The importance of soil solution chemistry to nickel toxicity on barley root elongation

Xiaoqing Zhang[a], Dongpu Wei[b], Bo Li[b], Yibing Ma[b*] and Zhanbin Huang[a]

[a]School of Chemical and Environmental Engineering, China University of Mining and Technology-Beijing, Beijing 100083, P.R. China

[b]National Engineering Laboratory for Improving Quality of Arable Land/Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 12 Southern Street of Zhongguancun, Beijing 100081, P.R. China

**E-mail: ybma@caas.ac.cn*

ABSTRACT

The influence of soil solution properties on nickel (Ni) toxicity and its effects on barley root elongation were investigated using 17 Chinese soils amended with Ni chloride, which may or may not have undergone leaching treatment. The Ni in the soil solution was recorded in the soil pore water and in the extraction with 0.01 M $CaCl_2$. The ranges of effective concentrations for $CaCl_2$ -extracted Ni that caused 10% root elongation inhibition (EC10) and for 50% inhibition (EC50) varied widely from 65 to 114 times between different soils. The EC10 and EC50 values for Ni toxicity thresholds in soil pore water for unleached and leached soils varied from 40 to 124 times. In most soils, the toxicity thresholds for Ni, based on 0.01 M $CaCl_2$ or soil pore water extractions, were not significantly affected by leaching. Regression models between toxicity thresholds and soil solution chemistry were developed and showed that Mg and S in soil pore water were the two most important factors affecting the extent of Ni toxicity on barley root elongation. However, pH showed only a weak correlation with toxicity thresholds.

Keywords: nickel, leaching, phytotoxicity, risk assessment, soil solution

INTRODUCTION

Current legislative frameworks for soil pollution focus predominantly on total metal content. However, the environmental risks posed by metals are not only a function of their overall presence in soils, but also of their chemical speciation. There have been a number of studies into the effects of metal toxicity on plants (Jolanta and Stanislaw, 2004; Daoust *et al*., 2006; Lock *et al*., 2007a; Warne *et al*., 2008; Papazoglou, 2011). For example, Rooney *et al*. (2007) reported that soil cation exchange capacity was the best single predictor for the median effective concentration (EC50) of added nickel (Ni) and the EC50 ranged from 52 to 1929 mg kg^{-1} and from 17 to 920 mg kg^{-1} in tests on barley and tomato plants, respectively. Li *et al*. (2011) also developed phytotoxicity models for Ni using 17 Chinese soils and showed that soil pH, soil organic carbon content and effective cation exchange capacity were good predictors of toxicity thresholds, with the EC50 ranging from 48 to 2519 mg kg^{-1} for barley root elongation. It has been shown that soil properties significantly influenced Ni toxicity on plants by altering its chemical forms/species in the soils and that soil solution chemistry was important for Ni speciation in soils. Dunemann *et al*. (1991) studied Ni speciation in three different soil solutions, with and without sludge amendments, in relation to the availability of Ni to oat plants. They showed that Ni availability was affected by the species of Ni that was present, which differed considerably between soil types. More recently, Van Laer *et al*. (2006) and Nolan *et al*. (2009) used the Donnan membrane technique (DMT) and Windermere humic aqueous model (WHAM) VI to determine the Ni speciation in surface water and in the pore water of contaminated soils, respectively, and their results showed that the Ni species distribution could be influenced by solution chemistry, especially the sulfate concentration and pH. In summary, artificial solutions, rather than real soil solutions, were used to investigate the relationships between the solution chemistry and Ni toxicity. Lock *et al*. (2007b) revealed that increased Mg^{2+} concentration linearly decreased soluble

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Ni toxicity while Ca^{2+}, Na^{+}, K^{+} and H^{+} did not significantly influence the Ni toxicity on barley root elongation in nutrient solution. Li *et al*. (2011) also reported the Ni toxicity to barley root elongation decreased with increases in Mg^{2+} and Ca^{2+} concentration but not for Na^{+} and K^{+} in solution culture. For European soils, Rooney *et al*. (2007) reported that the EC50, based on the Ni concentration in soil solution, showed little variation between soils and suggested that Ni solubility is, instead, a key factor influencing its toxicity to plants. However, there have been few studies into the influence of soil solution chemistry on Ni phytotoxicity.

Amending soils with soluble salts, such as chloride, nitrate, or sulfate salts, reduced the pH and led to a higher salinity, which could increase the toxicity of Ni added to soils. Oorts *et al*. (2007) and Li *et al*. (2011) found that leaching decreased Ni phytotoxicity significantly. Stevens *et al*. (2003) studied the phytotoxicity of anions and found that they indirectly increased both the metal concentrations in the soil solution and metal bioavailability. Therefore, to overcome this potential salinity effect, soils should be leached after contamination to achieve a more realistic exposure situation for assessing metal toxicity to plants, as would be more relevant under field conditions.

In the present study, bioassays of barley root elongation were undertaken in 17 Chinese soils with added Ni and with or without leaching. The aims of the present study were: (1) to quantify the effect of leaching on soluble Ni toxicity; (2) to establish empirical relationships between soil pore water properties and soluble Ni toxicity under leached and unleached conditions and (3) to determine the main factors in pore water influencing soluble Ni phytotoxicity.

MATERIALS AND METHODS

Soil samples and treatments

Seventeen soil samples (0–20 cm depth) were collected from latitude 19°55'N to 47°28'N and longitude 87º46'E to 126°57'E, which covered the main areas of China. The map of sampling sites and properties of the soils have been reported in detail by Li *et al*. (2011). The ranges of the main soil properties were as follows: pH 4.93–8.90; organic carbon content (OC) 0.60–4.28%; cation exchange capacity (CEC) 6.36–33.59 $cmol^{+}\ kg^{-1}$ and clay content 10–66%. The soils were representative of the major types of agricultural soils in China. The collected 17 soil samples before amendment with Ni solutions have not been polluted by other heavy metals (Cu, Zn, Pb, As, Cd and Cr).

The soil samples were air-dried and sieved through a < 2 mm screen. Then they were taken in triplicate, amended (50 mL kg^{-1} soil) with $NiCl_2$ solution with eight concentrations from 4.261×10^{-3} to 8.18×10^{-1} M separately and thoroughly mixed again to produce nominal concentrations of: 37.5, 75, 150, 300, 600, 1200 and 2400 mg Ni/kg for soils with pH > 7; 25, 50, 100, 200, 400, 800 and 1600 mg Ni/kg for soils with pH 5 to 7 and 12.5, 25, 50, 100, 200, 400 and 800 mg Ni/kg for soils with pH < 5. Then the amended soils were incubated for 2 days at 100% maximum water holding capacity (MWHC) (Jenkinson and Powlson, 1976), air-dried and sieved to < 2 mm using plastic mesh.

For each Ni treatment and each soil, samples of the amended soils were leached using artificial rainwater, prior to the barley root elongation bioassay, to overcome the potential salinity effect and to reduce the difference in Ni toxicity between laboratory treated and aged field soils (Stevens *et al*., 2003; Oorts *et al*., 2007; Li *et al*., 2011). A detailed description of the composition of the artificial rainwater and a protocol for the preparation of the leached samples have been reported by Li *et al*. (2011). All unleached and leached soil samples were air-dried, sieved through < 2 mm mesh and then stored at room temperature before bioassay.

Soil pore water samples (two replicates) were extracted according to Thibault and Sheppard (1992) by centrifugation of the soil samples after incubation overnight at 50 cm water tension and 20°C. The extracted soil pore water was passed through 0.45 mm filters. The Ni extraction, using 0.01 M $CaCl_2$ solution, was undertaken using an air-dried soil in a soil solution ratio of 1:5 (m/V). The soil and 0.01 M $CaCl_2$ solution mixtures were shaken for 30 minutes and centrifuged for 15 minutes at a speed of 3500 r/min. The supernatants were passed through 0.45 mm filters. The dissolved organic carbon (DOC), pH, electrical conductivity (EC), Na^{+}, K^{+}, Ca^{2+}, Mg^{2+} and SO_4^{2-} concentrations in the soil pore water samples were measured. The pH and EC of the pore water were measured using a microelectrode pH and EC meter (Thermo Fisher Scientific Inc., New York, USA). The concentration of DOC in the solutions was determined using a Formacs SERIES TOC/TN Analyser (Skalar Ltd., Breda, the Netherland). The concentrations of a number of positive ions (K^{+}, Ca^{2+}, Na^{+}, Mg^{2+}, Ni^{2+}) of the solutions were measured either by inductively coupled plasma–atomic emission spectrometry (Spectro flame Modula, Spectro, Boschstr, Kleve, Germany) or inductively coupled plasma–mass spectrometry (Spectro flame Modula, Spectro, Boschstr, Kleve, Germany) depending on their concentrations in the solutions (Zarcinas *et al*., 1996). Inorganic anions (Cl^{-}, NO_3^{-}, SO_4^{2-}) concentrations were measured by ion chromatography (IC; Dionex 4000i, AS9-HC column, Sunnyvale, CA, USA). The main properties of the unleached soil pore water samples are shown in Table 1. However, the leached soil pore water characteristics are not listed here.

Barley root elongation bioassay

The barley root elongation bioassay was performed according to ISO 11269-1(1993). Four pregerminated barley seeds (*H. vulgare cv.* Pinggu No.1) with radicles <2mm in length were planted in each of three replicate pots for each Ni treatment. After 5 days, plant roots were removed intact from soils and the length of the longest root of each plant recorded. All the detail procedures for the barley root bioassay have been described by Li *et al*. (2011). The percentage difference in barley root elongation compared to the controls (RE,%) in a test medium was calculated using the equation:

Table 1 Properties of the unleached soil pore water samples used in the present study before being amended with Ni

Soil no.	Location	pH	EC (mS cm^{-1})	DOC (mg L^{-1})	Ca (mg L^{-1})	K (mg L^{-1})	Mg (mg L^{-1})	Na (mg L^{-1})	S (mg L^{-1})
S1	Beijing	7.89	0.858	214	294	28.1	50	11.1	42
S2	Chongqing	7.88	0.976	235	187	2.4	15.8	20	75
S3	Gansu	8.29	1.443	302	310	12	99.7	99.4	150
S4	Guangzhou	8.05	1.83	313	390	36	23.2	59.3	210
S5	Hailun	7.41	0.543	131	114	0.8	28	20.6	46.5
S6	Hainan	6.47	1.081	98.4	60.9	53.6	20	17.6	3.66
S7	Hangzhou	7.32	2.675	280	525	40	92.3	155	272
S8	Hunan	5.11	1.266	79.1	202	17.7	23.1	45.5	29.1
S9	Jiaxing	7.48	2.502	163	369	8.19	85.7	155	125
S10	Jilin	8.15	0.926	226	246	4.4	22.8	15.7	75
S11	Langfang	8.3	0.835	143	140	18	21	33.1	24.2
S12	Neimeng	7.6	9.46	239	322	20	354	1925	690
S13	Shandong	8.17	2.192	207	295	3.2	108	285	120
S14	Shanxi	8.2	0.845	52.6	176	6.53	13.1	10.1	32.1
S15	Shijiazhuang	8.25	2.347	235	560	6	72	50.4	255
S16	Xinjiang	8.35	2.021	294	341	40	63.7	433	315
S17	Zhengzhou	8.2	0.97	94.3	118	<2	27	55	48

$$RE = \frac{RE_t}{RE_c} \times 100$$

where RE_t is the root length in the test medium and RE_c is the root length in the control without added Ni.

Chemical speciation

The chemical speciation model of WHAM VI was used in this study (Tipping, 1998a, b). Dissolved cation (Ni^{2+},Ca^{2+}, Mg^{2+}, K^+ and Na^+) and anion (CO_3^{2-}, Cl^-, NO_3^- and SO_4^{2-}) concentrations in the soil pore water samples were entered into the model with the exception of Fe^{3+} and Al^{3+}. The activities of Fe^{3+} and Al^{3+} were calculated according to Tipping *et al*. (2003). In order to input organic matter into the model, dissolved organic matter (DOM) was assumed to consist of a certain fraction of active fulvic acid (FA) and a fraction that was not available for ion binding. It was also assumed that the organic matter all consisted of FA (Tipping *et al*., 1998a and b). The concentration of DOM was roughly twice that of DOC in natural water and it was assumed that about 65% of DOM was active (Van Laer *et al*., 2006) and thus FA_{input} was calculated as DOC $\times$ 2 $\times$ 65%. The CO_2 pressure was maintained at 3.8×10^{-4} atm.

Data statistical analysis

The EC10 and EC50 values were calculated using the log–logistic dose–response curves based on the measured soluble Ni concentrations in both the $CaCl_2$ extraction solutions and in the pore water samples from unleached and leached soils. Their 95% confidence intervals were derived from the fitted curve parameters according to Haanstra *et al*. (1985). The regressions between toxicity thresholds (EC10 and EC50) and the properties of soil pore water were analysed using SPSS 19.0 for Windows (SPSS, Chicago, IL, USA). Parameters were only log transformed if it was found necessary after testing the data for normality and homogeneity of variance. Relationships were deemed significant at $P \leq 0.05$.

RESULTS

Toxicity thresholds for soluble Ni in unleached and leached soils

When considering the toxicity thresholds for Ni extracted by 0.01 M $CaCl_2$, it was found that the EC10 and EC50 values for unleached and leached soils varied widely from 65 to 114 fold differences (Table 2). For example, the EC50 ranged from 1.32 (S13) to 89.57 mg kg^{-1} (S9) in leached soils and from 0.94 (S14) to 60.87 mg kg^{-1} (S5) in unleached soils, respectively. Similarly, the toxicity thresholds for Ni in soil pore water showed that the EC10 and EC50 values for unleached or leached soils also varied considerably from 40 to 124 fold differences (Table 3). For example, the EC50 values varied from 0.8 (S13) to 70.8 mg L^{-1} (S12) in leached soils and from 1.6 (S14) to 64.2 mg L^{-1} (S9) in unleached soils, respectively. In the same set of soils, Li *et al*. (2011) reported that the EC50 values of soils spiked with Ni ranged from 48 to 2519 mg kg^{-1} (52.4 fold difference) in unleached soils. These results showed that the toxicity thresholds of both Ni extracted by 0.01 M $CaCl_2$ and in soil pore water ranged more widely than previously records and showed that the concentrations of Ni extracted by 0.01 M $CaCl_2$ and soil pore water were not a simple way to estimate the toxicity of Ni in a wide range of soils.

The toxicity thresholds for Ni based on 0.01 M $CaCl_2$ extraction and soil pore water were not strongly affected by

Table 2 Toxicity thresholds, measured by barley root elongation, for soil Ni extracted by 0.01 M $CaCl_2$ (mg kg^{-1}).

Soil no.	$CaCl_2$-extractable Ni（unleached soil）				$CaCl_2$-extractable Ni（leached soil）			
	EC10	95% CI[a]	EC50	95% CI	EC10	95% CI	EC50	95% CI
S1	6.5	4.5–9.4	27.4	23.6–31.8	17.3*	13.0–23.1	56.2*	47.5–66.5
S2	9.0	5.8–14.0	25.0	17.2–36.3	11.4	8.5–15.3	51.5*	45.4–58.4
S3	4.6	3.5–6.1	8.5	7.9–9.2	>20.86	NC	NC	NC
S4	4.5	2.3–8.6	16.1	8.9–28.8	25.0	5.6–112.4	58.1*	29.6–114.1
S5	26.2	16.9–40.5	60.9	50.6–73.3	23.8	13.3–42.8	84.5	61.4–116.4
S6	3.4	1.1–10.5	19.3	13.3–28.1	0.8	0.3–2.0	8.8	5.9–13.1
S7	13.6	9.8–18.8	51.7	42.6–62.7	15.9	11.0–22.9	66.6	57.4–77.2
S8	6.2	2.9–13.4	36.2	25.8–50.7	5.7	3.2–10.2	31.2	23.9–40.7
S9	20.0	14.0–28.5	59.6	50.5–70.4	23.3	15.0–36.3	89.6*	74.6–107.5
S10	2.7	0.4–18.6	8.9	5.5–14.4	4.8	2.9–8.2	11.4	9.7–13.6
S11	1.0	0.4–2.8	3.0	1.9–4.7	18.6*	9.3–37.1	43.2*	29.9–62.5
S12	13.6	4.1–44.9	48.5	29.2–80.5	25.4	17.2–37.6	82.1	67.6–99.8
S13	2.3	0.2–22.6	NC	NC	0.3	0.1–1.2	1.3	0.7–2.6
S14	0.2	0.1–0.6	0.9	0.6–1.4	0.4	0.0–69.0	NC	NC
S15	0.7	0.5–1.0	2.8	2.3–3.3	>3.29	NC	NC	NC
S16	0.6	0.5–0.9	1.8	1.5–2.1	0.4	0.0–475	NC	NC
S17	0.5	0.2–1.0	3.8	2.7–5.2	>2.08	NC	NC	NC

[a]Ranges given as 95% confidence intervals (CI).
NC: Toxicity thresholds could not be calculated because the highest soluble Ni dose measured did not result in 10% or 50% inhibition;
*Significant difference between unleached or leached EC10 and EC50 using a *t*-test at the $P \leq 0.05$ significance level.

Table 3 Toxicity thresholds, measured by barley root elongation, for Ni in soil pore water (mg L-1) for 17 Chinese soils.

Soil no.	Pore water Ni（unleached soil）				Pore water Ni（leached soil）			
	EC10	95% CI[a]	EC50	95% CI	EC10	95% CI	EC50	95% CI
S1	4.3	2.5–7.2	23.9	19.6–29.2	8.3	5.2–13.1	35.7	27.7–46.1
S2	5.5	3.0–10.1	20.9	12.6–34.6	2.1	1.1–4.3	18.8	13.7–25.7
S3	6.3	3.8–10.5	18.6	16.2–21.4	>18.1	NC	NC	NC
S4	1.9	0.8–4.3	12.3	6.3–23.7	14.8	0.0–396747	34.4	0.5–2427
S5	8.2	1.1–61.8	28.0	18.4–42.7	8.0	4.6–14.1	24.6	20.3–29.8
S6	0.9	0.0–28.0	4.9	1.9–12.4	0.2	0.0–35.4	1.9	0.4–8.2
S7	11.4	7.1–18.3	61.7	49.0–77.8	8.5	3.7–19.8	66.3	47.1–93.3
S8	2.1	0.5–8.6	21.1	11.5–39.1	2.8	1.1–7.4	17.9	11.7–27.3
S9	16.1	10.6–24.4	64.2	53.6–76.9	8.9	2.2–35.4	57.5	23.7–139.0
S10	0.3	0.1–0.9	4.1	2.4–7.3	1.9*	1.0–3.8	9.9*	7.5–12.9
S11	0.7	0.2–2.7	5.1	3.0–8.7	6.3*	2.9–13.9	14.7*	9.9–21.9
S12	14.3	4.4–46.2	52.9	29.6–94.5	22.1	14.0–34.7	70.8	56.7–88.5
S13	4.1	0.1–211.1	NC	NC	0.4	0.2–0.9	0.8	0.4–1.5
S14	0.1	0.0–1.3	1.6	0.6–4.2	0.2	0.0–209933.7	NC	NC
S15	0.7	0.3–1.4	6.4	4.7–8.9	>1.57	NC	NC	NC
S16	0.5	0.4–0.6	3.2	2.9–3.5	0.4	0.0–30.6	NC	NC
S17	0.4	0.1–1.2	7.2	4.5–11.3	>2.07	NC	NC	NC

[a]Ranges given as 95% confidence intervals (CI).
NC: Toxicity thresholds could not be calculated because the highest soluble Ni dose measured did not result in 10% or 50% inhibition;
*Significant difference between unleached and leached EC10 or EC50 using *t*-tests at the $P \leq 0.05$ significance level.

leaching. In $CaCl_2$ extraction solutions, leaching was found to significantly ($p \leq 0.05$) increase EC10 in only two soils (S1 and S11) and EC50 values in just five soils (S1, S2, S4, S9 and S11). For soil pore water, EC10 and EC50 values increased significantly in the S10 and S11 soil samples. In other soils, the EC10 or EC50 for Ni in the 0.01 M $CaCl_2$ extraction samples and in the pore water samples from leached soils were generally similar or larger than those from unleached soils (Figures 1 and 2) but the differences were not significant, which indicated that the toxicity of Ni extracted by the 0.01M $CaCl_2$ solutions and by the pore water did not significantly decrease after leaching.

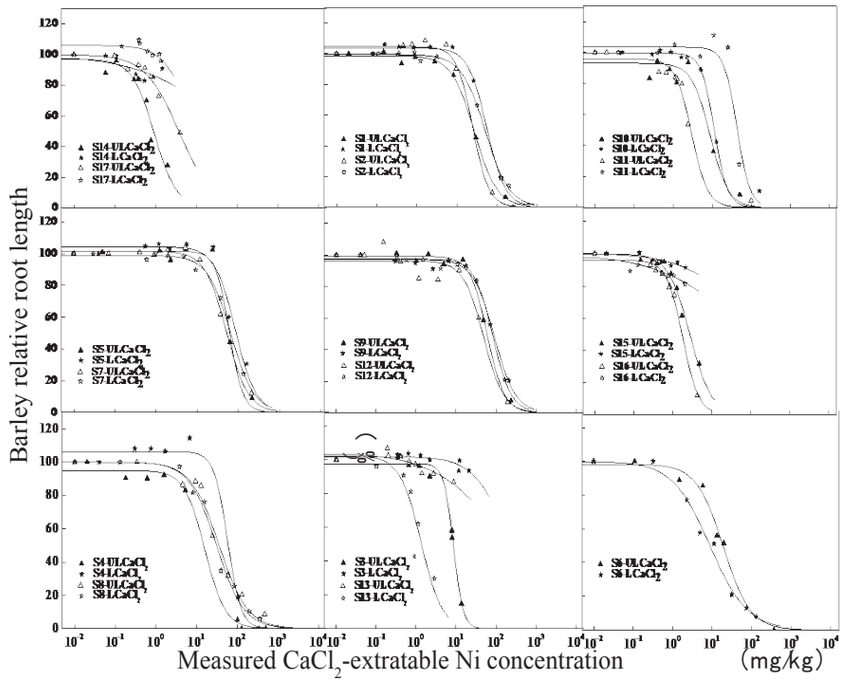

Figure 1 Dose–response curves for Ni concentrations in the 0.01 M $CaCl_2$ extraction samples for barley root elongation in 17 unleached and leached soils. Symbols represent all replicated data points, and lines are the fitted log–logistic curves. UL and L represent unleached and leached soils, respectively.

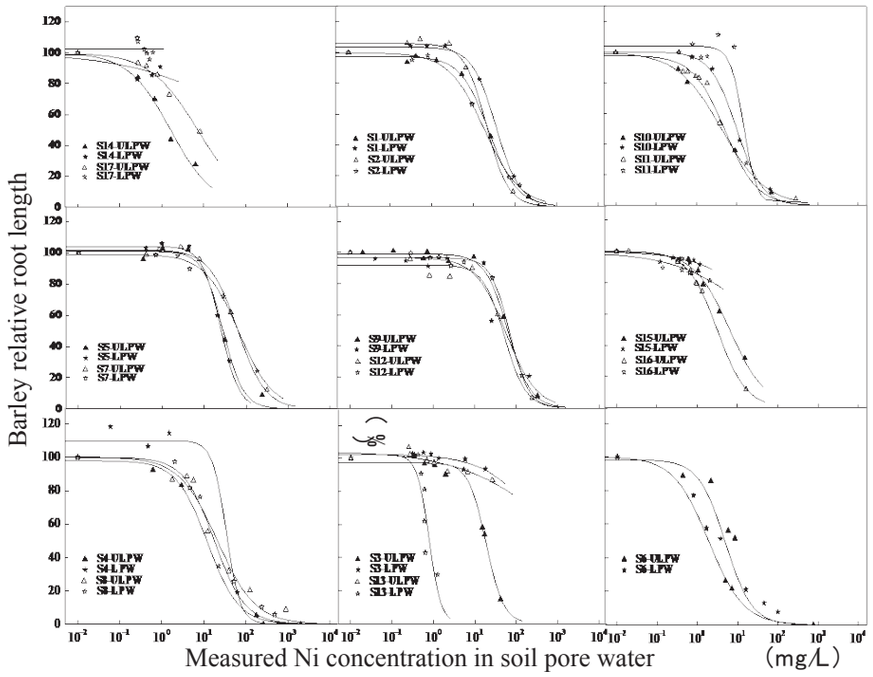

Figure 2 Dose–response curves for Ni concentrations in soil pore water samples for barley root elongation in 17 unleached and leached soils. Symbols represent all replicated data points, and lines are the fitted log–logistic curves. UL and L represent unleached and leached soils, respectively.

Soil solution factors influencing the soluble Ni toxicity on barley root elongation

Multiple regression analysis was applied in order to derive the relationship between pore water properties and soluble Ni toxicity thresholds under the leached and unleached treatments. Since not all the regression models provided significant factors in the prediction equations, the linear regression models are presented in Appendix Tables A1, A2, A3 and A4. The regression equations, 1 to 6, are presented in Table 4. The equations contain some major significant variables that help explain the thresholds. When pH, EC, DOC, Na^+, K^+, Ca^{2+}, Mg^{2+} and S data from the unleached and leached soil pore water samples, without addition of Ni, were incorporated into the regression models, together with corresponding unleached and leached toxicity thresholds for Ni from the 0.01M $CaCl_2$ extraction samples and pore water samples, the coefficient of determination (r^2) for EC50 or EC10 ranged from 0.31 to 0.91 in leached and unleached soil (Appendix Tables A1, A2, A3 and A4). The r^2 value for EC50 and EC10 for Ni from the 0.01 M $CaCl_2$ extraction samples ranged from 0.07 to 0.79 in the leached and unleached soils (Appendix Tables A3 and A4). The results showed that the r^2 values for EC10 and EC50 were considerably higher in the pore water samples than in the $CaCl_2$ extraction samples (Appendix Tables A1 and A2), which was probably due to the changes to the soil solution chemistry by the $CaCl_2$ extraction process. The toxicity thresholds for Ni in the pore water samples were significantly related to the properties of the soil pore water. Under the same conditions, the predictability of the soluble Ni by empirical models increased after leaching because the r^2 values for leached soils were appreciably larger than those for unleached soils, which implied that the soluble Ni thresholds in leached soils were strongly related to properties of the soil pore water.

In contrast, the results showed that the toxicity thresholds for Ni in the $CaCl_2$ extraction samples did not depend as much on the pore water properties. Therefore, this study only focused on the relationship between pore water chemistry and the toxicity thresholds for Ni in the pore water samples. The regression results showed that S, Mg and Na were found to be significantly correlated to Ni toxicity thresholds (EC50) in the pore water obtained from the leached soil samples (r^2 = 0.90). Furthermore, S could also explain 59% of the variance of EC50 seen in the leached soil samples and was also the best predictor for EC10 of Ni in soil pore water in the leached soil samples (r^2 = 0.67). Mg could explain 32% and 39% of variance of EC50 and EC10 of Ni in soil pore water from the unleached soil samples, respectively. From Eqn (1), S was the most important variable, followed by Mg, while Na was the least important variable. Furthermore, when Eqns (4), (5) and (6) were taken into account, Mg and S were the most significant predictors of Ni toxicity while the thresholds values for Ni were found not to depend on Na to any great extent. Therefore, Mg and S had the most significant protective effects against Ni toxicity in pore water obtained from leached and unleached soils.

Rooney *et al*. (2007) showed that EC10 or EC50 values based on soluble Ni were significantly inversely related to soil pH for barley and Oorts *et al*. (2006) reported that Ni thresholds based on free ion activity decreased as pH increased. However, in this study, there was only a weak correlation between pH and soluble Ni toxicity. When incorporating pH into the regression analysis, the EC10 or EC50 for Ni in the 0.01M $CaCl_2$ extraction samples and the pore water samples from the leached and unleached soils decreased as pH increased, with the exception of the EC10 value for Ni in the unleached soil pore water samples, similarly to the results of Rooney *et al*. (2007), which meant that Mg^{2+} and S concentrations varied widely and had a greater influence on soluble Ni toxicity than did pH.

DISCUSSION

Influence of soil leaching on soluble Ni toxicity

The results of this study showed that leaching led to a decrease in the soluble Ni concentrations and thus significantly decreased the toxicity thresholds based on added Ni (Li *et al*. 2011). However, leaching did not significantly influence

Table 4 Simple and multiple linear regressions between Ni toxicity thresholds based on Ni concentrations in soil pore water and pore water chemistry (mg L^{-1})

Regression equation	r^2	p		
Toxicity thresholds of Ni in leached soil pore water , compared with leached soil pore water chemistry				
1 EC50 = -3.62 + 0.44Mg–0.16Na + 0.45S （n = 12）	0.904	0.026	< 0.001	< 0.001
2 EC50 = 6.81-0.13Na + 0.50S（n = 12）	0.815	< 0.001	0.009	—
3 EC50 = 16.12 + 0.17S（n = 12）	0.585	0.004	—	—
4 EC10 = 2.40 + 0.05S（n = 14）	0.673	< 0.001	—	—
Toxicity thresholds of Ni in unleached soil pore water , compared with unleached soil pore water chemistry				
5 EC50 = 12.75 + 0.14Mg（n = 15）	0.313	0.03	—	—
6 EC10 = 2.18 + 0.04Mg（n = 16）	0.390	0.01	—	—

r^2, coefficient of determination (percentage of variance accounted for by the regression model); *P*, significant level; EC50, median effective concentration value and EC10, 10% of effective concentration value.

soluble Ni toxicity thresholds in most soils. Similarly, Stevens *et al*. (2003) reported that there were no differences in water-extractable Zn concentrations at the EC50 level for lettuce, except in one of the five soil samples. Leaching can change the properties of soil pore water. In the present study, the pH in soil pore water slightly increased and the Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and S concentrations decreased after leaching in most of the soils tested (Figure 3) but the disparity was not large. For example, the concentration of Na^{+}, which can lead to reduced plant growth, did not vary widely after leaching (Figure 3) and thus had little influence on the soluble Ni threshold values in the leached soils. The ECs of the soils were less than the salinity critical limits, with the exception of S12, so salt effects were not evident in most soils. Nevertheless, leaching had flushed away a small amount of the anions and cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) associated with the soil samples amended with Ni. This had negative effects on barley root elongation and consequently the toxicity thresholds for soluble Ni in the 0.01M $CaCl_2$ extraction samples and pore water samples increased slightly after leaching.

In theory, the effective soluble Ni in a soil solution should change to solid retainable Ni when additional Ni is initially added to soil. The effective soluble Ni in the soil solution should be prone to loss by leaching and this partly changes the dynamic process of absorption and desorption between effective soluble Ni and solid retainable Ni and thus the reaction is reversed. Therefore a difference in Ni distribution coefficients for solid state Ni and Ni in solution (Kd) existed between the leached and unleached soils. Kd has been found to be significantly related to pH (Tipping *et al*., 2003), increasing as the pH increased. This means that in high pH soils, the difference in Kd between leached and unleached soils is much higher than in low pH soils. In this study, leaching caused a slight pH increase (Figure 3) in the leached soils, which caused the soluble Ni^{2+} to decrease to a greater extent in the higher pH soils compared to the lower pH soils and the toxicity thresholds could not calculated in these higher pH soils.

The EC of the soil pore water was a reliable indicator of the solute (cation or anion) concentrations. Previous research indicated that there was a significant negative effect of EC on metal toxicity in unleached soils (Stevens *et al*., 2003). However, no such effect for EC was seen in this study. The reason may be that the cations and anions had different fractions in the pore water and functioned differently in influencing the soluble Ni toxicity thresholds. The variation in these cations and anions has been found to directly affect the change in EC (Stevens *et al*., 2003; Staunton, 2004).

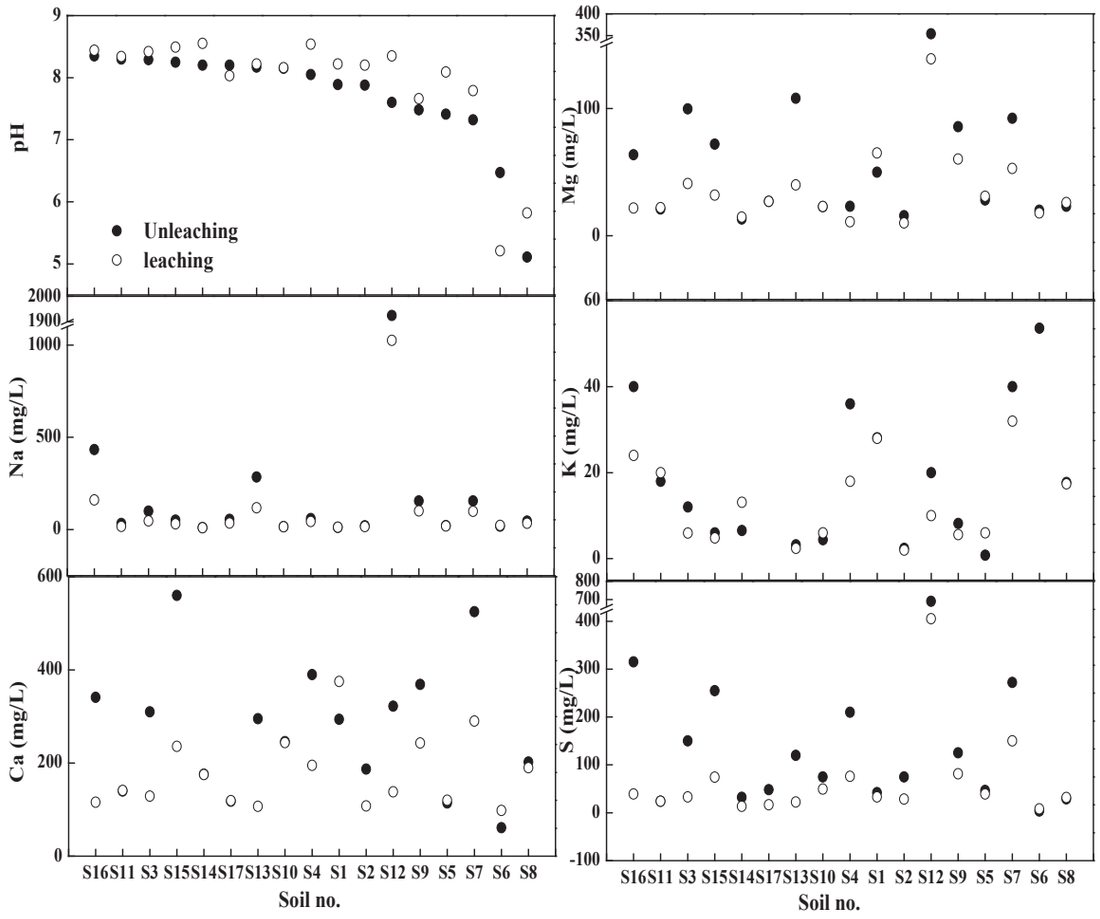

Figure 3 The relationship between pH, Mg, Na, K, Ca and S in soil solutions from the leached and unleached treatments.

Therefore, in an ideal soil solution, such as an artificial water solution culture with a simple proportion of cations and anions, the EC would probably show a strong relationship between with toxicity thresholds of unleached Ni in the $CaCl_2$ extraction samples and the pore water samples.

The effect of soil solution properties on soluble Ni toxicity

In the present study, the relationship between pH and EC10 or EC50 was not significant, although it was negative in the multi-factor regression analysis. However, Rooney *et al*. (2007) found consistent relationships between EC50 or EC10 based on soil solution Ni concentration and soil solution pH for barley root elongation. The coefficient was negative and significant (log EC50 = 2.05–0.11 soil pH, r^2adj = 0.49, n = 16; log EC10 = 1.68–0.15 soil pH, r^2adj = 0.38, n = 16). In a solution culture, the predictive ability of models was improved by incorporation of soil DOC, EC, pH, Na^+, K^+ and Ca^{2+}. Kozlova *et al*. (2009) found that Ca^{2+} and Mg^{2+} cations in solution had a protective effect on Ni toxicity with *Daphnia pulex*. Deleebeeck *et al*. (2008) also found that both Ca^{2+} and Mg^{2+} reduced acute Ni toxicity to *Daphnia magna* in a series of 48 h synthetic test solutions while Na^+ did not. Li *et al*. (2009) showed that Ni toxicity to barley root elongation in solution culture decreased with increases in Mg^{2+} and Ca^{2+} activities but not for Na^+ and K^+ activities. Higher H^+ activity decreased toxicity either through H^+ competition with Ni^{2+} bound to biotic ligands at pH < 7.0 or through changes to the Ni species found in solution at pH > 7.0, and meanwhile, H^+ could also decrease Ni^{2+} activities at the cell membrane surface (Wang *et al*., 2011).

A fraction of soluble Ni was complexed with DOM. While the DOM concentration was smaller in 0.01 M $CaCl_2$ extraction than that in soil pore water because it undergone the aggregation, dilution and sorption of Ca–DOM complexes on the solid phase in the process of $CaCl_2$ extraction. Moreover, the Ca^{2+} in $CaCl_2$ extraction would exchange the Ni^{2+} on the soil solid surface complexes and the Cl^- anions would form Ni-Cl complexes. Therefore, these could change soluble Ni speciation and also the properties of the soil solution, such as soil pH. While the properties of soil pore water in unleached and leached soils were found to be related to the soluble Ni thresholds. The relationships between the EC50 values and Mg^{2+} and S in leached and unleached soils are shown in Figure 4. The results revealed that Mg^{2+} was positively correlated with EC50, which implied that soluble Ni became less toxic with increasing soil solution Mg^{2+} concentration. However, although the Mg^{2+} and S concentrations in the S12 unleached soil were highest, the EC50 value was not the highest (Figure 4). This was because the EC of the soil was 9.46 mS cm^{-1}, which exceeded the salinity tolerant value of 4–5 mS cm^{-1} (Mass and Hoffman, 1977) and therefore the barley root elongation may have been inhibited by salinity damage. Mg^{2+} has been found to be a more important variable than the other cations (such as Na^+, Ca^{2+}, K^+) in affecting Ni toxicity to barley root elongation (*Hordeum vulgare*) (Lock *et al*., 2007; Li *et al*., 2011). Deleebeeck *et al*. (2009) investigated the protective effects of Mg^{2+} and increasing Mg concentrations were observed to decrease Ni toxicity to the green alga *Pseudokirchneriella subcapitata*. Wang *et al*. (2011) constructed an electrostatic toxicity model in order to predict the toxicity of Ni in hydroponic and soil solutions and found that Mg^{2+} also specifically alleviated Ni toxicity.

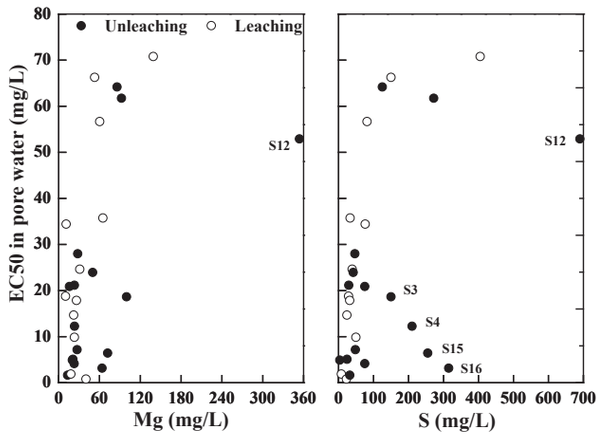

Figure 4 The relationship between soluble Ni EC50 values and Mg^{2+} and S in soil pore water from unleached and leached soils.

The concentrations of S in soil pore water were significantly positively related to EC50, which indicated that soluble Ni became less toxic with increasing S concentration, except for the unleached soils: S3, S4, S15 and S16. The toxicity thresholds for Ni in the pore water obtained from the unleached soils: S3, S15 and S16, may have been overestimated because of the increase in the anion (Cl^-) concentration when high doses of Ni are added to soils. There was no doubt that free metal ions were the dominant species available to biota. However, other Ni solution species, such as $NiCO_3^0$ and $NiHCO_3^+$, have also been found to be available to biota (Hoang *et al*., 2004; Li *et al*., 2009). In order to determine if Ni speciation could be used to explain the influence of S concentration on Ni phytotoxicity in pore water, three field soils with pH values 5.0, 7.2 and 8, respectively, were selected in order to analyse the influence of S on Ni species distribution in pore water. Soil pore water parameters were measured for predicting the Ni speciation concentration using WHAM (Tipping, 1998a, b; Weng *et al*., 2001; Nolan *et al*., 2009). The calculated distribution of Ni species (%) at the three sites is shown in Figure 5. When pH = 5.0, the free Ni fraction sharply decreased as the concentration of SO_4^{2-} in solution increased (Figure 5a). When pH = 7.2, the free Ni^{2+}, $NiCO_3^0$ and $NiHCO_3^-$ fractions decreased as the concentration of SO_4^{2-} increased (Figure 5b). When pH = 8.1, free Ni^{2+}, $NiCO_3$ and $NiHCO_3^-$ fractions declined slightly and the $NiSO_4$ fraction slightly increased (Figure 5c). These results could explain how S influences the Ni speciation distribution leading, in turn, to a decrease in soluble Ni toxicity. The results also suggested that S was not the only factor influencing the Ni species distribution because it was also changed significantly by the pH value.

Some studies about the protective abilities of cations against Ni toxicity were derived from nutrient solutions

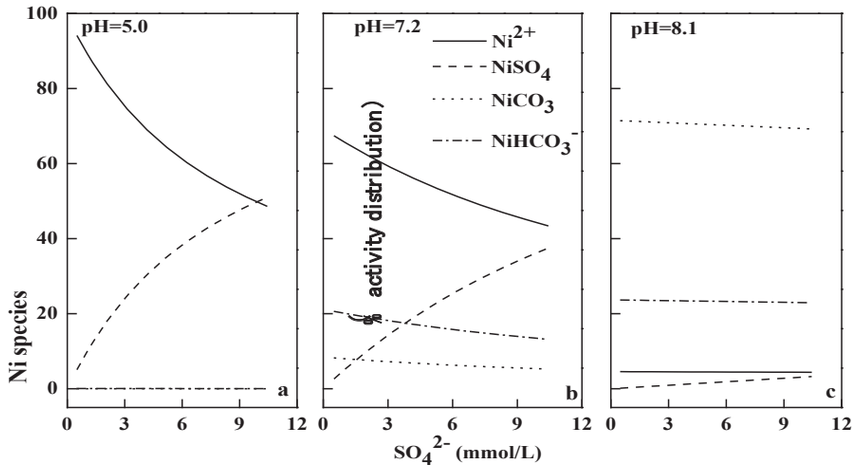

Figure 5 Calculated Ni species (% distribution) are influenced by the S concentration in pore water.

or from surface water (Li *et al*., 2009; Lock *et al*., 2007b; Deleebeeck *et al*., 2009). In the present study, the protective effect of Mg has been seen in real soil solutions. This indicated that the results acquired from nutrient solutions could be applied to real soil solutions and the results from the previous study could be used to provide references for real soil solution research.

CONCLUSIONS

Soluble Ni in the 0.01M $CaCl_2$ extraction samples and pore water samples did not predict Ni toxicity accurately because it was also influenced by the interaction between chemical substances in the soil solutions, particularly Mg^{2+} and S. The results showed that pH had a relatively weak relationship with soluble Ni toxicity compared with Mg^{2+} and S. Soluble Ni toxicity decreased with increasing concentration of Mg^{2+} and S. This study therefore recommends that $MgSO_4$ should be applied to soils to decrease the Ni toxicity in the field.

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Appendix Table A1 Simple and multiple linear regressions between leached Ni toxicity thresholds based on Ni concentrations in soil pore water and the leached soil pore water chemistry (mg L^{-1})

Regression equation	r^2					*P*				Significant factors
1 EC50 = 25.49–3.00pH–0.03K–0.08Ca–6.84EC + 0.80Mg–0.22Na + 0.52S + 0.03DOC (*n* = 12)	0.917	0.858	0.756	0.745	0.846	0.596	0.321	0.072	0.924	Mg，Na，S
2 EC50 = 16.09 − 1.55pH − 0.03K − 0.06Ca − 4.11EC + 0.67Mg − 0.20Na + 0.51S (*n* = 12)	0.917	0.784	0.725	0.537	0.811	0.181	0.072	0.025	—	□
3 EC50 = 9.88 − 1.11pH − 0.03K − 0.05Ca + 0.61Mg − 0.21Na + 0.50S (*n* = 12)	0.916	0.815	0.679	0.499	0.108	0.035	0.011	—	—	□
4 EC50 = 1.06 − 0.02K − 0.05Ca + 0.62Mg − 0.20Na + 0.50S (*n* = 12)	0.915	0.695	0.456	0.077	0.021	0.005	—	—	—	□
5 EC50 = -0.28 − 0.05Ca + 0.61Mg − 0.21Na + 0.50S (*n* = 12)	0.912	0.446	0.057	0.013	0.002	—	—	—	—	□
6 EC50 = -3.62 + 0.44Mg − 0.16Na + 0.45S (*n* = 12)	0.904	0.026	0.001	0.001	—	—	—	—	—	□
7 EC50 = 6.81 − 0.13Na + 0.50S (*n* = 12)	0.815	0.001	0.009	—	—	—	—	—	—	□
8 EC50 = 16.12 + 0.17S (*n* = 12)	0.585	0.004	—	—	—	—	—	—	—	□
9 EC10 = 1.64 + 0.19pH + 0.01K − 0.01Ca − 5.38EC + 0.15Mg − 0.02Na + 0.10S + 0.01DOC (*n* = 14)	0.779	0.937	0.835	0.759	0.467	0.450	0.537	0.127	0.681	S
10 EC10 = 3.22 + 0.01K − 0.01Ca − 5.60EC + 0.15Mg − 0.02Na + 0.10S + 0.01DOC (*n* = 14)	0.779	0.824	0.731	0.366	0.383	0.499	0.092	0.595	—	□
11 EC10 = 3.40 − 0.01Ca − 5.05EC + 0.14Mg − 0.02Na + 0.10S + 0.01DOC (*n* = 14)	0.777	0.715	0.333	0.359	0.468	0.069	0.599	—	—	□
12 EC10 = 2.67 − 4.74EC + 0.10Mg − 0.01Na + 0.09S + 0.01DOC (*n* = 14)	0.772	0.326	0.302	0.440	0.046	0.653		—	—	□
13 EC10 = 4.17 − 5.03EC + 0.10Mg − 0.01Na + 0.10S (*n* = 14)	0.766	0.271	0.298	0.472	0.019		—	—	—	□
14 EC10 = 5.76 − 6.54EC + 0.11Mg + 0.08S (*n* = 14)	0.751	0.107	0.236	0.014	—	—	—	—	—	□
15 EC10 = 5.11 − 3.42EC + 0.08S (*n* = 14)	0.712	0.250	0.014	—	—	—	—	—	—	□
16 EC10 = 2.40 + 0.05S (*n* = 14)	0.673	0.000	—	—	—	—	—	—	—	

r^2, coefficient of determination (percentage of variance accounted for by the regression model); *P*, significant level; EC50, median effective concentration value and EC10, 10% of effective concentration value; DOC, dissolved organic carbon; EC, electrical conductivity.

Appendix Table A2 Simple and multiple linear regressions between unleached Ni toxicity thresholds based on Ni concentrations in soil pore water and the unleached soil pore water chemistry (mg L^{-1})

Regression equation	r^2					*P*				Significant factors
1 EC50 = 46.05–7.56pH–0.08K + 0.06Ca + 7.95EC + 0.24Mg–0.02Na–0.13S+ 0.06DOC (*n* = 15)	0.585	0.422	0.839	0.690	0.694	0.499	0.884	0.592	0.665	Mg
2 EC50 = 45.84–7.69pH–0.08K + 0.07Ca + 6.59EC + 0.22Mg–0.16S + 0.06DOC (*n* = 15)	0.583	0.374	0.827	0.208	0.690	0.456	0.305	0.609	—	
3 EC50 = 39.54 − 6.96pH + 0.08Ca + 6.45EC + 0.23Mg − 0.16S + 0.06DOC (*n* = 15)	0.580	0.348	0.159	0.675	0.389	0.253	0.613	—	—	
4 EC50 = 52.22 − 7.90pH + 0.08Ca + 0.32Mg − 0.12S + 0.03DOC (*n* = 15)	0.570	0.243	0.145	0.061	0.181	0.717	—	—	—	
5 EC50 = 48.58 − 6.90pH + 0.08Ca + 0.30Mg − 0.11S (*n* = 15)	0.563	0.236	0.076	0.051	0.171	—	—	—	—	
6 EC50 = -1.91 + 0.08Ca + 0.34Mg − 0.13S (*n* = 15)	0.494	0.098	0.031	0.111	—	—	—	—	—	
7 EC50 = 4.71 + 0.03Ca + 0.12Mg (*n* = 15)	0.356	0.389	0.069	—	—	—	—	—	—	
8 EC50 = 12.75 + 0.14Mg (*n* = 15)	0.313	0.030	—	—	—	—	—	—	—	
9 EC10 = 9.57 − 1.30pH − 0.05K − 0.01Ca + 2.60EC + 0.08Mg − 0.02Na + 0.001S + 0.01DOC (*n* =16)	0.553	0.557	0.654	0.722	0.588	0.337	0.484	0.989	0.715	Mg
10 EC10 = 9.49 − 1.30pH − 0.05K − 0.01Ca + 2.61EC + 0.08Mg − 0.02Na + 0.01DOC (*n* = 16)	0.553	0.528	0.628	0.592	0.554	0.231	0.297	0.651	—	
11 EC10 = 7.90 − 0.93pH − 0.03K − 0.01Ca + 1.96EC + 0.09Mg − 0.02Na (*n* = 16)	0.541	0.602	0.715	0.721	0.620	0.182	0.311	—	—	
12 EC10 = 10.28 − 1.27pH − 0.04K + 1.00EC + 0.08Mg − 0.01Na (*n* = 16)	0.534	0.383	0.622	0.715	0.173	0.217	—	—	—	
13 EC10 = 11.61 − 1.38pH − 0.03K + 0.10Mg − 0.01Na (*n* = 16)	0.528	0.319	0.648	0.043	0.187	—	—	—	—	
14 EC10 = 9.77 − 1.23pH + 0.10Mg − 0.01Na (*n* = 16)	0.518	0.340	0.028	0.138	—	—	—	—	—	
15 EC10 = 0.54 + 0.09Mg − 0.01Na (*n* = 16)	0.478	0.033	0.162	—	—	—	—	—	—	
16 EC10 = 2.18 + 0.04Mg (*n* = 16)	0.390	0.010	—	—	—	—	—	—	—	

r^2, coefficient of determination (percentage of variance accounted for by the regression model); *P*, significant level; EC50, median effective concentration value and EC10, 10% of effective concentration value; DOC, dissolved organic carbon; EC, electrical conductivity.

Appendix Table A3 Simple and multiple linear regressions between leached Ni toxicity thresholds based on Ni concentrations in 0.01M $CaCl_2$ extraction and the leached soil pore water chemistry (mg kg^{-1})

Regression equation	r^2					*P*				Significant factors
1 EC50 = 179.28–14.68pH–0.08K–0.41Ca–77.50EC + 2.99Mg–0.39Na + 0.85S–0.20DOC (*n* = 12)	0.794	0.665	0.683	0.400	0.316	0.349	0.360	0.111	0.730	Mg，EC，S
2 EC50 = 110.42 − 4.01pH − 0.07K − 0.27Ca − 57.49EC + 2.03Mg − 0.27Na + 0.78S (*n* = 12)	0.784	0.728	0.684	0.184	0.154	0.073	0.177	0.058	—	
3 EC50 = 75.84 − 0.03K − 0.27Ca − 53.50EC + 1.99Mg − 0.28Na + 0.77S (*n* = 12)	0.776	0.789	0.139	0.116	0.047	0.127	0.035	—	—	
4 EC50 = 76.04 − 0.27Ca − 56.67EC + 2.03Mg − 0.28Na + 0.78S (*n* = 12)	0.773	0.107	0.054	0.025	0.096	0.018	—	—	—	
5 EC50 = 54.97 − 51.50EC + 1.07Mg − 0.07Na + 0.49S (*n* = 12)	0.637	0.105	0.087	0.527	0.060	—	—	—	—	
6 EC50 = 67.03 − 61.39EC + 1.11Mg + 0.40S (*n* = 12)	0.614	0.026	0.064	0.046	—	—	—	—	—	
7 EC10 = 20.13 − 0.20pH − 0.001K − 0.08Ca − 17.72EC + 0.62Mg − 0.09Na + 0.24S + 0.03DOC (*n* = 14)	0.704	0.965	0.981	0.241	0.215	0.120	0.166	0.061	0.615	EC，S
8 EC10 = 19.57 − 0.13pH − 0.08Ca − 17.74EC + 0.62Mg − 0.09Na + 0.24S + 0.03DOC (*n* = 14)	0.704	0.968	0.196	0.170	0.084	0.125	0.038	0.579	—	
9 EC10 = 18.53 − 0.08Ca − 17.48EC + 0.62Mg − 0.09Na + 0.24S + 0.02DOC (*n* = 14)	0.704	0.160	0.089	0.050	0.095	0.024	0.503	—	—	
10 EC10 = 21.67 − 0.07Ca − 17.86EC + 0.56Mg − 0.08Na + 0.25S (*n* = 14)	0.683	0.184	0.070	0.048	0.100	0.015	—	—	—	
11 EC10 = 13.82 − 15.23EC + 0.31Mg − 0.03Na + 0.18S (*n* = 14)	0.599	0.120	0.116	0.303	0.030	—	—	—	—	
12 EC10 = 18.60 − 19.80EC + 0.34Mg + 0.14S (*n* = 14)	0.546	0.031	0.085	0.043	—	—	—	—	—	

r^2, coefficient of determination (percentage of variance accounted for by the regression model); *P*, significant level; EC50, median effective concentration value and EC10, 10% of effective concentration value; DOC, dissolved organic carbon; EC, electrical conductivity.

Appendix Table A4 Simple and multiple linear regressions between unleached Ni toxicity thresholds based on Ni concentrations in 0.01M $CaCl_2$ extraction and the unleached soil pore water chemistry (mg kg^{-1})

Regression equation	r^2					*P*				Significant factors
1 EC50 = 122.43–14.41pH–0.26K–0.02Ca + 4.44EC + 0.22Mg–0.04Na–0.02S + 0.05DOC (*n* = 15)	0.455	0.209	0.598	0.923	0.850	0.597	0.802	0.939	0.767	None
2 EC50 = 124.78–14.51pH–0.26K–0.03Ca + 4.28EC + 0.23Mg–0.04Na + 0.04DOC (n= 15)	0.454	0.168	0.559	0.795	0.842	0.494	0.616	0.748	—	
3 EC50 = 130.57–15.05pH–0.26K–0.01Ca + 0.26Mg–0.03Na + 0.03DOC (*n* = 15)	0.451	0.115	0.537	0.856	0.376	0.539	0.771	—	—	
4 EC50 = 129.88 − 14.99pH − 0.26K + 0.23Mg − 0.03Na + 0.02DOC (*n* = 15)	0.448	0.095	0.507	0.330	0.522	0.807	—	—	—	
5 EC50 = 123.38 − 13.77pH − 0.22K + 0.26Mg − 0.03Na (*n* = 15)	0.444	0.047	0.514	0.227	0.430	—	—	—	—	
6 EC50 = 111.68 − 12.86pH + 0.27Mg − 0.03Na (*n* = 15)	0.419	0.048	0.180	0.354	—	—	—	—	—	
7 EC50 = 112.85 − 12.36pH + 0.10Mg (*n* = 15)	0.369	0.053	0.121	—	—	—	—	—	—	
8 EC50 = 115.22 − 11.83pH (*n* = 15)	0.223	0.075	—	—	—	—	—	—	—	
9 EC10 = 39.61 − 3.84pH − 0.16K − 0.05Ca + 1.27EC + 0.11Mg − 0.04Na + 0.06S + 0.01DOC (*n* = 16)	0.307	0.349	0.398	0.439	0.884	0.474	0.440	0.508	0.930	None
10 EC10 = 39.52 − 3.74pH − 0.15K − 0.05Ca + 0.99EC + 0.12Mg − 0.04Na + 0.07S (*n* = 16)	0.306	0.306	0.362	0.406	0.895	0.391	0.392	0.396	—	
11 EC10 = 41.36 − 3.95pH − 0.15K − 0.04Ca + 0.12Mg − 0.04Na + 0.07S (*n* = 16)	0.305	0.200	0.329	0.368	0.320	0.319	0.367	—	—	
12 EC10 = 33.91 − 3.55pH − 0.13K + 0.05Mg − 0.01Na + 0.01S (*n* = 16)	0.235	0.235	0.385	0.577	0.653	0.823	—	—	—	
13 EC10 = 32.02 − 3.28pH − 0.12K + 0.06Mg − 0.01Na (*n* = 16)	0.231	0.207	0.372	0.484	0.678	—	—	—	—	
14 EC10=32.64-3.21pH-0.13K+0.03Mg (*n* = 16)	0.219	0.198	0.302	0.306	—	—	—	—	—	
15 EC10 = 32.79 − 3.02pH − 0.13K (*n* = 16)	0.144	0.224	0.313	—	—	—	—	—	—	
16 EC10 = 25.51 − 2.38pH (*n* = 16)	0.072	0.316	—	—	—	—	—	—	—	

r^2, coefficient of determination (percentage of variance accounted for by the regression model); *P*, significant level; EC50, median effective concentration value and EC10, 10% of effective concentration value; DOC, dissolved organic carbon; EC, electrical conductivity.