected by serpentinite

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Abstract

Nickel (Ni) is an essential micronutrient for plants although it is considered toxic when present in excess in the soil. This study investigated the transfer of Ni from the soil to rice in terraced paddy fields affected by serpentinite, which contains an anomalously higher Ni content compared with other geological materials. Soils, soil solutions, and rice plants were collected at several different growing stages from three adjacent terraced paddy fields subject to the same water and fertilizer management. Temporal changes in their elemental compositions revealed that a higher concentration of Ni was dissolved in the soil solution during flooded conditions, probably due to the co-solubilization with Mn oxides under low redox potential conditions. However, rice accumulated Ni at a higher rate during the drainage period than in the flooding period. Although the Ni concentration in the soil solution was lowest in the drainage period, the relative concentration to Fe (i.e., Ni/Fe ratio) was much higher than that in flooded conditions. These relationships suggest that a potential measure to counter the transfer of Ni from the soil to rice in serpentinite-affected paddy fields is to increase Fe phytoavailability during the drainage period.

Introduction

Serpentinite is an ultramafic rock that is widely distributed worldwide along tectonic zones because it is formed by the subduction and alteration of peridotite and pyroxene along plate boundaries (Coleman and Jove 1992; Oze et al., 2004). It is present in less than 1% of the total land surface area of the earth, but can be found in Japan (Mizuno et al., 2009). Serpentinite contains anomalously large proportions of heavy metals such as chromium (Cr), cobalt (Co), and nickel (Ni) (Proctor and Woodell, 1975; Vithanage et al., 2019; Kierczak et al., 2021). Relatively recently, Ni was discovered to be an essential micronutrient that supports enzyme activities in plants and humans (Eskew et al., 1983; Brown et al., 1987; Al-Fartusie and Mohssan, 2017; County, 2020; Islam et al., 2023). However, the optimal concentration range of Ni in living organisms is typically narrow. The excessive uptake and accumulation of Ni from serpentinite soils in edible plants has often led to reduced plant yields and jeopardized human health security (Hseu and Lai, 2017; Hassan et al., 2019; Infante et al., 2021). Therefore, it is important to understand the factors controlling the transfer of Ni to plants, especially crops, from agricultural soils developed from serpentinite. Previous studies have shown that rice (Oryza sativa L.), one of the most important staple foods worldwide (Kyuma 2004), takes up Ni from the soil more readily than wheat (Chen et al., 2018).

The total Ni content of serpentinite soils is approximately 1000–4000 mg kg$^{-1}$, more than 10 times higher than that of non-serpentine soils (Mizuno et al., 2009; Alves et al., 2011; Aziz et al., 2015; Bini et al., 2017; Kierczak et al., 2021). The Ni in serpentinite soils can be conceptually considered as labile or non-labile forms with different phytoavailability (Echevarria et al., 1998; Nkrumah et al., 2016). Non-labile Ni pools, i.e., those with the lowest phytoavailability, are typically present in the mineral lattice as an isomorphic substitution for magnesium (Mg) and Fe at the octahedral sites of primary and secondary silicates, such as serpentinite, talc, and chlorite (Ratié et al., 2015; Kierczak et al., 2021). Labile Ni pools in the solid phase, i.e., those with moderately high phytoavailability, can be further separated into those in the solid phase as an adsorbent on phyllosilicates or Fe/Mn oxides, those in the solid phase as co-precipitates
with Fe/Mn oxides and sulfides, and those bound to organic matter (Cheng et al., 2011; Aziz et al., 2015). Labile Ni pools in the liquid phase, i.e., those with the highest phytoavailability, mostly exist as free Ni\(^{2+}\) ions in the soil solution, but also partly as chelates with dissolved organic matter, both of which have the potential to be rapidly absorbed by rice as long as there are no limitations in the root transport systems (Reddy and DeLaune 2008; Chen et al., 2009; Yusuf et al., 2011). These Ni fractions can not only be separated conceptually, but also technically using several wet chemistry methods (Yang et al., 2022), which can provide important insights into how Ni is transferred from the soil to the plant. However, it should be noted that the chemical state of Ni is highly dependent on the background chemistry of the soil and soil solution and varies among the different phytoavailable fractions, especially in relation to changes in redox conditions during the crop growing season (Echevarria et al., 2006). Variations in the reduction–oxidation (redox) conditions are the greatest in the paddy fields compared with other typical agroecosystems, but their impact on Ni transfer from serpentinitic paddy fields to rice grains have not been sufficiently elucidated.

Temporal changes in redox potential (Eh) in paddy fields are caused by intermittent flooding and drainage, a traditional water management practice in rice production (Liesack et al., 2000; Kögel-Knabner et al., 2010; Husson, 2013). The flooding period prevents the paddy soil from taking oxygen from the atmosphere, and changes to reduced conditions (low Eh). In contrast, in the drainage period, the paddy soil is supplied with oxygen from the atmosphere, and returns to oxidized conditions (high Eh). With this change in soil redox conditions, redox-sensitive species, such as Fe, Mn, S, and organic carbon, dissolve or precipitate (Schulz, 2000). In contrast, Fe oxides, Mn oxides, and organic carbon dissolve during the flooding period, and sulfides dissolve during the drainage period. It is known that labile Ni pools in the solid phase coprecipitate mainly with these redox-sensitive species and is then released into the soil solution upon their dissolution (Ponnamperuma, 1972; Patrick and Jugsujinda, 1992; Yu et al., 2007; Shaheen et al., 2014). Thus, drastic changes in Ni phytoavailability are expected in paddy fields during the growing season; however, these cannot be predicted only from single soil sampling and in vitro extraction experiments. Furthermore, it is still unclear which redox-sensitive species exert the most control over the precipitation and dissolution behavior of Ni during rice growth in actual paddy field conditions.

Therefore, the objective of this study was to elucidate the factors controlling the temporal patterns of Ni transfer from the soil to rice through the analysis of the relationship between the concentration of the labile Ni pools in both the solid and liquid phases and the Ni uptake by rice.

**Materials And Methods**

**Research site**

The field experiment was conducted between 15 May 2022 and 8 September 2022, inclusive, in three terraced paddy fields in Yosano town, Kyoto Prefecture (35°29’ N, 135°07’ E; Fig. 1-(a)(b)(c)), with a mean annual temperature of 14.6°C and annual precipitation of 1788 mm. The fields are located on a gentle slope at the northwestern foot of Mt. Oe in the serpentinite area at an altitude of approximately
110–124 m. The same management of planting, fertilization, and water was used in all the fields. Rice seedlings (*Oryza sativa* L. cv. Koshihikari) were transplanted in all fields on 15 May (0 days after transplanting [DAT]). The type and amount of fertilizers in these fields were 81 kg N ha$^{-1}$, 21 kg P$_2$O$_5$ ha$^{-1}$, and 30 kg K ha$^{-1}$, all of which were applied once on 11 May, 2022. These fields were flooded from 0 DAT until 40 DAT (24 June), drained from 40 DAT until 75 DAT (29 July), re-flooded from 75 DAT until approximately 105 DAT (28 August), and then re-drained until harvesting (8 September). Irrigation water was drawn from the Atsue river flowing from Mt. Oe, and the average Ni concentration of the irrigation water from June to September was 0.01 µmol L$^{-1}$.

**Sampling methods for soils, plants, and soil solutions**

Bulk soil samples were collected from the plowed layer (0–15 cm depth) before rice was transplanted on 4 December 2021. Five sub-samples were collected from the three terraced paddy fields using a trowel and mixed to obtain a composite sample. Bulk soil samples were dried at approximately 40°C for more than 1 week and then sieved until particle sizes were less than 2 mm before analysis.

Seedling samples were collected before transplanting. Rice plants, including both shoots and roots, were collected at four timepoints in total: active tillering (29 DAT), panicle initiation (59 DAT), heading (89 DAT), and maturity (116 DAT) (Hussain et al., 2014) (Table 1). The rice plants were collected from three locations in each of the three fields; thus, in total, there were nine replicate samples for each growth stage. The samples were rinsed with tap water and deionized water to remove attached soil particles, and then were partitioned into roots, shoot (leaves and stems), and grains. These samples were dried in a ventilated oven at 65°C for a minimum of 48 h; then, the dry weight was determined.

Rhizosphere soil samples were collected from the soil remaining after rice samples were shaken to remove the loosely adherent soil surrounding the root. The number of samples collected and the sampling dates were therefore the same as for the rice samples. Rhizosphere soil samples were dried at approximately 25°C in an anaerobic chamber to avoid artificial oxidation during the drying process (Coy Laboratory Products Inc., Grass Lake, MI), which took more than 1 week. Afterwards, the soils were sieved until particles were less than 2 mm before analysis (Emmett et al., 2019; Feng et al., 2005).

Soil solution samples were collected in accordance with the method used by Nishikiori et al. (2020). An acrylic box with three holes at a depth of 5 cm from the soil surface was installed in the three paddy fields approximately 3 m from the outlet to obtain soil solution. A porous cup (DIK-301A-A1; Daiki Rika Kogyo Co., Ltd., Saitama, Japan) was placed into each hole inside of the box to collect soil solution from the outside of the box. Using this device, 15 mL of soil solution was collected every 2 weeks, for a total of eight samples during the growing season. Nine replicates of soil solutions were collected at each sampling campaign and immediately filtered through a 0.45 µm Millipore filter (Table 1). Soil solution sampling was performed twice per day. The first sample (soil solution A) was treated with 1 mL of HNO$_3$ and the second soil solution (soil solution B) was left untreated. Both samples were stored at 4°C and protected from light.
### Table 1

Sampling date of rice, bulk soil, rhizosphere soil, and soil solution.

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td></td>
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<td>5/23</td>
</tr>
<tr>
<td></td>
<td>(8 DAT)</td>
<td>(17 DAT)</td>
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<tr>
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<td>+</td>
</tr>
<tr>
<td>Bulk soil</td>
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<td>+</td>
</tr>
<tr>
<td>Rhizosphere soil</td>
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<td>+</td>
</tr>
<tr>
<td>Soil solution</td>
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<td>+</td>
</tr>
</tbody>
</table>

<table>
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<th>6/29</th>
<th>7/13</th>
<th>7/27</th>
<th>8/10</th>
<th>8/24</th>
<th>9/8</th>
</tr>
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<td>(29 DAT)</td>
<td>(45 DAT)</td>
<td>(59 DAT)</td>
<td>(73 DAT)</td>
<td>(87 DAT)</td>
<td>(101 DAT)</td>
<td>(116 DAT)</td>
<td></td>
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<td>Rice</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Rhizosphere soil</td>
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<td>+</td>
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<td></td>
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<tr>
<td>Soil solution</td>
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<table>
<thead>
<tr>
<th></th>
<th>(87 DAT)</th>
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<tr>
<td>Rice</td>
<td>(101 DAT)</td>
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<tr>
<td>Bulk soil</td>
<td>(116 DAT)</td>
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<tr>
<td>Rhizosphere soil</td>
<td></td>
</tr>
<tr>
<td>Soil solution</td>
<td></td>
</tr>
</tbody>
</table>

Physical, chemical, and mineralogical properties of the bulk soils

The soil pH was measured using the glass electrode method with a soil-to-water ratio of 1:5 (pH/Ion Meter F-23; HORIBA, Japan). The soil electrical conductivity (EC) was measured with an EC meter (CM-30R, DKK-TOA, Japan) at a soil-to-water ratio of 1:5. The exchangeable Na\(^+\), K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) ions were extracted using 1 mol L\(^{-1}\) ammonium acetate and their levels were determined using an atomic absorption spectrophotometer (AA6200; SHIMADZU, Japan). The cation exchange capacity (CEC) of the soil was determined by extracting each sample with 10% NaCl solution after extraction of the exchangeable bases and then evaluating the NH\(^{4+}\) concentration using the indophenol method (Sparks et al. 1996; Rhine et al., 1998). Total C content was determined with the dry combustion method, whereby finely ground soil samples were oven-dried at 110°C for approximately 24 h and then analyzed with an NC analyzer (Sumigraph NC95A; Sumika Chemical Analysis Center, Japan) (Sparks, 1996). The size distribution of soil particle was assessed by pipette and sieving methods after organic matter was removed by the addition of 30% H\(_2\)O\(_2\) adjusted to pH 5 at 80°C (Gee and Bauder 1986).

The elemental composition of bulk soil samples was measured with an energy dispersive X-ray fluorescence spectrometer (EDXRF) to confirm whether the upstream serpentinite area is affected. Approximately 2 g of the finely ground bulk soil sample was filled into an aluminum ring (φ 22 mm, depth 1.5 mm) and pelletized by pressurizing to approximately 400 kgf cm\(^{-2}\) for 10 min using a hydraulic press machine (AS ONE Corporation). The Si, Al, Fe, Na, K, Ca, Mg, P, Zn, Mn, Cr, and Ni content in the pelleted bulk soil samples were determined by an EDXRF spectrometer (SPECTRO XEPOS C, SPECTRO Analytical Instruments, GmbH, Kleve, Germany). The analytical conditions were measurement time of 600 s, using 45 kV and 0.89 mV for Fe, Ni, and Zn; 17.5 kV and 1.80 mV for Ca, Cr, K, and Mn; and 22.5 kV and 1.78 mA for Al, Na, Mg, P, and Si.
The mineral composition of bulk soil samples was measured with X-ray diffraction (XRD), which can semi-quantify the minerals, to confirm the presence of serpentinite-specific minerals in the bulk soils. The preparation of bulk soil samples followed the method of Butler and Hillier (2021). Bulk soil samples were mixed with 20 wt% internal standard Al$_2$O$_3$ (Baikalox3.0CR, Baikowski, France) and wet-milled to a fine powder (approximately 5 µm) for 10 min using a McCrone micronizing mill (XRD-Mill McCrone, Verder Scientific, Toyo, Japan). After air-drying, the powders were analyzed using a Rigaku MiniFlex600 X-ray diffractometer. XRD patterns were collected using Cu Kα radiation (40 kV, 15 mA) from 2θ values of 5º to 65º in 0.01º steps with a scan rate of 10º per minute. The XRD data were analyzed using powdR, a package available in R statistical software that was developed based on the USGS RockJock program (Butler and Hillier, 2020). This is a computer program that uses the overall diffraction data from an analysis to qualify minerals with reference to the diffraction patterns of each mineral accumulated in the past and to quantify minerals by comparing the integrated diffraction intensities with the intensities of internal standards (Eberl et al., 2003).

Chemical composition of soil solutions

Concentrations of Ni, Fe, and Mn in soil solution A were measured using inductively coupled plasma atomic emission spectroscopy (SPS-1500VR, Seiko Instruments). The concentration of dissolved organic carbon (DOC) in soil solution B was measured with a TOC analyzer (TOC-V CSH, SHIMADZU). Sulfate (SO$_4^{2−}$) concentration in soil solution B was measured using ion chromatography (SHIMADZU). The soil solution pH was measured with a pH meter (LAQUAtwin-pH-22B, HORIBA Advanced Techno Co., Ltd., Kyoto, Japan).

Calculation of the redox potential (Eh)

The redox potential (Eh) can be used as a measure of soil redox conditions (Husson et al., 2013). The electron activity in soil (pe) controls the ratio of Fe$^{3+}$ to Fe$^{2+}$ in the soil solution. The equilibrium reaction relating these two oxidation states is given by (Lindsay, 1979):

$$\text{Fe}^{3+} + e^- \leftrightarrow \text{Fe}^{2+} \quad \log K^\circ = 13.04$$

This relationship can be rearranged to give:

$$\log \frac{\text{Fe}^{2+}}{\text{Fe}^{3+}} = 13.04 - p_e$$

Combining this reaction with the solubility expression for soil-Fe gives:

$$\text{Fe}^{3+} + e^- \leftrightarrow \text{Fe}^{2+} \quad \log K^\circ = 13.04$$

$$\text{Fe (OH)}_3 \quad (\text{soil}) + 3\text{H}^+ \leftrightarrow \text{Fe}^{3+} + 3\text{H}_2\text{O} \quad \log K^\circ = 2.70$$

$$\text{Fe (OH)}_3 \quad (\text{soil}) + 3\text{H}^+ + e^- \leftrightarrow \text{Fe}^{2+} + 3\text{H}_2\text{O} \quad \log K^\circ = 15.74$$
from which

\[
\log [\text{Fe}^{2+}] = 15.74 - (\text{pe} + \text{pH}) - 2\text{pH} \quad \text{(Eq. 1)}
\]

where \text{pe} is the negative log of electron activity (e\text{−}). The following conversion is useful (Lindsay, 1982): \[\text{Eh (mV)} = 5.92 \times \text{pe} \quad \text{(Eq. 2)}\]

By substituting soluble Fe and pH in the soil solution into Eq. (4), \text{Eh} was calculated from Eqs. 1 and 2.

**Phytoavailable elements in rhizosphere soils**

The phytoavailable Ni content in rhizosphere soils was determined by diethylenetriamine pentaacetic acid (DTPA) extraction as described by Lindsay and Norwell (1978). A 10 g rhizosphere soil sample was shaken with 20 mL of DTPA extract solution (0.005 mol L\textsuperscript{−1} DTPA with 0.01 mol L\textsuperscript{−1} CaCl\textsubscript{2} and 0.1 mol L\textsuperscript{−1} triethanolamine at pH 7.3) for 2 h. The extracted solution was filtered through a 0.45 µm Millipore filter. The Ni, Fe, and Mn concentration in the filtered extracts was determined by inductively coupled plasma atomic emission spectroscopy (SPS-1500VR, Seiko Instruments).

**Elemental composition of straw and brown rice**

The elemental analysis of rice samples was based on the methods of Hadif et al. (2015), Ramzani et al. (2016), and Zhang et al. (2021). First, 0.2 g of straw and grain samples was mixed with 10 mL of HNO\textsubscript{3} in a conical beaker and left undisturbed for 12 h. The mixture was then heated gently on a hot plate (EA-DC10, ZOJIRUSHI, Osaka, Japan) at nearly 90°C for 2 h. After that, 1 mL of 30% H\textsubscript{2}O\textsubscript{2} was added to digest the sample completely. After cooling, the digested solution was filtered through a 0.45 µm Millipore filter. The content of Ni, Fe, and Mn in the filtered solution was determined by inductively coupled plasma atomic emission spectroscopy (SPS-1500VR, Seiko Instruments).

**Statistical analysis**

R version 4.2.2 (R Core Team, 2022) was used to compute the average, the standard deviation, the significance of differences, and the correlation coefficients among the parameters. The average and standard deviation (\(n = 9\)) for three terraced paddy fields per DAT were calculated. The significant differences between the DAT for each parameter were determined by Tukey–Kramer's multiple range test with a 95% confidence level. Associations between soluble Ni and redox-sensitive species and between DTPA-Ni and other elements were examined using the Pearson correlation coefficient.

**Results**
Soil properties

The arithmetic mean (± standard deviation) values of physical and chemical properties of the bulk soils are presented in Table 2. The bulk soils consisted of 23% ± 2.3% clay (< 2 µm), 25% ± 2.8% silt (2–20 µm), 11% ± 0.1% fine sand (20–50 µm), 19% ± 1.2% medium sand (50–200 µm), and 22% ± 4.0% coarse sand (200–2000 µm). The pH was 5.6 ± 0.2 (H₂O). The EC was 35 ± 0.4 µS cm⁻¹. The CEC was 14 ± 1.0 cmol c kg⁻¹. The total C content was 19 ± 0.8 mg kg⁻¹. The total N content was 1.8 ± 0.1 mg kg⁻¹. These values were similar to the average values for paddy soils in Japan (Honda et al., 2007; Yamada et al., 2009; Takahashi et al., 2020). Exchangeable Na content was 0.15 ± 0.02 (cmol c kg⁻¹), the exchangeable K content was 0.29 ± 0.03 (cmol c kg⁻¹), the exchangeable Ca content was 2.5 ± 0.3 (cmol c kg⁻¹) and the exchangeable Mg content was 3.3 ± 0.50 (cmol c kg⁻¹). The exchangeable Ca/Mg ratio (0.75 ± 0.05) was quite low, in the range of 0.1–1.5, which was comparable with that reported for serpentinite soils (McGahan et al., 2009).
Table 2
Chemical and physical properties of bulk soils. (n = 3)

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.6 ± 0.2</td>
</tr>
<tr>
<td>EC (µS cm⁻¹)</td>
<td>35 ± 0.4</td>
</tr>
<tr>
<td>Exchangeable Na (cmol_c kg⁻¹)</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Exchangeable K (cmol_c kg⁻¹)</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Exchangeable Ca (cmol_c kg⁻¹)</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Exchangeable Mg (cmol_c kg⁻¹)</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Exchangeable Ca/Mg ratio</td>
<td>0.75 ± 0.0.5</td>
</tr>
<tr>
<td>CEC (cmol_c kg⁻¹)</td>
<td>14 ± 1.0</td>
</tr>
<tr>
<td>Total C (mg kg⁻¹)</td>
<td>19 ± 0.8</td>
</tr>
<tr>
<td>Total N (mg kg⁻¹)</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>&lt; 2 µm (%)</td>
<td>23 ± 2.3</td>
</tr>
<tr>
<td>2–20 µm (%)</td>
<td>25 ± 2.8</td>
</tr>
<tr>
<td>20–50 µm (%)</td>
<td>11 ± 0.1</td>
</tr>
<tr>
<td>50–200 µm (%)</td>
<td>19 ± 1.2</td>
</tr>
<tr>
<td>200–2000 µm (%)</td>
<td>22 ± 4.0</td>
</tr>
</tbody>
</table>

The arithmetic mean (± standard deviation) values of total element content in bulk soils are shown in Table 3. The total Ni content was 1,730 ± 414 (mg kg⁻¹), the total Cr content was 2,479 ± 837 (mg kg⁻¹), the total Mg content was 33.0 ± 10.5 (g kg⁻¹), the total Fe content was 62.0 ± 8.9 (g kg⁻¹) and the total Mn content was 823 ± 134 (mg kg⁻¹) in bulk soil. These elements are abundant in serpentine soils (Whittaker, 1954; Proctor and Woodell, 1975; Mizuno and Kirihata, 2015), and were found considerably more abundant than in typical Japanese soils (Yamasaki et al., 2001; Yanai et al., 2012). In particular, total Mg and total Ni were approximately 4 and 100 times higher, respectively.
Table 3
Elemental composition of the bulk soils. (n = 3)

<table>
<thead>
<tr>
<th>Element</th>
<th>This study</th>
<th>Japan^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni (mg kg(^{-1}))</td>
<td>1,730 ± 0.29</td>
<td>17.9</td>
</tr>
<tr>
<td>Cr (mg kg(^{-1}))</td>
<td>2,479 ± 837</td>
<td>25.7^b</td>
</tr>
<tr>
<td>Mg (g kg(^{-1}))</td>
<td>33.0 ± 10.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Fe (g kg(^{-1}))</td>
<td>62.0 ± 8.9</td>
<td>35.1</td>
</tr>
<tr>
<td>Mn (mg kg(^{-1}))</td>
<td>823 ± 134</td>
<td>640</td>
</tr>
</tbody>
</table>

^a Yanai et al. (2012)

^b Mizuno and Kirihata (2015)

The arithmetic mean (± standard deviation) values of the mineral content of soils are shown in Table 4. The bulk soils contained magnesite, talc, brucite, spinel, chlorite, amphibole, and olivine, which are derived from serpentine (Whittaker, 1954; Proctor and Woodell, 1975; Christensen, 2004). The contents of these minerals were considerably higher than those in Japanese paddy soils. The bulk soils contained elements and minerals that are abundant in serpentinite.

Table 4
Mineral composition of bulk soils (wt.%). (n = 3)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>This study</th>
<th>Japan^a</th>
</tr>
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<tbody>
<tr>
<td>magnesite</td>
<td>0.97 ± 0.29</td>
<td>0.86</td>
</tr>
<tr>
<td>talc</td>
<td>2.7 ± 1.4</td>
<td>0.01</td>
</tr>
<tr>
<td>brucite</td>
<td>0.44 ± 0.32</td>
<td>N.D.</td>
</tr>
<tr>
<td>spinel</td>
<td>0.38^b</td>
<td>0.03</td>
</tr>
<tr>
<td>chlorite</td>
<td>4.6 ± 0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>amphibole</td>
<td>3.5 ± 0.8</td>
<td>0.43</td>
</tr>
<tr>
<td>olivine</td>
<td>0.91 ± 0.31</td>
<td>0.63</td>
</tr>
</tbody>
</table>

^a Azuma et al. (under review)

^b Observed in only one field

N.D. = Not detected
Changes in pH, Ni, Fe, Mn, S, dissolved organic carbon (DOC), and Eh in soil solutions

The pH and the Ni, Fe, Mn, S, and organic matter content in the soil solution are shown in Fig. 2a–f. These results changed significantly during the rice growth period. The pH of the soil ranged from 6.3 to 7.0; it was significantly higher at 29, 73, and 116 DAT and significantly lower at 17, 59, and 87 DAT. The concentration of soluble Ni ranged from 0.35 to 5.6 µmol L\(^{-1}\), that of soluble Fe from 0.77 to 656 µmol L\(^{-1}\), soluble Mn from 0.76 to 145 µmol L\(^{-1}\), DOC from 9.0 to 52.3 mg L\(^{-1}\), and soluble S from 2.8 to 11.9 µmol L\(^{-1}\), respectively. The increasing or decreasing trend in the concentration of Ni was similar to that of Fe, Mn, and DOC. The values decreased significantly from 29 to 59 DAT and then increased slightly in the soil solution. The opposite trend was observed for S.

The redox potential (Eh), as estimated based on Eqs. 1 and 2, decreased sharply and reached a minimum value of −111 mV at 29 DAT and increased to 153 mV at 59 DAT (Fig. 3). Subsequently, the Eh decreased gradually and increased again. The Eh was lower during flooding periods and higher during drainage periods (from 40 DAT until 75 DAT).

Changes in DTPA-extractable Ni, Fe, and Mn in rhizosphere soil

The DTPA-extractable Ni (DTPA-Ni), Fe (DTPA-Fe), and Mn (DTPA-Mn) content in rhizosphere soil, which can be used as indicators of the amount of labile pools for each element, respectively, in the solid phase, are shown in Fig. 4a–c. The content of DTPA-Ni ranged from 30.1 to 37.9 mg kg\(^{-1}\), DTPA-Fe from 210 to 285 mg kg\(^{-1}\), and DTPA-Mn from 38.3 to 91.1 mg kg\(^{-1}\), respectively. The Ni, Fe, and Mn content extracted by DTPA was relatively higher during the drainage period (59 DAT), which was in contrast to the trend of relatively lower concentrations in the soil solutions for the same period.

Relationships between Ni and the redox-sensitive species in soil solution and solid phase

Pearson correlation analyses between the content of Ni and the redox-sensitive species (Fe, Mn, S, DOC) in the soil solution revealed that the soluble Ni concentration was positively correlated with the concentrations of soluble Fe (\(r = 0.78, p < 0.01\)), soluble Mn (\(r = 0.92, p < 0.01\)), and DOC (\(r = 0.74, p < 0.01\)) in the soil solution (Fig. 5). The soluble Ni content had the highest correlation coefficient with the soluble Mn content. No significant correlation was found between the concentration of soluble Ni and soluble \(\text{SO}_4^{2-}\). Similarly, the DTPA-Ni content was positively correlated with DTPA-Mn (\(r = 0.72, p < 0.05\)), but did not correlate with DTPA-Fe in rhizosphere soil (Fig. 6).

Changes in dry weight (DW) and the content of Ni, Fe, and Mn in shoots of rice

Time-course changes in the DW of rice straw and grain (i.e., shoot) during the growing season are presented in Fig. 7. The shoot DW continued to increase for up to 87 DAT, followed by a slight increase from 87 DAT to 116 DAT (Fig. 7). Consequently, the shoot DW at harvest (116 DAT) was 47.1 ± 7.1 g. During this period, the percentage of grain DW in the shoot DW increased, reaching approximately 50%.
The concentrations of Ni and Fe in straw increased sharply from 0 DAT to 29 DAT, reaching maximum values of $14.4 \pm 2.5$ and $2015 \pm 573$ mg kg$^{-1}$, respectively, after which they decreased (Fig. 8a, b). The Mn concentration in straw decreased sharply from 0 DAT to 29 DAT and then remained relatively unchanged (Fig. 8c). The final Ni, Fe, and Mn concentrations in straws at 116 DAT were $14.4 \pm 2.5$ mg kg$^{-1}$, $2015 \pm 573$ mg kg$^{-1}$, and $394 \pm 189$ mg kg$^{-1}$, respectively. The Ni, Fe, and Mn concentrations in grains at 116 DAT were $6.2 \pm 1.8$ mg kg$^{-1}$, $22.1 \pm 14.3$ mg kg$^{-1}$ and $20.5 \pm 3.4$ mg kg$^{-1}$, respectively (Fig. 8d, e, f). The Ni concentration of grains in this study was considerably higher than that growing on non-serpentine soil (Tsukada et al., 2007; Khan et al., 2013), and comparable with that grown on serpentine soil in previous studies (Hseu and Lai, 2017; Infante et al., 2021). Thus, in this study, it is clear that the rice is affected by serpentine soil.

**Discussion**

Relationships between Ni and redox-sensitive substances in soil solution and solid phase

In this study, it was observed that the Fe, Mn, and DOC concentrations in the soil solution increased during the flooding period, whereas the SO$_4^{2-}$ concentration decreased (Fig. 2; Fig. 3). This trend corresponded well with the theoretical redox chemistry of soil in a paddy field. The soil oxidation–reduction (redox) potential (Eh) started to decrease gradually after flooding, which suggested that the Eh was affected by water management (Fig. 3). The Eh values are altered by the transport of electrons from the electron donator to the electron acceptor owing to the activity of soil microorganisms (Kjaergaard, 2006). In the anoxic conditions occurring during the flooding period, the Eh decreases as the soil microorganisms use electron acceptors, in the order Mn (III/VI) oxide, Fe (III) oxide, and SO$_4^{2-}$, to generate Mn$^{2+}$, Fe$^{2+}$, and sulfide (Ponnampерuma, 1972; Patrick and Jugsuinda, 1992; Yu et al., 2007; Shaheen et al., 2014). Under the low Eh conditions, the DOC content increases because DOCs are released from OM bound to reductively dissolved Fe and Mn oxides and/or produced by reducing bacteria (Yu et al., 2007; Grybos et al., 2009).

Similar to these redox-sensitive species (Fe, Mn, S, DOC), the concentration of Ni in the soil solution was increased or decreased as the Eh changed. Analysis revealed that the Ni concentration was positively correlated with that of Fe, Mn, and DOC but negatively correlated with that of SO$_4^{2-}$ in the soil solution (Fig. 5). These relationships agree with those shown in previous studies for serpentine soil (Rinklebe et al., 2016). However, Ni does not change oxidation state when changes in Eh occur, but instead exists generally as a divalent cation (Takeno, 2005). The possible mechanisms behind the Eh-dependent changes in Ni solubility are adsorption or coprecipitation with Fe and Mn oxides in oxic conditions, and precipitation as Ni sulfides in anoxic conditions (Rinklebe and Shaheen, 2017; Antić-Mladenović et al., 2011; Du Laing et al., 2009; Shaheen et al., 2014). Among these redox-sensitive species, the main sinks contributing to labile Ni are considered to be Fe and Mn oxides. Previous studies (Friedrich et al., 2011; Friedrich et al., 2019) show that the labile Ni in Fe oxide was released into the soil solution upon the dissolution of Fe oxide (e.g., ferrihydrite and hematite) during reduction processes, with Ni adsorbed on
the surface of Fe oxide and incorporated into structural sites when Fe oxide recrystallizes during oxidation processes. Similar Eh-dependent mechanisms were also observed in Mn oxide (e.g., birnessite) (Heyden and Roychoudhury, 2015; Lefkowitz and Elzinga, 2017). DOC is considered the main solubilizer for Ni$^{2+}$. Previous studies (Antić-Mladenović et al., 2010; Antić-Mladenović et al., 2016) indicated that the co-solubilization of Ni and DOC and the formation of soluble Ni-DOC complexes might occur under anoxic conditions. The formation of soluble Ni-DOC complexes has been implicated to decrease the sorption on Fe and Mn oxides and to increase the solubility of Ni (Uren, 1992; Lockwood et al., 2015). Thus, our results indicated that increasing the concentration of Ni in soil solution during the flooding period might be due to the reductive dissolution of Fe and Mn oxide and the formation of soluble complexes with DOC. In addition, DTPA-Ni content in the rhizosphere soil decreased during the flooding period, possibly because DTPA extracts Ni adsorbed on Fe and Mn oxides and organic matter.

The highest correlation coefficient suggests that Mn was the most important variable for controlling Ni solubility in terraced rice paddies in this study (Fig. 5, 6). However, this was not the case for other studies. Some studies (Antić-Mladenović et al., 2011; Rinklebe et al., 2016; Shaheen et al., 2022) of serpentine soil reported that DOC was the most important factor determining Ni solubility. The concentration of DOC in the aforementioned studies was higher than that in this study (Antić-Mladenović et al., 2011; Rinklebe et al., 2016; Shaheen et al., 2022; therefore, we suggest that DOC was not the most important variable controlling Ni solubility. Hseu et al. (2015) reported that the solubility of Ni was influenced more by Fe oxide than by Mn oxide because Ni was predominantly retained by amorphous and crystalline Fe oxides. Antić-Mladenović et al. (2017), who studied serpentine soil with similar pH values (6.54–6.78) to those in this study, indicated that redox-induced transformations of Fe oxides, rather than Mn oxides, affect Ni solubility.

Two hypothetical mechanisms can be proposed to explain why the solubility of Ni depends more closely on Mn than on Fe or other redox-sensitive species in this study. First, it is possible that Ni coprecipitated and dissolved preferentially in Mn oxides rather than Fe oxides, which could be attributable to the higher valence of Mn and larger surface area (Covelo et al., 2007; Borch et al., 2010; Friedich and Catalano, 2012; Frohne et al., 2014). Second, the Ni that coprecipitated with Fe oxides might not have been sufficiently dissolved during the flooding period in this study. We considered the latter hypothesis to be more appropriate for the environmental situation of this study field. The minimum value of Eh in this study was higher than that in previous studies (Antić-Mladenović et al., 2017; Shaheen et al., 2022) using serpentine soils under microcosm systems. The reason for the relatively high minimum Eh in this study may involve the high infiltration rate of terraced paddy fields (Huang et al., 2003). Soil Eh is higher in terraced paddy fields than in plain paddy fields and microcosm systems because of water runoff out of the system with downward infiltration. As an electron acceptor at the higher Eh stage by soil microorganisms, Mn oxides are used earlier than Fe oxides. The soil Eh may have been too high to enhance the complete dissolution of Fe oxides during the flooding period. We therefore considered it necessary to confirm the soluble Fe and soluble Ni concentrations in the soil solution when the soil Eh was further reduced.
Based on these results, we concluded that the maximum-soluble Ni concentration in the soil solution occurs during the flooding period when the soil is in the reduced state, and that this phenomenon is mainly caused by the dissolution of Mn oxides.

Factors determining the Ni uptake of rice

There is a general trend that the higher the concentration of dissolved essential nutrients in the soil solution, the greater the uptake by rice plants (Vlamis and Williams, 1964; Fageria, 1976; Pasricha, 1983; Uchida, 2000). Some previous studies (Robinson et al., 1999; Puschenreiter et al., 2005) suggested that Ni uptake by plants depends on Ni concentration in the soil solution as plants mainly take up Ni as Ni\textsuperscript{2+} (Deng et al., 2018). Altaf et al. (2022) reported that the Ni content of pepper increased as the Ni concentration in the nutrition solution increased. Thus, we first assumed that Ni uptake by plants would also increase with higher Ni concentrations in the soil solution. However, in this study, Ni uptake by shoots was increased during the drainage period rather than during the flooding period (Fig. 9), even though the concentrations of soluble Ni during the drainage period were more than ten times lower. Pinson et al. (2015) also reported that the Ni content in rice was higher under drained soil conditions than under flooded soil conditions. In any case, these results provide a clear indication that the concentration gradient of soluble Ni in the soil solution is not a primary factor in promoting Ni uptake by rice.

Two hypotheses can be proposed to explain Ni uptake in rice. First, the increased availability of Ni content in rhizosphere soils during the drainage period extracted by DTPA (DTPA-Ni) may contribute to Ni uptake by rice (Fig. 4). DTPA extraction is widely used for assessing the amount of Ni available for plant uptake (Echevarria et al., 1998; Echevarria et al., 2006). The DTPA-Ni fraction is in equilibrium with Ni in the soil solution and is readily soluble in the soil solution (Echevarria et al., 1998). Thus, because it is possible for DTPA-Ni to be taken up by plants, decreasing the soluble Ni concentration in soil solutions during the drainage period might not necessarily decrease the availability of Ni in whole soil.

Second, soluble Fe and/or soluble Mn in soil solution might competitively inhibit Ni influx into plant roots. This would occur because Ni is mainly absorbed via the low-affinity transport systems of other divalent micronutrient elements (Körne et al., 1987; Deng et al., 2018). Thus, we calculated the soluble Ni/Fe and Ni/Mn ratios for the soil solutions and bioavailable Ni/Fe and Ni/Mn ratios for DTPA extraction (Fig. 10). The results showed that only the soluble Ni/Fe ratio for the soil solution during the drainage period was significantly higher than that during the flooding period. The Ni/Fe ratio increased despite the decrease in the concentration of soluble Ni and Fe in the soil solution, possibly because Ni is indirectly affected by Eh via redox-sensitive species. Thus, not all the Ni\textsuperscript{2+} ions coprecipitate with Fe or Mn, and therefore remained at much higher concentration than Fe in the soil solution. The Ni uptake by shoots was enhanced during the period when the Ni/Fe ratio of the soil solution increased, indicating that the Ni/Fe ratio is more important for Ni uptake than the absolute concentration of available Ni in the soil. This indicates that Ni uses the Fe transport system and not an Ni-specific transport system. Some previous studies have suggested that Ni can be taken up by the Fe transport system in plants (Cataldo et al. 1978, Yang et al. 1996, Pandey and Sharma 2002) and that Ni antagonizes Fe when uptake occurs (Yang et al., 1996; Chen
IRT1 is one of the iron-regulated membrane proteins, i.e., an Fe transporter, and has been reported to be used for Ni uptake by some plants (Nishida et al., 2011; Halimaa et al., 2014). As rice also takes up Fe by IRT1 during the drainage period, it probably also takes up Ni. In contrast, the soluble and bioavailable Mn/Ni ratios were not related to Ni uptake in rice. Körne et al. (1987) also reported that Mn did not influence Ni influx into plant roots. Thus, we suggested that Ni uptake in rice may use the Fe transport system and therefore be enhanced during the drainage period, when the Ni concentration is relatively high compared with Fe.

Therefore, we considered that increasing the soluble Ni/Fe ratio in soil solutions and/or DTPA-Ni content in rhizosphere soil would determine the Ni uptake by rice; the former theory is particularly plausible because soluble Ni in soil solutions is more easily taken up by plants than DTPA-Ni (Adams and Kissel, 1989; Estrade et al., 2015).

Concluding remarks focusing on human exposure to Ni in rice

The transfer of Ni from the soil to rice in terraced paddy fields was found to be closely related to water management practices, which directly alter the solubility of Mn and Fe in soil. Nickel is an essential micronutrient and has the narrowest concentration range for optimal growth. Even though the biological mechanisms to enhance Ni transport within root systems remain elusive, these relationships are valuable for controlling the Ni concentration in rice grains.

The risk of human exposure to Ni from serpentine soil through rice consumption depends on the ability of rice plants to take up soil Ni and transfer it to the rice grain, as well as on the daily consumption of rice grown in that soil. The World Health Organization (WHO) recommends a provisional tolerable weekly intake (PTWI) of Ni of 0.035 mg kg$^{-1}$ body weight for adults (WHO 1994). In Japan, the daily rice consumption per adult is approximately 0.14 kg. By considering the average Ni concentrations of brown rice (6.2 mg kg$^{-1}$) in the present study fields (Fig. 8), the weekly Ni intake for an adult of 60 kg is 0.10 mg kg$^{-1}$ body weight through brown rice, considerably exceeding the PTWI. This assumption is not realistic in the research site because the landowner cultivates more than 50 terrace paddies, most of which are not affected by serpentinite and mixes rice harvested from all these fields before packaging. However, this assumption is important to predicting the risk of human exposure through rice consumption in other areas heavily affected by serpentinite (Hseu and Lai., 2017). In the agricultural areas located close to serpentinite geology, Ni intake from water and other foods (vegetables, meat, and milk) is also relevant, and the total Ni intake would be even higher (Miranda et al., 2009; Susaya et al., 2010; Toman et al., 2022). However, the effect of serpentinite on Ni concentration in rice grains has not been comprehensively investigated in Japan, and requires further investigation.

If there is a need to reduce Ni transfer to rice in serpentine-affected paddy fields, two possible countermeasures can be proposed based on the findings of this study: 1) prolonging the waterlogged period to maintain low Eh conditions to solubilize more Fe$^{2+}$ in the soil solution; and 2) supplementing fertilizers with Fe$^{2+}$ during the drainage period to increase the phytoavailable Fe content in soil. As the
former strategy causes a trade-off with methane emission from paddy fields, the latter is our first recommendation.

**Declarations**

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**Conflicts of interest** The authors declare that they have no conflicts of interest.

**References**


49. Lindsay, W. L. (1979). *Chemical equilibria in soils*. John Wiley and Sons Ltd.


**Figures**
Figure 1

Map of Yosano town, Kyoto Prefecture, Japan (a). A more detailed map of Yosano town (b). A picture of the three terraced paddy fields used in this study (c). The yellow boundary marks Yosano town; the red square indicates the terraced paddy fields used in this study; the purple areas indicate the serpentinite area; the blue lines indicate rivers; the blue areas show the three fields used for this study.
Figure 2

Solubility changes in Ni (µmol L\(^{-1}\)) (a), Fe (µmol L\(^{-1}\)) (b), Mn (µmol L\(^{-1}\)) (c), SO\(_4^{2-}\) (µmol L\(^{-1}\)) (d) and organic carbon (DOC) (mg L\(^{-1}\)) (e), and changes in pH (f) in soil solution. Nine samples collected per day; on some days, insufficient sampling was performed. Different letters indicate a significant difference on different days after transplanting (DAT). Gray areas indicate drainage periods.
Figure 3

Change in Eh (mV). Nine samples per day; on some days, sampling was insufficient. Different letters indicate significant differences between days. Gray areas indicate drainage periods.
Figure 4

Bioavailability changes in Ni (mg kg\(^{-1}\)) (a), Fe (mg kg\(^{-1}\)) (b), and Mn (mg kg\(^{-1}\)) (c) in rhizosphere soil extracted by DTPA. Nine samples per day. Different letters indicate a significant difference between values at different days after transplanting (DAT). Gray areas indicate drainage periods.
Figure 5

Relationship between the concentration of Ni (µmol L$^{-1}$) and the concentrations of Fe (µmol L$^{-1}$) (a), Mn (µmol L$^{-1}$) (b), SO$_4^{2-}$ (µmol L$^{-1}$) (c), and dissolved organic carbon (DOC) (mg kg$^{-1}$) (d) in the soil solution. Nine samples per day; on some days, insufficient sampling was performed. Different colored plots indicate the different sampling dates.
Figure 6

Relationship between the Ni content (mg kg\(^{-1}\)) and the content of Fe (mg kg\(^{-1}\)) (a) and Mn (mg kg\(^{-1}\)) (b) in rhizosphere soil extracted by DTPA. Nine samples per day. Different colored plots indicate the different sampling dates.
Figure 7

Change in shoot dry weight (DW) (g plant$^{-1}$). Fill indicates straw DW and shading indicates grain DW. Nine samples per day. Different letters indicate a significant difference between values AT days after transplanting (DAT).
Figure 8

Changes in the concentration of Ni (mg kg\(^{-1}\)) in straw (a), in grain (b), Fe (mg kg\(^{-1}\)) in straw (c), in grain (d), Mn (mg kg\(^{-1}\)) in straw (e) and Mn (mg kg\(^{-1}\)) in grain (f). Nine samples per day. Different letters indicate significant differences between days after transplanting (DAT). Gray areas indicate drainage periods.
Figure 9

Change in Ni uptake (g plant$^{-1}$) (a), Fe uptake (g plant$^{-1}$) (b), and Mn uptake (g plant$^{-1}$) (c) by shoot. Green indicates straw DW and pink indicates grain DW. Nine samples per day. Different letters indicate a significant difference between days after transplanting (DAT).
Figure 10

Changes in the Ni/Fe ratio in the soil solution (a), the Ni/Fe ratio in rhizosphere soil (b), the Ni/Mn ratio in the soil solution (c), and the Ni/Mn ratio in rhizosphere soil. Different letters indicate a significant difference between days after transplanting (DAT). Nine samples per day; on some days, insufficient sampling was performed. Gray areas indicate drainage periods.