Advanced Materials Research Vols. 750-752 (2013) pp 1441-1447
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doi:10.4028/www.scientific.net/AMR.750-752.1441

Evaluation the influence of soil solution chemistry on soluble nickel toxicity to bok choy

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Keywords: soil; nickel; phytotoxicity; bok choy; leaching

Abstract. Nickel (Ni) is an essential element for plants but it is toxic at large concentrations. In the present study, bioassays of bok choy were taken in 17 Chinese soils with different properties and climate characteristics to evaluate the toxicity of soil soluble nickel (Ni) toxicity in soil pore water to bok choy. The tested soils were spiked with 8 levels of soluble Ni chloride with or without leaching treatments. The effective soluble Ni concentrations that caused 10% root growth inhibition (EC10) and 50% inhibition (EC50) varied widely from 0.05 to 2.1 mg/L and from 0.13 to 7.6 mg/L in 17 unleached soils, represented 41.8 to 58.5 folds differences, and from 0.08 to 2.2 mg/L and from 0.47 to 4.5 mg/L in leached soils, represented 27 to 9.6 folds differences. It indicated that the soil soluble properties greatly influenced Ni toxicity to bok choy. However, soluble Ni toxicity thresholds were not significantly decreased in 15 soils. Regression models between soil solution properties and phytotoxicity threshold values were developed. The model showed that soil solution Mg^{2+}, K^+ and electrical conductivity (EC) were the important factors affecting Ni toxicity on bok choy, and meanwhile they were positively related to the toxicity thresholds. These quantitative relationships could be used for the risk assessment of Ni in terrestrial environment in China.

Introduction

Bioavailability/toxicity of Ni was determined by various factors, for example, total soil metal contents, soil properties, soil solution properties. The soil properties have been taken into account in the risk assessment added Ni phytotoxicity [1-4]. Li et al.[4] found that soil pH was the most important factor controlling Ni toxicity to barely in soils, explaining approximately 68% of the variance in EC50 values (the Ni concentration that caused 50% root growth inhibition). Similarly, Rooney et al. reported that soil cation exchange capacity was the best single predictor for the EC50 of added Ni to barley and tomato. However, the added Ni toxicity thresholds changed widely in different types soils and the total metal concentration could not better assess its potential availability to plants [5-7]. While, compared to total soil metal contents, soil soluble metal concentration was more reliable and closer to the soil-to-plant transfer. Therefore, it is essential to evaluate the soluble Ni phytotoxicity. There have been some studies on the influence of solution properties on soluble metal toxicity based on nature water or artificial solution [8, 9]. For example, Lock et al. [8] revealed that the dissolved cations, including Mg^{2+}, Ca^{2+}, Na^+, K^+, performed different extent of influences on Ni toxicity to barley root elongation in nutrient solution. The effects of dissolved cations on Ni toxicity were based on the biotic ligands model (BLM), which assumed that cations may compete with metal ions for these binding sites of biotic ligands and decrease the toxicity. Thus, it is necessary to investigate whether it was workable in the soil solution by the existence of solid phases.

The present study was to evaluate the influence of soil solution properties on soluble Ni toxicity to bok choy in a wide range of Chinese soil. Our objectives were to investigate whether variation in toxicity thresholds among soils can be better explained by the solubility of Ni and meanwhile to establish empirical relationships between soil solution properties and soluble Ni toxicity under leached and unleached conditions.

Experimental

Soil samples and treatments. Seventeen soils were sampled from locations throughout the main areas of China at the surface soil (0~20 cm). These soils are representative of the major soil types in the region and the main properties of the soils could refer to Li et al. [9].

After sampling, the soil samples were air-dried and sieved to < 2 mm screen. Each soil was amended with $NiCl_2$ solution to obtain a range of eight Ni concentrations including controls. According to soil pH, the nominated concentration were divided into three ranges: 12.5, 25, 50, 100, 200, 400 and 800 mg Ni/kg for soils with pH < 5; 25, 50, 100, 200, 400, 800 and 1600 mg Ni/kg for soils with pH 5 to 7; 37.5, 75, 150, 300, 600, 1200 and 2400 mg Ni/kg for soils with pH > 7. After that, soil samples were incubated for 2 d at 100% maximum water holding capacity (MWHC) [10], then air-dried and sieved to < 2 mm.

For the leached soil samples, the prepared artificial rainwater was used to leach soil samples in order to decrease the difference in Ni toxicity between laboratory and field soils [9, 11, 12]. The composition of artificial rainwater were 5×10^{-4} mol/L $CaCl_2$, 5×10^{-4} mol/L $Ca(NO_3)_2$, 5×10^{-4} mol/L $MgCl_2$, 10^{-4} mol/L Na_2SO_4 at pH 5.9 . The detailed leaching process was described by Li et al. [11]. Then these soil samples were air-dried, sieved through < 2 mm mesh.

Soil solutions were collected by centrifugation according to Thibault and Sheppard [13].The deionized water was added to the soil sample (25 g) in according to the 50 cm water tension and the mixture was incubated overnight. Then soil samples were centrifuge at the speed of 3 500 r/min for 45 min, after that at the higher speed of 15 000 r/min for 45 min. Each soil was extracted and analyzed in duplicate. Immediately after extraction, soil solution samples were passed through 0.45 μm filters. 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 concentrations of Ni and other major elements (K^+, Ca^{2+}, Na^+, Mg^{2+}, S) were determined by ICP-AES. Dissolved organic carbon (DOC) was measured using a DOC analyzer (Skalar Ltd., Breda, the Netherland). The measured soil chemistry characters were listed in Table 1.

Table 1 Soil solution properties of 17 soil samples before being spiked with Ni

Location (longitude and latitude)	pH	EC (mS/cm)	DOC (mg/L)	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Na (mg/L)	S (mg/L)
Beijing (39°55'N116°8'E)	7.89	0.858	214	294	28.1	50	11.1	42
Chongqing (30º26'N106º26'E)	7.88	0.976	235	187	2.4	15.8	20	75
Gansu (38°56'N100°27'E)	8.29	1.443	302	310	12	99.7	99.4	150
Guangzhou (23°10'N113°18'E)	8.05	1.83	313	390	36	23.2	59.3	210
Hailun (47°28'N126°57'E)	7.41	0.543	131	114	0.8	28	20.6	46.5
Hainan (19°55'N111°29'E)	6.47	1.081	98.4	60.9	53.6	20	17.6	3.66
Hangzhou (30º26'N120º25'E)	7.32	2.675	280	525	40	92.3	155	272
Hunan (26°45'N111°52'E)	5.11	1.266	79.1	202	17.7	23.1	45.5	29.1
Jiaxing (30º77'N120º76'E)	7.48	2.502	163	369	8.19	85.7	155	125
Jilin (42º40'N124º88'E)	8.15	0.926	226	246	4.4	22.8	15.7	75
Langfang (39°31'N116°44'E)	8.3	0.835	143	140	18	21	33.1	24.2
Neimeng (46°03'N22°03'E)	7.6	9.46	239	322	20	354	1925	690
Shandong (37°20'N116°29'E)	8.17	2.192	207	295	3.2	108	285	120
Shanxi (34°19'N108°0'E)	8.2	0.845	52.6	176	6.53	13.1	10.1	32.1
Shijiazhuang (38°03'N114°26'E)	8.25	2.347	235	560	6	72	50.4	255
Xinjiang (43º95'N87º46'E)	8.35	2.021	294	341	40	63.7	433	315
Zhengzhou (34º47'N112º40'E)	8.2	0.97	94.3	118	<2	27	55	48

Bok choy shoot bioassay. The bok choy (*Brassica chinensis L.*) shoot bioassay was performed according to ISO 11269-2 (1995). Five pre-germinated bok choy seeds (radicles < 5 mm) were planted in three replicate pots of each Ni treatment of each unleached and leached soil. During the growth period, the soil moisture content was maintained at 60~65% of water holding capacity by additions of deionized water and the nutrient solution was also added to ensure the plant growth. After 21 days, the bok choy shoots were cut just above the soil surface and dried for 48 h in dry oven (70 °C), and then the dry biomass was recorded.

Statistics. The log - logistic dose - response curves based on the soluble Ni concentrations and toxicity effects were used to calculated toxicity thresholds [14]. The equation was listed as following:

$$Y_1 = \frac{Y_0}{1 + e^{(b(X-M))}} \tag{1}$$

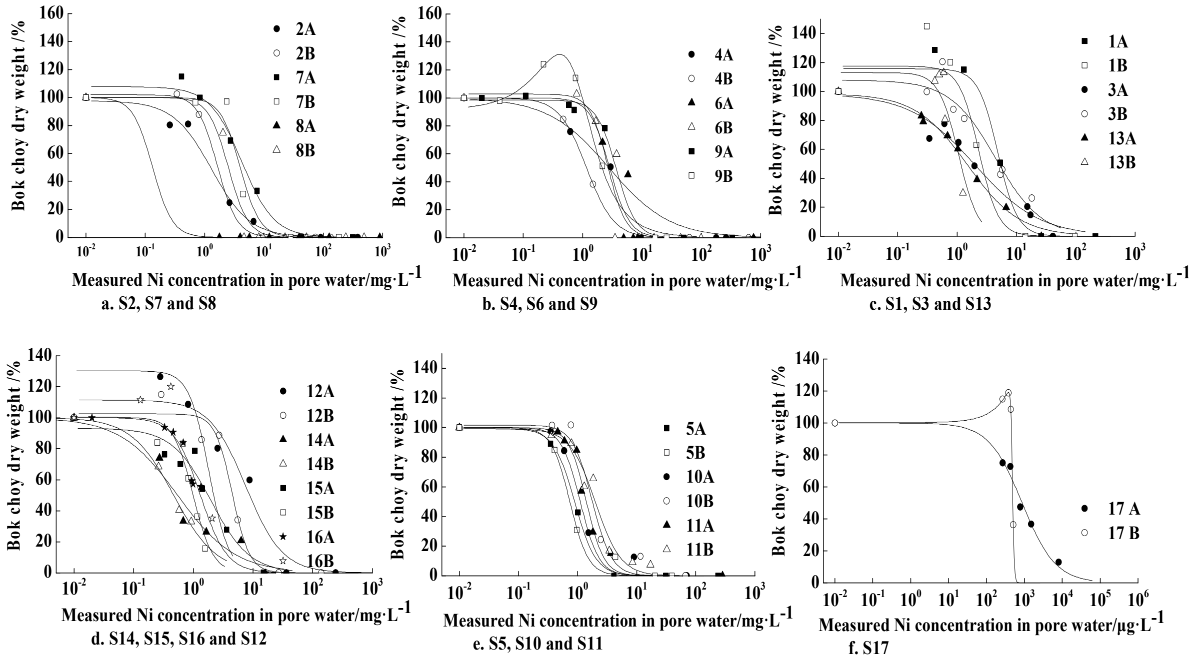
Where Y_1 = bok choy shoot biomass, X_1 = log10 (actual concentration of soluble Ni), and Y_0, M and b were parameters to be fitted. The 95% confidence intervals of thresholds were also derived from the fitted curve parameters.

The hormesis effect of low soluble Ni levels was referred to Schabenberger et al. [15] and the 95% confidences intervals of toxicity thresholds were fitted by Table Curve 2D v5.01. The multiple regressions were analyzed by SPSS 19.0 for Windows (SPSS, Chicago, IL, USA).

Results and Discussion

Dose - response curves and toxicity threshold values for soluble Ni. The results from dose and response fitting showed that bok choy shoot growth was significantly inhibited at high soluble Ni concentrations (Fig. 1). The soil solution chemistry obviously influenced toxicity thresholds. For instance, in 17 unleached soils, the EC10 and EC50 ranged from 0.05~2.09 and 0.13~7.60 mg/L, which represented 41.8 and 58.5 differences between the maximum and minimum value, respectively (Table 2). Similarly, in the leached soils, the EC10 and EC50 ranged from 0.08~2.16 and 0.47~4.49 mg/L, which represented 27 and 9.6 differences, respectively (Table 2). These results indicated that the ranges of toxicity thresholds were largely reduced by leaching treatment. However, the difference was still more than 9.6, suggesting that the soluble Ni toxicity was depended on soil solution properties. For the added Ni toxicity to bok choy was also determined by soil properties and the toxicity thresholds in unleached or leached soils varied considerably from 25.6 to 55.5 fold differences [16]. It concluded that the soluble Ni in the unleached soils could not better explain variance of toxicity threshold, whereas that in the leached narrowed down the differences caused by different soil solution properties.

From the dose - response curve, it showed that the bok choy shoot was simulated in the low level of soluble Ni in the soil S9 and S17, where the largest bok choy dry weights were 124% and 119% to the corresponded controls. There were several mechanisms which could account for hormetic effects, including overcompensation, overcorrection and DNA repairing [17-19]. However, these phenomena were only observed in two soils and hence little data was appropriate for further consideration of hormesis effects.



a. S2, S7 and S8
b. S4, S6 and S9
c. S1, S3 and S13
d. S14, S15, S16 and S12
e. S5, S10 and S11
f. S17

Fig.1 The dose - response curves for soluble Ni concentrations for bok choy shoot in 17 unleached and leached soils.

Table 2 Toxicity thresholds of soluble Ni to bok choy shoot (mg/L)

No.	Location	EC10	EC50	EC10	EC50
S1	Beijing	0.09 (0.35-12.58)[a]	4.87 (2.80-8.45)	1 (0.260-3.87)	2.33 (1.05-5.13)
S2	Chongqing	0.25 (0.08-0.77)	1.31 (0.79-2.16)	0.71 (0.55-0.91)	1.65 (1.09-2.49)
S3	Gansu	0.13 (0.02-0.90)	2.01 (0.81-5.01)	0.64 (0.11-3.80)	4.46 (2.05-9.67)
S4	Guangzhou	0.21 (0.07-0.60)	2.77 (1.78-4.31)	0.36 (0.36-0.37)	1.16 (1.16-1.17)
S5	Hailun	0.36 (0.27-0.48)	0.89 (0.81-0.99)	0.34 (0.12-0.90)	0.74 (0.51-1.08)
S6	Hainan	1.22 (0.22-6.78)	2.84 (1.20-6.70)	1.23 (0.24-6.13)	2.64 (1.36-5.15)
S7	Hangzhou	1.07 (0.44-2.61)	4.26 (2.80-6.46)	1.71 (0.54-5.48)	3.98 (2.35-6.74)
S8	Hunan	0.06 (0.04-0.09)	0.13 (0.09-0.20)	1.08 (0.52-2.27)	2.51 (1.78-3.54)
S9	Jiaxing	1.58 (1.10-2.28)	3.68 (2.96-4.57)	1.27 (0.93-1.61)[b]	2.30 (1.48-3.13)[b]
S10	Jilin	0.49 (0.18-1.33)	1.11 (0.73-1.68)	0.76 (0.26-2.22)	1.76 (1.05-2.95)
S11	Langfang	0.66 (0.40-1.10)	1.41 (1.15-1.73)	0.67 (0.26-1.75)	2.00 (1.40-2.87)
S12	Neimeng	1.61 (0.18-14.79)	7.60 (2.75-21.0)	2.16 (0.61-7.61)	4.49 (3.09-6.53)
S13	Shandong	0.16 (0.08-0.35)	1.50 (1.09-2.08)	0.46 (0.15-1.42)	1.06 (0.51-2.20)
S14	Shanxi	0.05 (0.00-1.48)	0.55 (0.18-1.67)	0.08 (0.03-0.24)	0.47 (0.34-0.65)
S15	Shijiazhuang	0.38 (0.04-3.54)	1.97 (0.86-4.49)	0.43 (0.19-0.95)	0.99 (0.73-1.35)
S16	Xinjiang	0.43 (0.26-0.69)	1.31 (1.07-1.62)	0.79 (0.15-4.24)	1.83 (0.51-6.63)
S17	Zhengzhou	0.10 (0.02-0.38)	0.87 (0.51-1.49)	0.46 (0.45-0.48)[b]	0.5 (0.49-0.51$^{)b}$

a: Ranges given as 95% confidence intervals (C. I.); EC10 or EC50: soluble Ni concentrations resulted in 10 % or 50 % inhibition; b: Significant difference between unleached or leached EC10 and EC50 using a T-test at the $p \leq 0.05$ significance level.

Multiple linear regression models to predict soluble Ni toxicity. The relationship between pore water properties (pH, EC, K^+, Na^+, Ca^{2+}, Mg^{2+}, S, DOC) and soluble Ni toxicity thresholds was analyzed through multiple regressions. Soil solution Mg^{2+} and EC were the two best single predictors of toxicity thresholds (Equation 4, 7, 9). Soil solution Mg^{2+} explained 60% and 39% of the variance for EC50 in unleached and leached soils, respectively. Soil solution EC explained 61% of variance for EC10. Moreover, other soil properties also significantly influenced the soluble Ni toxicity, for example K^+ and pH values. The determination coefficient of the equations (r^2) of equation inclusion of K^+ and Mg^{2+} increased to 0.72 (Equation 3), and r^2 increase to 0.71 by incorporation of pH and EC (Equation 6).

In Table 3, when more soil solution properties were taken into account, the r^2 for EC10 and EC 50 in unleached soil was 0.56 to 0.84, respectively, and those was 0.85 and 0.74 in leached soil, respectively (Equation 1, 2, 5, 8), which were further enhanced. These empirical models based soil solution properties could provide reasonable estimation for soluble toxicity for bok choy. It was obviously that the difference of r^2 and significant factors between the unleached and leached soil was quite large, which may be caused by the discrepancy of soil solution properties and toxicity threshold.

Table 3 Simple and multiple linear regressions for unleached and leached soils from bok choy shoot bioassay between soluble Ni toxicity thresholds and soil pore water chemistry

No.	Regression equations	r^2	p
	Unleached soils		
1	EC10= -1.8+0.18pH+0.024K+0.003Ca+0.26EC+0.002Mg+0.002Na-0.009S +0.0002DOC	0.56	-
2	EC50=-5.3+0.48pH+0.053K+0.002Ca+1.6EC+0.011Mg-0.001Na-0.015S+0.005DOC	0.84	-
3	EC50=0.41+0.018Mg+0.041K	0.72	*
4	EC50= 1.2+0.018Mg	0.60	**
	Leached soils		
5	EC10=2.3-0.29pH+0.001K-0.016EC+0.009Mg-0.001Na+0.004S+0.001DOC	0.85	-
6	EC10 =1.728-0.185pH+0.421EC	0.72	*
7	EC10 =0.266+0.422EC	0.61	***
8	EC50= 7.1-0.76pH+0.002K-0.004Ca-1.7EC+0.058Mg-0.004Na+0.013S+0.007DOC	0.74	*
9	EC50=1.2+0.025Mg	0.39	**

r^2: coefficient of determination (percentage of variance accounted for by the regression model); p: significant level, *, ** and *** represented 5%, 1% and 1‰ significant level; EC10 and EC 50 represented the soluble Ni concentration caused 10% and 50% inhibition of bok choy; DOC: dissolved organic carbon; EC: electrical conductivity.

Discussion

No consistent significant major factors were found in the unleached and leached soils except Mg^{2+}. In the equation 4 and 9, the Mg^{2+} was positively correlated with EC50. As the Mg^{2+} concentration increased 100 mg/L, the EC50 increased 1.8 and 2.5 in unleached and leached soils, respectively. It was more apparently in Fig. 2 where the EC50 increased as Mg^{2+} concentration increased. The protective effects of Mg^{2+} against soluble Ni toxicity to barley and tomato were also revealed in real soil solution [20, 21]. The mechanism for this effect may be ascribed to the competition of Mg^{2+} with Ni^{2+} for the ligand sites [8]. Similarly to Mg^{2+}, the positive relationship was found between K^+ and EC50, exhibited with the increased trend of EC50 as K^+ increased. Except the protective effect, K^+ was an important cation in most biological systems [22, 23]. It was reported that the quality and yield of bok choy were improved by K^+ [24]. Therefore, the influence of K^+ was caused by the interaction between Ni toxicity and plant nutrients in the plant growth.

In the leached soil, the soil solution pH and EC10 exhibited relatively poor linear correlation and the relationship between them was more complicated (Fig.2). Especially for the soil pH of 8~9, EC10 decreased as pH increased in most soils and the trend was opposite in the rest few soils. The EC was significantly influenced Ni toxicity, with a quite stable growth of EC10 as EC increased. The bok choy was sensitive to salt damage with limit value of 1.8 mS/cm. In present assay, the EC of soil solution was more than the limits in Jiaxing, Hangzhou and Dezhou. However, the bok choy maintained the normal production without being inhibited by salinity stress. The EC was determined by the dissolved cations and anions concentrations. While the K^+ and Mg^{2+} were proved to decrease Ni toxicity. Moreover, Ca^{2+} played an important role in adjusting the osmotic pressure, protecting cell structure and promoting photosynthesis. Therefore, the effect of EC on Ni toxicity was indirectly influenced by the dissolved cation concentrations. Although the EC could relieve the Ni toxicity, it may induce potential salinity threaten to bok choy shoot.

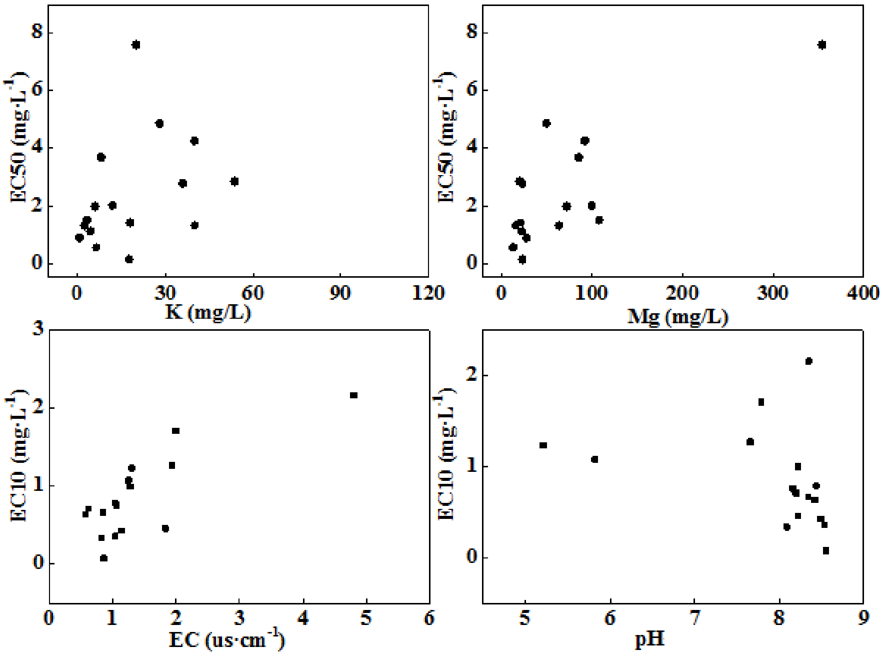


Fig. 2 Relationships between soil solution properties of K^+, Mg^{2+}, EC, pH values and toxicity thresholds of soil soluble Ni in pore water obtained from the bok choy shoot assays

Conclusions

In conclusion, the soluble Ni toxicity thresholds to bok choy varied widely among 17 typical Chinese soils. The leached treatment reduced the variation. However, it also represented larger than 9.6 differences and thus the soluble Ni would not accurate explain the variation among different types of soils. Soil soluble Ni phytotoxicity was controlled byMg^{2+} and EC, while pH exhibited less closely with toxicity thresholds.

Acknowledgements

The authors were appreciated to the financial support from the Natural Science Foundation of China (project no.40971262), and the Special Fund for Public Industry in China (Agriculture, project no.200903015).

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