Leaching impacts Ni toxicity differently among soils but increases its predictability according to nitrification assay

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Abstract

Purpose Deriving toxicity thresholds through bioassays has become the scientific basis for the risk assessment of metal contamination in soils, but few studies have been reported for Ni toxicity in Chinese soils. In addition, although leaching has been recommended to increase the ecological relevance of the output of soil toxicity bioassays, its impacts on Ni threshold and soil solutions in soils remained to be shown. Therefore, in this study, 17 soils were collected throughout China and were assessed for Ni toxicity using substrate-induced nitrification (SIN) assay for both un-leached and leached treatments. Meanwhile, soil solutions of all Ni treatments were extracted and analyzed. This study provides information for the development of a terrestrial biotic ligand model (TBLM) for Ni in Chinese soils.

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Materials and methods Soils were artificially amended with NiCl_2 solution to generate nominal Ni contamination levels, and aliquot of each Ni treatment was leached with artificial rain to prepare leached soil samples. All the 17 un-leached and the 17 leached soils were subjected to the standard SIN protocol. A total of 816 microcosm tubes were established. Soil solution was extracted, and the solution physiochemical properties were analyzed for each Ni treatments. Both the total-Ni-based and the solution-Ni-based median effective concentrations (EC_{50}) in un-leached soils and leached soils were calculated from dose–response curves. Free- Ni^{2+} -based EC_{50} was also calculated based on solution chemical properties. Leaching effect was assessed based on these results.

Results and discussion Ni EC_{50} varied largely among soils for both the total-Ni-based and the solution-Ni-based EC_{50} in un-leached soils. In un-leached soils, the total-Ni-based EC_{50} values ranged from 52.8 mg kg^{-1} (Hulunber soil) to $2,385.4 \text{ mg kg}^{-1}$ (Wulumuqi soil), and the solution-Ni-based EC_{50} ranged from 0.70 mg L^{-1} (Jiaxing soil) to 293.35 mg L^{-1} (Wulumuqi soil). Stepwise multiple regressions showed that soil pH and total Ca were the best predictors for the total-Ni-based EC_{50} . Protective effects of Ca^{2+} and Mg^{2+} but not H^{+} against Ni toxicity in soil solution were found. Leaching impacted Ni EC_{50} differently among soils, with leaching factor ranging from 0.2 (Haikou soil) to 19.7 (Hulunber soil) for the total-Ni-based EC_{50} and from 0.1 (Dezhou soil) to 35.7 (Jiaxing soil) for the solution-Ni-based EC_{50} . In addition, predictability of Ni EC_{50} was higher in leached soils. The variations in Ni EC_{50} were in agreement with the findings in previous studies. Soil pH and Ca may play important roles in the partition of Ni in Chinese soils and thus impact the Ni toxicity. Protective effects of Ca^{2+} and Mg^{2+} in soil solutions against Ni toxicity may exist, but the role of H^{+}

remained to be justified. The inconsistent impact of leaching on the free Ni^{2+} concentration at low Ni doses and the two competing effects of loss of salts in soil solution on Ni EC50 can partly account for the variation in leaching factor among soils.

Conclusions Ni toxicity varied largely among Chinese soils, and this variation can be largely explained by soil parameters. Leaching-impacted soil solution compositions and Ni EC50 values heavily. The impacts, however, were apparently different among soils. The results in this study can be used to develop a TBLM for Ni in Chinese soils. Furthermore, a need exists to quantitatively investigate the leaching effect on soil solution chemistry before leaching is used as a standard protocol in toxicity assays.

Keywords Leaching · Ni toxicity · Nitrification assay · Soil · Soil solution

1 Introduction

Most of the current guidelines for heavy metals in soil are still based on total metal concentration; this is also the case in China (GB 15618-1995, <http://www.cnemc.cn/images/temp/aa19741f-b768-4b33-8352-3ee774599e99.pdf>). However, the total concentration has been demonstrated not to be directly responsible for the exposure risk of metals to soil biota (Allen et al. 2008; Giller et al. 1998; Lofts et al. 2004; Nriagu and Pacyna 1988). Thus, there is an increasing need to incorporate toxicity tests in risk assessments and the establishment of site-specific guidelines for metals in soils (Fernandez et al. 2005).

Ni is an essential element for organisms, but excess Ni in soil can exert toxic effect on soil biota and expose indirectly risks to human beings (Allen et al. 2008; Berton et al. 2006; Fargasova 2008; Lock and Janssen 2002). Efforts have been made to investigate the Ni toxicity thresholds in European soils using various toxicity assays including barley root elongation (Rooney et al. 2007) and microbial assays (Oorts et al. 2006, 2007). Moreover, these results-based toxicity assays were used to establish a terrestrial biotic ligand model (TBLM; Thakali et al. 2006a, b), which incorporated the concepts of free ion activity model and the theory of protective cations (Allen et al. 2008; Paquin et al. 2002). TBLM has been shown to be a promising method to predict EC50 values for Ni in soils in less time and at lower cost. However, few of such studies have been systematically done for Ni toxicity in Chinese soils.

Presently, modeling of metal toxicity thresholds in soils largely relies on not only the method of toxicity assay but also the metal speciation and the chemo-physical environment in soil solution. Accordingly, analysis of the partition behavior of metal after addition to soil is a critical step.

Although a TBLM presented by Thakali et al. (2006a, b), on the basis of solution chemistry analysis, largely explained the variation in the solution-Ni-based EC50 in European soils, prediction of Ni EC50 in alkaline soils is still difficult (Wang et al. 2009). Therefore, it is necessary to couple the toxicity assay with a detailed investigation of the soil solution.

In addition, leaching has been recommended to increase the ecological relevance of the output of soil toxicity bioassays (Lock et al. 2006; Oorts et al. 2007). Yet, that how leaching affects Ni-amended soil solution and Ni EC50 in soils remains unrevealed on a larger scale before leaching is used as a standard protocol in soil toxicity assay.

Therefore, the aims of this study were to (1) screen Ni toxicity in Chinese soils using substrate-induced nitrification (SIN) assay with the objective of generating information for the development of a TBLM for Ni in Chinese soils, and (2) investigate the leaching effect on Ni toxicity thresholds in soils. To this end, 17 soils were collected throughout China, and the widely used nitrification assay (Broos et al. 2005; Oorts et al. 2006; Smolders et al. 2001, 2004) was used for screening Ni toxicity in both un-leached and leached soils. In addition, soil solutions of each Ni treatment were analyzed.

2 Materials and methods

2.1 Soils

Seventeen uncontaminated topsoils were collected throughout China. The soils represent the major soil types, soil pH, and organic matter content (OMC) of arable soils in China. Selected properties of the soils were listed in Table 1. The soils were air-dried and sieved through 2 mm mesh and then were measured for soil physicochemical properties. Briefly, soil pH was measured with a soil/water ratio of 1:5. Soil OMC was determined by combustion analysis (Marriott and Wander 2006). Total nitrogen content was determined as N_2 by thermal conductivity detection (Matejovic 1997). Cation exchanging capacity (CEC) was measured with 1 M ammonium chloride at pH 7.0 after pretreatment to remove soluble salts. Soil texture was analyzed following the method of Tan (2005). Elemental concentrations were determined by inductively coupled plasma (ICP) atomic emission spectrometry (AES) or ICP-mass spectrometry (MS) after dissolving with aqua regia ($\text{HNO}_3:\text{HCl}=1:3$; Zarcinas et al. 1996).

2.2 Soil Ni addition and leaching

Sieved, air-dried soil was amended with NiCl_2 (Shenyang Reagent Company, China, analytical grade) solution to

Table 1 Soil type, location, and selective soil properties of the 17 soils used in this study

Site	Location	PNR0 (mg N/kg/day)	Soil type	pH		EC ($\mu\text{S}/\text{cm}$)	CEC (cmol^+/kg)	OMC		CaCO_3 %	Total N %	Soil texture <2 μm /2–20 μm / 20 μm –2mm	Citrate-dithionite extractable (mg/kg)		
				ST ^b	pH			%	%				Al	Fe	Mn
Haikou	19°55'N, 111°29'E	1.3	Humid-thermo ferralitic	Ultisols	4.93	110.8	8.75	1.51	–	0.12	66/18/16	9478.4	83920.3	574.2	
Guangzhou	23°80'N, 113°17'E	9.2	Paddy soils	Inceptisols	7.30	136.7	8.30	1.50	0.2	0.13	25/13/62	1488.1	11410.6	48.2	
Qiyang	26°45'N, 111°52'E	1.5	Red earths	Ultisols	5.31	74.1	7.47	0.87	–	0.09	46/35/19	3292.9	26153.9	421.9	
Hangzhou	30°26'N, 120°25'E	7.6	Paddy soils	Inceptisols	6.80	203.3	12.83	2.46	–	0.25	41/42/17	630.9	8412.7	152.6	
Chongqing	30°26'N, 106°26'E	4.8	Purplish soils	Inceptisols	7.12	71.0	22.30	0.99	–	0.09	27/25/48	369.8	7726.8	314.7	
Jiaying	30°77'N, 120°76'E	4.6	Paddy soils	Inceptisols	6.70	158.8	19.33	1.42	–	0.15	39/36/25	794.1	10823.9	297.0	
Yangling	34°19'N, 108°0'E	11.2	Loessial soils	Inceptisols	8.83	83.2	8.46	0.62	8.92	0.08	16/14/70	460.6	7192.9	349.6	
Zhengzhou	34°47'N, 112°40'E	12.1	Fluvo-aquic soils	Inceptisols	8.86	108.7	8.50	1.57	0.15	0.07	28/41/31	360.9	4288.7	166.1	
Dezhou	37°20'N, 116°29'E	9.6	Fluvo-aquic soils	Inceptisols	8.90	111.8	8.33	0.69	6.17	0.08	18/18/64	369.5	4965.1	219.2	
Shijiazhuang	38°03'N, 114°26'E	4.2	Gray-cinnamon soils	Alfisols	8.19	302.0	11.71	1.0	3.84	0.11	10/4/86	579.1	7544.2	261.3	
Zhangye	38°56'N, 100°27'E	8.6	Irrigated desert soils	–	8.86	151.8	8.08	1.02	7.75	0.10	20/24/56	451.1	8356.3	331.0	
Langfang	39°31'N, 116°44'E	10.9	Fluvo-aquic soils	Inceptisols	8.84	5.7	6.36	0.6	2.42	0.06	21/22/57	272.7	3729.0	111.6	
Lingshan	39°55'N, 116°8'E	4.1	Brown earths	Alfisols	7.48	92.5	22.65	4.28	4.27	0.37	20/21/59	705.9	6949.7	276.1	
Gongzhuling	42°40'N, 124°88'E	9.4	Black soils	Mollisols	7.82	146.9	28.76	2.17	0.27	0.20	45/26/29	1066.6	6932.3	365.9	
Wulumuqi	43°95'N, 87°46'E	10.4	Gray desery soils	–	8.72	226.5	10.31	0.87	5.08	0.10	25/23/52	396.0	4794.6	305.4	
Hulunber	46°03'N, 122°03'E	6.9	Chernozems	Mollisols	7.66	888.0	22.74	2.66	0.27	0.25	37/16/47	955.5	5258.9	322.4	
Hailun	47°28'N, 126°57'E	2.9	Black soils	Mollisols	6.56	153	33.59	3.03	–	0.25	40/27/33	1244.1	6558.7	396.5	

‘–’ means undetermined or non-detected; pH was measured with a water/soil ratio of 5:1

PNR0 potential nitrification rate in control soils, EC electrical conductivity, CEC cation exchanging capacity, OMC organic matter content

^a Soil type according to genetic soil classification of China (GSCC)

^b Corresponding soil order according to American soil taxonomy (ST)

produce nominal concentrations of 37.5, 75, 150, 300, 600, 1,200, and 2,400 mg Ni kg⁻¹ dry soil for soils with pH>7; 25, 50, 100, 200, 400, 800, and 1,600 mg kg⁻¹ dry soil for soils with pH<7; and 12.5, 25, 50, 100, 200, 400, and 800 mg kg⁻¹ dry soil for soils with pH<5. The amended soils were left to equilibrate overnight at 100% maximum water holding capacity (MWHC), then air-dried and sieved again through 2-mm mesh.

Aliquot of the freshly spiked soil was leached for each Ni treatment and for each soil by artificial rainwater before use. The detailed composition of the artificial rainwater and protocol for the preparation of the leached samples were as described by Oorts et al. (2007). Briefly, the soils were leached with two pore volumes of artificial rainwater (CaCl₂ 5×10⁻⁴ M, Ca(NO₃)₂ 5×10⁻⁴ M, MgCl₂ 5×10⁻⁴ M, Na₂SO₄ 10⁻⁴ M, and KCl 10⁻⁴ M, pH 5.9). Leached samples were also air-dried and sieved through 2-mm mesh. All un-leached and leached soils were stored at room temperature before use.

2.3 Substrate-induced nitrification assay

The un-leached and leached soils were used to establish a standard SIN assay (Oorts et al. 2006; Smolders et al. 2001, 2004). For pre-incubation, three replicates of 7 g amended soil for each Ni treatment were incubated under dark aerobic conditions at 25±2°C in a 50-ml centrifuge tube maintained at 50±5% of MWHC by addition of deionized water. Totally, 816 microcosm tubes were established. After the 14-day pre-incubation, one tube of each Ni treatment was extracted with 35 ml of 1.0 M KCl, and NH₄⁺, NO₂⁻-N, plus NO₃⁻-N were colorimetrically determined using a flow injection auto-analyzer (SKALAR, Holland). To each of the remaining tubes in each treatment, 0.5 ml of 0.044 M (NH₄)₂SO₄ was added, and then incubation was continued for 7 days for soils with pH>5 and 28 days for soils with pH<5. The remaining two replicates were analyzed for NH₄⁺, NO₂⁻-N, plus NO₃⁻-N again as above. Data were used to calculate potential nitrification rate (PNR).

2.4 Soil solution extraction and analysis

One replicate from each Ni treatment was used to extract soil solution at the beginning of SIN assay using the method of Thibault and Sheppard (1992). Soil pH, electrical conductivity (EC), and dissolved organic carbon (DOC) were measured following conventional procedures (Tan 2005). Dissolved elemental concentrations including Ni in soil solutions were measured either by ICP-AES or by ICP-MS depending on their concentrations in the solutions according to the methods of Khan et al. (2008).

2.5 Data analysis

PNR values were calculated from the linear increase in soil NO₃⁻-N during the period after ammonium addition (Oorts et al. 2006; Smolders et al. 2001). PNR data was used to generate log–log dose–response curves automatically by SigmaPlot 9.0 (SPSS Inc., Chicago, IL, USA) for each soil. Ni EC50 based on both the measured total Ni concentration (total-Ni-based EC50) and the dissolved Ni concentration in soil solution (solution-Ni-based EC50) was calculated for both the un-leached and leached soils. Some dose–response relationships between PNR and solution Ni cannot be fitted well by any logical model. Hence, solution-Ni-based EC50 values for six un-leached and seven leached soils were absent.

Toxicity thresholds were related to soil properties (e.g., pH, CEC, clay, OMC, and background Ni) with enter or stepwise multiple regressions (SPSS 11.5). The corresponding pH and concentrations of Ca, Mg, K, Na, and Zn in soil solutions at Ni EC50 were also estimated by linear interpolation between the measured values (Oorts et al. 2006). The EC50-based free-Ni²⁺ in soil solution (free-Ni²⁺-based EC50) was calculated based on corresponding solution-Ni-based EC50 and solution composition at EC50 using MINTEQ 2.6 following assumptions by Oorts et al. (2007) and Thakali et al. (2006a). Briefly, the input parameters included solution-Ni-based EC50, and corresponding soil solution pH, DOC, Ca, Mg, K, Na, Al, Fe, SO₄²⁻, and Cl⁻·SO₄²⁻ was calculated from dissolved S, and Cl⁻ was set at a concentration estimated to balance the cations. It was assumed that 70% of the dissolved organic matter (DOC/2) was fulvic acid since most of the soils used in this study were alkaline.

3 Results

3.1 Soil physico-chemistry

The 17 soils used in this study represent the majority of Chinese soil types and cover a wide range of soil properties in pH (4.9–8.9), CEC (7.5–33.6 cmol⁺ kg⁻¹), clay content (10–66%), background Ni (4.8–118.1 mg kg⁻¹), total Ca (<0.1–3.5%), etc. The total concentrations of other potentially toxic elements (e.g., Cu, Cr, Cd, Zn, As, and Pb; data not shown) in the studied soils generally fall within the normal range of background levels in Chinese soils (Wei et al. 1991).

3.2 Ni concentration

Measured total Ni concentrations (Ni_{total}) were in good agreement with the nominal dose applications in almost all

treatments. Dissolved Ni concentration [Ni] in un-leached soil solution generally increased with applied Ni dose exponentially (data not shown). For all Ni treatments, [Ni] ranged from <0.25 to 1898.9 mg L^{-1} (Guangzhou soil) in un-leached soil solutions and from <0.25 to 627.8 mg L^{-1} (Guangzhou soil) in leached soil solutions. The un-leached soils can be clearly divided into three groups according to the [Ni] at the highest Ni doses: first is the lower-solution-Ni soils with $[\text{Ni}] < 100 \text{ mg L}^{-1}$, including Dezhou, Gongzhuling, Shijiazhuang, Yangling (the lowest, 6.31 mg L^{-1}), Zhangye, and Zhengzhou soils; second is the higher-solution-Ni soils with $[\text{Ni}] > 380 \text{ mg L}^{-1}$, including Chongqing, Guangzhou (the highest, $1,898.90 \text{ mg L}^{-1}$), Haikou, and Qiyang soils; the others are median-solution-Ni soils with [Ni] ranging from 216.3 mg L^{-1} (Lingshan soil) to 330.8 mg L^{-1} (Wulumuqi soil).

Higher Ni amendments heavily impacted the chemical composition of the soil solutions in un-leached treatments (data not shown), leading to increases in H^+ , Ca^{2+} , and Mg^{2+} for all soils. The largest decrease in pH at the highest Ni doses occurred in Guangzhou soil (2.20 units) and the lowest decrease occurred in Haikou soil (0.30 units).

Multiple regression showed that up to 78% ($P=0.005$) of the variation in [Ni] in soil solution at highest Ni doses can be explained by clay content, CEC, and total Ca. No significant correlation was found between [Ni] in soil solution at highest Ni doses and OMC.

3.3 Leaching effect on Ni concentration

Leaching effects on Ni_{total} in soils and [Ni] in soil solutions were shown by plotting scatter graphs of the Ni_{total} in the un-leached against the leached soils, and [Ni] in the un-leached against the leached soil solutions, respectively. To assess the leachability and thus the sorption behavior of Ni at the highest Ni doses, removal rate (RR) is defined as the ratio of the loss of Ni_{total} or [Ni] due to leaching to original Ni_{total} or [Ni] before leaching, respectively. As shown in Fig. 1a, for total Ni in soil, leaching effect was not apparent throughout the range of Ni dosage except for the highest Ni doses in some soils, like Langfang with the largest RR of 51%. Leaching apparently impacted [Ni] in soil solutions (see Fig. 1b). At higher [Ni] ($>20 \text{ mg L}^{-1}$), [Ni] was significantly reduced, while at lower [Ni], leaching even increased [Ni] in some soil solutions. RR values of [Ni] at highest Ni doses varied from 5% (Gongzhuling soil) to 95% (Dezhou soil).

The other chemical compositions of the soil solutions were also strongly impacted by leaching (data not shown), leading to increase in pH and reduction in EC and salinity. For instance, up to 93.0% of Ca and Mg in soil solution at the highest Ni dose was removed by leaching in Langfang soil.

Fig. 1 Leaching effect on the total Ni concentration (a) and dissolved Ni concentration ([Ni]) (b) in the 17 studied soils. The solid line is 1:1 line

3.4 Ni toxicity threshold

Both the total-Ni-based EC50 (based on Ni_{total}) and solution-Ni-based EC50 (based on [Ni] in soil solution) for Ni in un-leached and leached soils were calculated (Fig. 2). In un-leached soils, the total-Ni-based EC50 values ranged from 52.8 mg kg^{-1} (Hulunber soil) to $2,385.4 \text{ mg kg}^{-1}$ (Wulumuqi soil), and the available solution-Ni-based EC50 ranged from 0.70 mg L^{-1} (Jiaxing soil) to 293.35 mg L^{-1} (Wulumuqi soil). In leached treatments, the total-Ni-based EC50 values ranged from 78.9 mg kg^{-1} (Qiyang soil) to $2,304.1 \text{ mg kg}^{-1}$ (Zhengzhou soil), and the available solution-Ni-based EC50 ranged from 0.67 mg L^{-1} (Yangling soil) to 173.37 mg L^{-1} (Wulumuqi soil).

In order to investigate the protection effect of major cations against Ni toxicity, the free- Ni^{2+} -based EC50 values were calculated and related to pH, Na^+ , K^+ , Ca^{2+} , and Mg^{2+} concentration in soil solutions at EC50. Solution-Ni-based EC50 for un-leached soils was not correlated with pH, Na^+ , and K^+ in soil solution at EC50 but significantly correlated with Ca^{2+} and Mg^{2+} in soil solution at EC50 (Fig. 3).

Fig. 2 Total-Ni-based and solution-Ni-based EC50 of Ni in the 17 unleached and leached soils subjected to substrate-induced nitrification assay. Bars represent $\pm$ standard errors of prediction. The *absent bars* are unacceptable standard errors of prediction

3.5 Leaching effect on Ni toxicity threshold

Leaching factor is defined as the ratio of EC50 in leached soil to EC50 in un-leached soil (Oorts et al. 2007). Results showed that leaching inconsistently impacted the EC50 values for Ni among soils, with leaching factor ranging from 0.2 (Haikou soil) to 19.7 (Hulunber soil) for the total-Ni-based EC50 and from 0.1 (Dezhou soil) to 35.7 (Jiayang soil) for the solution-Ni-based EC50. In addition, significant correlation ($R^2=0.81$, $P<0.001$) was found between the leaching factor for total-Ni-based EC50 and EC (Fig. 4), and up to 93% ($P<0.001$) of the variation in leaching factor for total-Ni-based EC50 was explained by EC, total Ca, clay content, and CEC. EC was also the

controlling factor of leaching factor for solution-Ni-based EC50 ($R^2=0.31$, $P=0.031$).

Linear regressions between the Ni EC50 values and the soil parameters were done for both un-leached and leached soils (Table 2). Soil pH and total Ca was the best predictor for total-Ni-based EC50 in un-leached soils, while soil pH was the best predictor for solution-Ni-based EC50 in leached soils. Generally, a better prediction for total-Ni-based and solution-Ni-based EC50 in leached soils was obtained than those in un-leached soils.

4 Discussion

4.1 Variation and prediction of Ni EC50

EC50 values varied largely among soils for both the total-Ni-based and solution-Ni-based EC50 in un-leached soils. This was in good agreement with Oorts et al. (2006), who found a 90-fold variation for Ni based on microbial assays

Fig. 3 Regression between free-Ni²⁺-based EC50 and dissolved Ca and Mg concentration in soil solution at EC50

Fig. 4 Positive correlation between leaching factor for total-Ni-based EC50 in soils and soil electrical conductivity

in European soils, and with Rooney et al. (2007), who found a 37-fold variation based on barley root elongation assay and 54-fold variation based on tomato shoot growth assay for Ni in European soils. These results generally meant that it is more practical to establish a site-specific rather than a single guideline for Ni in soil. Therefore, efforts were also made to predict the EC50 for Ni in soils using easily determined soil variables in this study. As shown in Table 2, predicting ability of the equations for Ni EC50 in leached samples was generally better than that for un-leached samples. With less than three soil variables, a

best predicting equation ($R^2=0.69$) was obtained for Ni EC50 based on solution Ni for leached soils. The predicting ability of the linear models in this study was generally at the same level as those developed for metals in other studies based on microbial assays (Broos et al. 2007; Oorts et al. 2006; Smolders et al. 2004), however, was obviously less powerful than those based on plant assays (Rooney et al. 2007). A linear model based on barley root elongation assay using the same soils as in present study was able to predict the EC50 within a twofold range of the measured values. Furthermore, involving more variables could increase the predicting ability of the equations for Ni EC50, indicating that Ni toxicity to nitrification process in test soils should be controlled by multi-variables but not a single variable. Since higher sensitivity of microbial assays generally means higher variability, the prediction of Ni EC50 based on SIN assay may need a further investigation on a larger scale and require comparisons between models for diverse soils from different parts of the world.

For total-Ni-based EC50, it tended to be close after leaching in some soils within the same soil type. For example, the total-Ni-based EC50 was around 860 mg kg^{-1} in paddy soils from Jiaying, Hangzhou, and Guangzhou, and around 800 mg kg^{-1} in black soils from Gongzhuling and Hailun. This implied it may be a possible way to establish soil guidelines for Ni according to soil type and even soil order instead of using a single predicting model.

Table 2 Multiple regression between Ni EC50 based on substrate-induced nitrification assay in the 17 soils and soil parameters

	Regression equation	R^2	P
Un-leached soils	Based on total Ni ($n=17$)		
	EC50=373.1 (134.0) Ca+335.0 (220.4)	0.34	0.014
	log EC50=0.3 (0.1) pH+0.8 (0.7)	0.33	0.016
	EC50=388.3 (195.9) pH+325.9 (214.1) Ca+28.9 (13.3) clay–3504.3 (1619.6)	0.56	0.012
	Based on soil solution Ni ($n=11$)		
	No single predictor	–	–
	EC50=5.81 (2.28) pH+0.74 (0.21) clay–6.91 (3.19) OMC+0.60 (0.22) Ni–64.99 (23.55)	0.71	0.080
Leached soils	EC50=11.41 (5.32) pH–7.87 (6.56) Ca–0.45 (0.43) CEC+0.67 (0.22) clay–8.69 (4.53) OMC+0.84 (0.30) Ni–92.02 (34.30)	0.79	0.190
	Based on total Ni ($n=16$)		
	EC50=402.03 (90.65) pH–2037.61 (692.73)	0.58	0.001
	EC50=503.67 (142.60) pH+11.04 (11.92) clay–3184.45 (1420.13)	0.61	0.002
	EC50=315.52 (170.08) pH+402.34 (199.18) Ca+0.53 (0.61) EC–19.45 (12.45) clay+118.77 (126.32) OMC–2828.77 (1465.33)	0.73	0.011
	Based on soil solution Ni ($n=10$)		
	EC50=0.56 (0.18) clay–8.29 (6.94)	0.48	0.013
	EC50=0.61 (0.15) clay–0.58 (0.23) CEC+0.001 (6.61)	0.69	0.006
	EC50=0.88 (2.87) pH+0.73 (0.23) clay–0.59 (0.26) CEC+0.24 (0.27) Ni–16.70 (30.01)	0.72	0.041

Values in parentheses are standard errors. ‘R’ indicates correlation coefficient and ‘P’ for significant level

Linear regression (see Table 2) showed that soil pH and total Ca governed the Ni toxicity in un-leached soils, which was different from Rooney et al. (2007) who found CEC was the best single predictor for the EC50 of Ni phytotoxicity in European soils, and was also different from Oorts et al. (2006) who found CEC, background Ni, and clay content were the dominant factors controlling thresholds of Ni microbial toxicity in European soils. This result was not surprising considering the special chemical properties of Chinese soils. Since there is a strong correlation between soil pH and total Ca content of the test soils, it is not able to discriminate between an effect of total Ca and the pH effects in this study. Basically, soil pH is a key factor determining the adsorption of metals in soil (Sauve et al. 2000). On the other hand, soil pH is largely controlled by soil chemical compositions and soil texture, particularly Ca minerals (e.g., calcite and gypsum) and Fe-Al oxides. High-pH soil environment (accordingly high Ca and low clay content in this study) generally leads to a high retention of soluble Ni added to soil. Therefore, as shown in Table 1, ultisols soils from Haikou and Chongqing containing a large amount Fe-Al oxides (expressed as Citrate-dithionite extractable content) were of a pH <5.5 and had a lower total-Ni-based EC50 in un-leached soils. In contrast, inceptisols soils from Dezhou, Langfang, Zhan-gye, Yangling, and Zhengzhou containing a large amount of Ca minerals were of a pH >8.8 and had a higher total-Ni-based EC50 in un-leached soils. In addition, Ca-rich minerals in soil can significantly influence the retention and movement of chemicals and thus their toxicity especially in alkaline environment (Lal and Shukla 2004). That more than 70% of the test soils in present study were with a pH >7 may be the reason why Ca but not Fe-Al minerals content became the predictor of the total-Ni-based EC50 in un-leached soils. Such mechanisms have also been shown in previous studies which found liming (Everhart et al. 2006) and amendment of Ca-rich clay mineral (Usman et al. 2005) greatly decreased the Ni bioavailability in soils. Furthermore, high total Ca generally means high Ca^{2+} in soil solution (Fig. 5), and thus may impact the Ni toxicity through competitive binding on biological membranes. However, when comparing correlations with soil properties between the present study and Oorts et al. (2006) or Rooney et al. (2007), it should be noted that the CEC in these previous studies was measured at pH of the soil and at low ionic strength, resulting in an effective CEC compared to a potential CEC in the present study. The differences in CEC analysis can strongly affect the resulting correlations. Therefore, whether effective CEC also plays important role in predicting the Ni EC50 in test soils in present study remains to be investigated.

The variation in total-Ni-based EC50 was not explained by solution-Ni-based EC50 in un-leached soils, since a

Fig. 5 Positive correlation between total Ca in soil and dissolved Ca concentration in soil solutions at EC50

larger variation in solution-Ni-based EC50 was found in this study. This was generally consistent with previous studies and made it impossible to explain the variation in metal toxicity among soils by using free metal-ion activity alone (Broos et al. 2007; Oorts et al. 2006). Free- Ni^{2+} -based EC50 was not involved in the prediction for Ni EC50 in this study, since it was calculated based on many assumptions and not an easily determined parameter. Furthermore, many studies revealed a remarkable diversity of DOC in binding capacity from different soils (Kolts et al., 2008) and free ion activity theory has been challenged for Ni in soil (Sarathy and Allen 2005). A strong correlation between dissolve Ni in soil solution and calculated free- Ni^{2+} in soil solution was found in present and previous studies (Oorts et al. 2007; Fig. 6), with the percentage of calculated free- Ni^{2+} in dissolved Ni at EC50 being around 30%. This result implied free- Ni^{2+} in soil solution might be not sensitive as Cu to soil solution chemistry and controlled mainly by dissolved Ni concentration. However, this conclusion was based on results by model prediction (MINTEQ 2.6 or WHAM 6) and required confirmation by mechanistic study.

Competitive binding of Ca^{2+} and Mg^{2+} on biological membranes against free Ni^{2+} was indicated by the significant correlations between free- Ni^{2+} -based EC50 and dissolved Ca and Mg concentrations at EC50 (see Fig. 3). This may largely but not completely explain the variation in solution-Ni-based EC50 according to the concept of TBLM (Lock et al. 2007a, b; Simpson and Batley 2007; Thakali et al. 2006a, b). In addition, the competitive effect of H^{+} on Ni^{2+} toxicity was not observed in this study. This is consistent with the studies for Ni toxicity by Lock et al. (2007b) and also for some other metals (de Schampelaere

Fig. 6 Strong correlation between $-\lg$ dissolved Ni in soil solution ($p[\text{Ni}]$) and $-\lg$ calculated free Ni^{2+} in soil solution ($p[\text{Ni}^{2+}]$) in present study and previous study

and Janssen 2002; Heijerick et al. 2002b; Lock et al. 2007a). However, more previous studies have observed a significant correlation between EC50 and pH at EC50 (Erickson et al. 1996; Heijerick et al. 2002a; Parker et al. 1998; Thakali et al. 2006a, b; Voigt et al. 2006). Hence, incorporation of proton competition into the concept of BLM remains to be justified (Wang et al. 2009).

4.2 Leaching effect on Ni concentration and EC50 in soils

Leaching heavily impacted soil solution compositions and Ni EC50 values. The impacts on both the solution chemistry and Ni EC50, however, were apparently inconsistent among soils (see Figs. 1 and 2).

As suggested by some authors (Lock et al. 2006; Oorts et al. 2007), leaching obviously removed the “excess” salts including $[\text{Ni}]$ at higher Ni doses (see Fig. 1). This may increase the ecological relevance of the toxicity assay for soil metals, since the loss of “excess” salts including $[\text{Ni}]$ by leaching simulated the natural leaching and aging process of metals in soil (Keller et al. 2002; Kiikkila et al. 2002; Nemeth and Kadar 2005) and reduced the potential toxicity of the counterions (Bongers et al. 2004). Loss of free metal ion in soil solution induced by leaching was also observed for Ni (Oorts et al. 2007) and for Pb (Lock et al. 2006) in European soils. In addition, the significant increase of the correlation between total-Ni-based EC50 and original soil pH in leached soils (see Table 2), together with the correlation between solution-Ni-based EC50 and soil clay content, might point to a return of the soil solution chemistry to a pH-controlled equilibrium from the state of overloading of soluble Ni in some soils. As indicated by the analysis of the variation in $[\text{Ni}]$ at largest Ni doses among soils and also previous studies (Agbenin and Atin 2003; Yu

et al. 2002), the adsorption mechanisms of soluble metals added to soil might be quite different at high Ni doses and could not simply be explained by the soil pH, since the immediate increase in $[\text{Ni}]$ and $[\text{H}^+]$ may remarkably exceed the maximum adsorption for Ni in some soils especially for those with low pH and high salinity. Thus, removal of excess Ni^{2+} might increase the predictability of Ni EC50 with soil parameters. Taken together, leaching increased the ecological relevance of the SIN assay for Ni.

Although leaching caused an obvious loss of $[\text{Ni}]$ at higher Ni doses in all soils, the impacts of leaching on both the total-Ni-based EC50 and the solution-Ni-based EC50 were inconsistent among soils as indicated by the leaching factor. Generally, the inconsistent effect of leaching on the total-Ni-based EC50 can be attributed to the inconsistent impact of leaching on $[\text{Ni}]$ at low Ni doses (see Fig. 1) and the two counteracting effects of leaching on the soil solution compositions (Oorts et al. 2007). Although it was concluded that leaching can decrease the free ion concentration of a given metal in soil solution, an increase in $[\text{Ni}]$ at low Ni doses in Haikou, Qiyang, Gongzhuling, and Hailun soils was observed, implying a complicated influence of leaching on soil solution at low Ni doses. This may partially explain why these soils had a leaching factor of <1 for total-Ni-based EC50. Moreover, loss of free Ni^{2+} , which can positively increase the total-Ni-based EC50, was simultaneously accompanied by the loss of a large amount of protective cations, which may negatively decrease the total-Ni-based EC50. This may also contribute to the variation in leaching factor for total-Ni-based EC50.

For solution-Ni-based EC50, the inconsistent impact of leaching cannot be simply explained by the loss of major cations in soil solution. Generally, as observed by Oorts et al. (2007), in all of the three test soils, loss of protective cations in soil solution may decrease solution-Ni-based EC50. In this study, however, leaching factor was found to be >1 in four soils, although the solution-Ni-based EC50 were decreased by leaching in most soils. One possible reason for this could be that high salinity induced by spiking was significantly toxic to nitrification process, and leaching alleviated such toxicity. Yet, this explanation refers to the nature of the indigenous nitrifying communities and remains to be shown, since in most soils, leaching factor was <1 for solution-Ni-based EC50, and the incorporation of the toxicity of counterions seemed not necessary.

The original purpose of introducing leaching to bioassays is to remove the excess counterions which can exert a direct toxic effect or an indirect effect by raising the salinity. However, as natural process, the partition of the soluble metal added to soils, together with the covarying solution chemistry, are controlled by soil properties (Alumaa et al. 2002; Ponizovsky et al. 2008; Yu et al. 2002), while the artificial leaching may disturb the entire

soil solution except for counterions and generate temporary thermodynamic equilibria which might be largely determined by leaching process (e.g., the duration of leaching, the composition of leaching solution, and the total volume of leaching solution). Hence, to what extent the leaching is sufficient, that is, whether the leaching effect following a conventional leaching protocol is comparable among soils and whether the leaching effect can be predicted with soil parameters, are critically important. Leaching factor for the total-Ni-based EC50 was well predicted by EC, total Ca, clay content, and CEC in this study. All of the factors determining the leaching factor for both total-Ni-based EC50 and solution-Ni-based EC50 are related to the immediate adsorption capacity of soil and thus the leachability of Ni added as soluble salt to soil. Particularly, EC is an indicator for soil salinity (Rhoades and Corwin 1990; Rhoades et al. 1989), and salinity can largely impact the retention of soluble metals added to soil through competitive adsorption and salt effect enhancing the solubility of the added salt. Therefore, in Hulunber soil with highest EC high leachability of dissolved Ni in soil solution may be responsible for the highest leaching factor. However, it should be stressed that for soils with low leaching factors, EC effect was not significant, and Ca and clay content together with CEC may play an important role. In addition, similar with the study by Lock et al. (2006), conductivities of more than $4 \text{ mS}^{-1} \text{ cm}$ were observed in the highest Ni treatments of five soils. Furthermore, RR of [Ni] at highest Ni doses varied largely among soils, and no correlation was found between RR values and soil properties. This probably implied that the leaching effect on the [Ni] was controlled by the leaching process itself but not soil properties. Taken together, these results highlight the need for further quantitative investigation of leaching effect on the Ni partition in soils before leaching is used as a standard protocol in toxicity assays.

5 Conclusions

In conclusion, large variation was found for the total-Ni-based EC50 in un-leached Chinese soils. The variation in total-Ni-based EC50 was not explained by the solution-Ni-based EC50 since a larger variation in the solution-Ni-based EC50 was observed. This may make it difficult to establish a single guideline for Ni in Chinese soils and, alternatively, site-specific guidelines should be more practical. Linear models based on soil variables were tried to predict both total-Ni-based and solution-Ni-based EC50. Soil pH, clay content and total Ca content were found to be important factors affecting Ni EC50. Protective effects of Ca^{2+} and Mg^{2+} but not H^+ , Na^+ , or K^+ at EC50 against free Ni^{2+} toxicity were found. In addition, leaching heavily

impacted soil solution compositions and both the total-Ni-based EC50 and the solution-Ni-based EC50 in the studied soils, but its impacts were inconsistent among soils. To some extent, leaching increased the ecological relevance of the SIN assay for Ni, considering that the predictability of the total-Ni-based EC50 in leached soils increased, and the variation in leaching factor among soils was largely explained by soil properties. However, a need still exists to quantitatively investigate the leaching effect on soil solution chemistry before leaching is used as a standard protocol in toxicity assays.

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