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Assessing availability, phytotoxicity and bioaccumulation of lead to ryegrass and millet based on 0.1 mol/L $Ca(NO_3)_2$ extraction

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Abstract: This study was conducted to assess availability, phytotoxicity and bioaccumulation of lead (Pb) to ryegrass (*Lolium perenne* L.) and millet (*Echinochloa crusgalli*) based on the 0.1 mol/L $Ca(NO_3)_2$ extraction. Effect of soil properties on availability, phytotoxicity and bioaccumulation of Pb to the two plants was also evaluated. Five soils with pH values varying from 3.8 to 7.3, organic carbon (OC) contents from 0.7% to 2.4%, and clay contents from 11.6% to 35.6% were selected. Soils were spiked with Pb to achieve a range of concentrations: 250, 500, 1000, 3000 and 5000 mg/kg. Pb availability in the spiked soils was estimated by extracting soil with 0.1 mol/L $Ca(NO_3)_2$. The results indicate that plants yield decreased with decreasing soil pH and increased with increasing soil clay and OC content. Negative relationship between available Pb and the relative dry matter growth (RDMG) of the two plants were significantly related. Available Pb used to assess EC_{20} (20% effective concentration) and EC_{50} (50% effective concentration) of millet was 119 and 300 mg/kg, respectively. Available Pb used to assess EC_{20} and EC_{50} of ryegrass was 63 and 157 mg/kg, respectively. Bioaccumulation, expressed as bioconcentration factors of Pb, was inversely related to soil pH, soil OC and clay content. Strong relationships were found between available lead and uptake by the two plants (r^2 was 0.92 and 0.95 respectively). In general, 0.1 mol/L $Ca(NO_3)_2$ available Pb may be used to assess the availability, phytotoxicity and bioaccumulation of lead to the two plants tested.

Keywords: heavy metals; bioconcentration factor; spike; extraction; lead (Pb)

Introduction

Lead (Pb) is not a plant nutrient. High content of Pb in soil is toxic to plants. Small amount of Pb in soil may not result in toxicity because most plants can tolerate small amount of Pb. However, high concentrations of Pb in soil can be absorbed and accumulated by plants, and may cause toxicity to occur. Soil physicochemical conditions, such as pH, and the content of clay and organic carbon affect Pb availability, phytotoxicity and bioaccumulation. Pb is found in different forms in soils. Some forms are more soluble and available than others. The different forms of Pb have different effects on plants in different soils. Schroder (2003) reported that the bioaccumulation and phytotoxicity of Pb to lettuce differed with soil properties. Accordingly, the ecotoxicological end-points were greatly affected by soil properties. For same EC_x (x% effective concentration), where x is the percentage reduction, the total concentration of metals may be different. For example, 1000 mg/kg of Pb may result in 20% reduction of plants yield in high clay soil, but 50% reduction in sandy soil. Similar results can be obtained in terms of germination rate and bioaccumulation to plants. So, it is very hard to assess a plant yield reduction and Pb bioaccumulation just according to total Pb concentrations in soil without knowing the soil property. Therefore, total amount of Pb concentration in different soils is not a good indicator for the Pb availability, phytotoxicity and bioaccumulation. An effective sentinel to reflect the real effects of Pb in soils with different properties to plants is needed. Basta and Gradwohl (2000) indicated that metal levels extracted by 0.1 mol/L $Ca(NO_3)_2$ were well correlated with metal availability and toxicity to lettuce. The objective of this study was to evaluate whether the availability, phytotoxicity and bioaccumulation of Pb to ryegrass and millet can be assessed based on 0.1 mol/L $Ca(NO_3)_2$ extraction across different soil properties with a large range of Pb concentrations. Effect of soil properties on the availability, phytotoxicity and bioaccumulation of Pb to the two selected plants was also evaluated.

1 Materials and methods

1.1 Selection of soils

Five soils with different physical/chemical properties including soil pH, organic carbon (OC) content and clay content were collected from Oklahoma State and Iowa State of USA. The soil physical/chemical properties (Table 1) showed a wide range including soil pH (3.8 to 7.3), organic carbon (0.7% to 2.4%) and clay content (11.6% to 35.6%). The Teller and Sassafras soils are acid and sandy soils with low absorption capacity. The Webster and Kirkland soils are neutral soils with similar clay content while the organic carbon content is very high in the Webster soil and very low in the Kirkland soil. The clay content in Richfield soil is close to that of Webster soil and Kirkland soil while the pH is much higher. The OC content in Richfield soil is higher than that of Kirkland soil while lower than that of Webster soil. All soils were air-dried and sieved to pass a 2-mm screen prior to analysis.

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Table 1 Physical and chemical properties of soils

Soil	Soil pH ($CaCl_2$)[a]	Soil pH (H_2O)[b]	OC[c], %	Clay, %
Teller	3.80	4.50	0.72	12.3
Sassafras	4.40	5.19	0.41	11.6
Webster	5.50	6.01	2.39	35.6
Kirkland	5.78	6.27	0.66	27.5
Richfield	7.30	7.60	1.43	30.9

Notes: a. Soil pH measurement in 1∶1 (soil:0.01 mol/L $CaCl_2$ solution); b. soil pH measurement in 1∶1 (soil:deionized water); c. OC (organic carbon) content in soil

1.2 Soil physical and chemical properties

Soil pH was measured with soil:0.01 mol/L $CaCl_2$ (1∶1) and soil∶water (1∶1), respectively (Sparks *et al.*, 1996). Acid dichromate digestion was used to determine soil organic carbon content (Heanes, 1984). The hydrometer method was used to determine soil texture (Gee and Bauder, 1986). Duplicate soil pH and soil organic carbon content were analyzed and triplicate analyses were conducted in the determination of soil texture.

1.3 Pb spiking and incubation

Soils were spiked at the desired concentrations with reagent grade $Pb(NO_3)_2$. All spikes were calculated on a metal basis and 0.5 L of spiking solution was prepared using the metal salt and deionized distilled water. 200 ml spiking solution was added and mixed with 1 kg of soil in an aluminum pan. Additional deionized distilled water was added and thoroughly mixed with the soil with a glass stick to make a saturated paste. The soils in the aluminum pans were oven-dried at 60℃ for 20 h. Then the dried soils were removed from the oven and rewetted with deionized distilled water followed by being dried under the same condition. All soils underwent three wet-dry cycles to achieve adequate reaction with the soil matrix. Each spiked soil in an aluminum pan was split into five 200 g subsamples. Four of the five 200 g soil subsamples were put into four-inch pots for the range finder studies and the other one was left for later analysis.

1.4 Range finder test

Range finder tests were used to determine the soil metal concentrations that result in little to 100% phytotoxicity for the plants in the growth study. Selected plants were perennial ryegrass and millet in this study. For each soil, Pb was nominated in six concentrations: 0 (control), 250, 500, 1000, 3000 and 5000 mg/kg. The concentrations were based on literature values for sub-lethal toxicity in the test organism of concern. 200 g spiked soil was weighed out and put into the four-inch plant pot. Four replicates were conducted in the range finder study. Each soil was tested for available N-P-K prior to planting and fertilized with the needed nutrients of an equivalent 120 lbs/acre N (60 mg/kg), 60 lbs/acre P (30 mg/kg), 60 lbs/acre K (30 mg/kg). Twenty seeds were sowed in each pot. The test conditions were set as: temperature: 25℃ (light) and 20℃ (dark) ± 3℃; photoperiod: 16 h (light) and 8 h (dark); light intensity: (5000 ± 500)lx; soil moisture: 75% of water holding capacity. The pots were wrapped with a plastic bag to keep the soil moisture. The luminosity level was measured weekly using a photometer to make sure the light intensity was as designed. After the germination incubation time, the emerged seeds were counted for each pot. Then, the shoots of the plants were harvested two weeks later. The fresh shoot biomass was placed in a drying oven set at 70℃ for 24 h, and immediately weighed out and recorded.

1.5 Definitive test

As the concentrations from no effect on plants to lethal were obtained from the range finder tests, the same metal concentrations were used in the definitive tests. The test conditions and procedures were similar to those of range finder test. However, the plants were grown for 40 d. After the fresh biomasses were oven-dried and recorded, the plants were ground and placed in paper sample bags and stored at room temperature. Acid wet digestion using HNO_3 and $HClO_4$ was conducted on all plant materials and Pb in the solutions were analyzed by inductively coupled plasma (ICP).

1.6 Measurement of available Pb using 0.1 mol/L $Ca(NO_3)_2$ extractant

All the soil samples were extracted with neutral salt extraction (0.1 mol/L $Ca(NO_3)_2$) to determine the Pb availability. Soil (1.0 g) was weighed out in a 50-ml centrifuge tube and 20.0 ml of 0.1 mol/L $Ca(NO_3)_2$ was added. The samples were shaken on a reciprocal shaker for 16 h. The solution was then centrifuged at 7190 g for 15 min. The centrifuged supernatants were filtered with 0.45-mm syringe filters into 20-ml glass scintillation vials. 1.0 ml of trace metal concentrated hydrochloric acid (HCl) was added to each sample. All the samples were then stored at 4℃ until the analysis of metal was conducted by inductively coupled plasma atomic emission spectrometry (ICP-AES).

1.7 Quality assurance and quality control

Blanks, spikes and certified/standard reference materials were digested and analyzed for quality assurance and quality control of Pb in soil and plant tissue. Examples of certified/standard reference materials for different sample types include: Soil (CRM020-050, RTC Corporation, Laramie, WY, USA) and plant tissue (*Lagarosiphon* major BCR No 60). Blanks, spikes and certified/standard reference materials were evaluated for every six samples of soil or plant tissue. Mean recoveries of Pb in certified reference soil (CRM020-050, RTC Corporation, Laramie, WY, USA) was 98% with relative standard deviation 3.7% , while mean recoveries of Pb in certified plant materials (Aquatic Plant BCR) was 95% with relative standard deviation 2.6%. Another certified reference material (Benson soil from North American Proficiency Testing Program) was analyzed to validate the texture measurement. The clay content just was less than 1% difference between our measurement (53.6%) and the data provided by North American Proficiency Testing Program (53.1%).

2 Results and discussion

2.1 Effect of soil types on available Pb

Available Pb varied among the five soils across all the spiked concentrations (Table 2). Available Pb increased with decreasing soil pH. In the alkaline soil (Richfield soil), available Pb were always the lowest while those in the acidic soils (Teller and Sassafras soils) were always the highest and those in the neutral soil (Webster and Kirkland soils) were between them. Pb precipitation and adsorption could cause decreasing Pb availability with increasing soil pH, for more and more ionic and exchangeable Pb were precipitated as Pb hydro-oxide and Pb carbonate and adsorbed to soil components, which are not available to plants any more. The results are comparable with former researches. Deiss *et al.* (2004) found that Pb's enhanced-mobility in soil was facilitated by the high acidity of soil. The sorption of Pb increases with increasing pH and decreased with decreasing pH (Young *et al.*, 1993). Elzahabi and Yong (2001) concluded that the solubility of Pb was highly pH dependent and increased with decrease in the soil pH. It was also reported that one of the most important method to decrease lead availability to plants was liming which increased soil pH and enhanced Pb retention in the solid phase (Tsadilas, 2000).

In addition to the effect of pH, high clay content was another important factor that contributed to the difference of available Pb in soil. High clay content decreased Pb availabilities. Available Pb in sandy soils (Teller and Sassafras soils) was higher than those in high clay content soils (Kirkland, Webster and Richfield). High organic matter content also decreased the available metal. Available Pb in Webster was lower than those in the Kirkland soil, which was due to the higher soil organic matter content in the Webster soil. Strawn and Sparks (2000) reported that soil organic matter was an important factor that affected the sorption and desorption of Pb in soil. McBride *et al.* (1997) also stated that Pb solubility depended on clay and organic matter content.

Table 2 Mean available Pb in soils

Unit: mg/kg

Soil	Spiked Pb concentration					
	Control	250	500	1000	3000	5000
Teller	<1.05[a]	31.0±0.72	96.5±2.04	204±6.03	926±22.5	1702±60.3
Sassafras	<1.05	26.0±0.70	84.1±2.40	185±4.11	790±25.6	1795±53.9
Webster	<1.05	1.22±0.03	2.86±0.05	8.76±0.15	39.9±0.74	96.5±1.87
Kirkland	<1.05	1.17±0.03	3.16±0.06	13.6±0.27	120±2.64	320±7.77
Richfield	<1.05	<1.05	<1.05	1.33±0.03	7.21±0.18	14.3±0.36

Note: [a] Detection limits

2.2 Plants germination

Germination rates (*Tf*) (Table 3) decreased with decreasing soil pH and increased with increasing soil clay and OC content in the high Pb spiked soils (>500 mg/kg in this study). In 250 mg/kg Pb spiked soils, both two plants did not show big *Tf* differences across all soils. *Tf* decreased sharply in the acid sandy soil while slowly in the clayey soils.

Germination rate was not a sensitive enough indicator of the metal phytotoxicity. One reason was *Tf* just decreased a little before they were sharply down to zero. So, it was very hard to get the precise metal EC_{20} and EC_{50} of germination rate. Another reason was that most of *Tf* were similar while plant yields were greatly different. In the same soil with different metal concentrations, germination rates were close while plants yields differed more than 50%. In this case, it was impossible to tell the metal phytotoxicity by the germination rate. Schroder (2003) also indicated the germination was not a sensitive indicator of metal availability and toxicity.

2.3 Plants yield

Generally, relationships between plants dry biomass and spiked metal concentrations were very strong (Fig.1). Yields decreased with soil pH and increased with clay and organic matter content of soil. Both two plants yields in sandy acidic soils (Teller and Sassafras soil) were lower than those in soils with higher pH, higher clay and OC content (Webster, Richfield and Kirkland soil). In general, the yield across the two plant species followed the trend

Webster $\geqslant$ Richfield, Kirkland $>$ Sassafras $\geqslant$ Teller

These results were consistent with the effect of soil chemical properties on availability of Pb. With higher pH in soil, more precipitation reaction would occur, which may decrease the Pb availability and phytotoxicity. In the soil with high clay and organic matter content, there are a large number of sorption sites, which could adsorb high concentration of Pb and decrease the Pb toxicity to plants. However, in the sandy soil, there are fewer sorption sites and the metal

Table 3 Mean germination rates (*Tf*) of ryegrass and millet in the definitive study

Soil	Spiked Pb concentration, mg/kg					
	Control	250	500	1000	3000	5000
			Tf of ryegrass, %			
Teller	80.0±1.41	82.0±1.41	86.0±4.24	63.0±1.41	0.0	0.0
Sassafras	85.0±2.83	81.0±2.83	82.0±2.83	70.0±2.83	0.0	0.0
Webster	88.0±4.24	89.0±4.24	88.0±1.41	86.0±4.24	85.0±1.41	72.0±1.41
Kirkland	83.0±1.41	90.0±5.66	83.0±2.83	86.0±2.83	78.0±2.83	0.0
Richfield	90.0±4.24	92.0±4.24	90.0±5.66	88.0±4.24	84.0±4.24	78.0±2.83
			Tf of millet, %			
Teller	83.0±1.41	87.0±5.66	86.0±2.83	62.0±1.41	0.0	0.0
Sassafras	88.0±2.83	88.0±4.24	86.0±5.66	63.0±1.41	0.0	0.0
Webster	92.0±4.24	88.0±1.41	90.0±1.41	91.0±2.83	86.0±4.24	79.0±1.41
Kirkland	92.0±2.83	90.0±5.66	91.0±4.24	86.0±4.24	84.0±2.83	85.0±2.83
Richfield	90.0±5.66	92.0±2.83	90.0±2.83	86.0±5.66	87.0±5.66	80.0±4.24

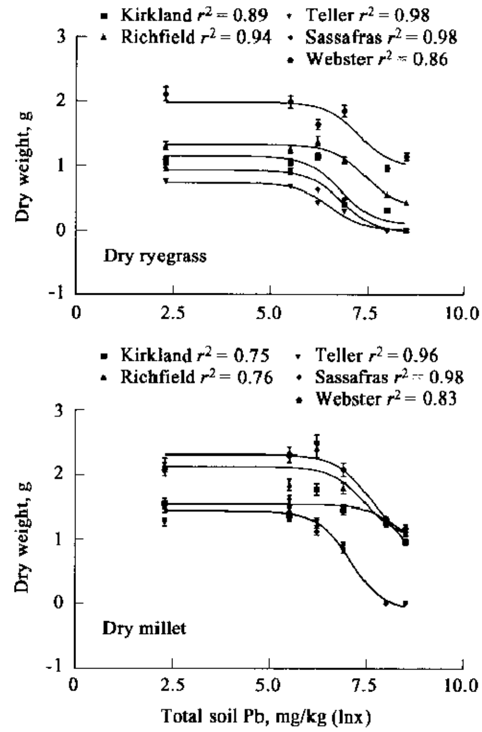

Fig.1 Relationships between soil Pb contents and the biomass of ryegrass and millet

would have higher availability and toxicity to plants. Peralta-Videa *et al.* (2002) also reported that plant yields were significantly higher at pH 7.1 than at pH 4.5. Under normal conditions, plants grow better at pH higher than 6.5 (Brady and Weil, 1999).

2.4 Relationships between available Pb and RDMG

As the dry matter growth was different in different soils, relative dry matter growth (RDMG), which refers to the relative dry matter growth, was calculated for each plant in each soil across all the five levels of Pb. RDMG is equal to the dry matter growth of metal treatment for a soil/average dry matter growth of control for that soil as described by Equation (1):

$$\text{RDMG}=\frac{r_{\text{metal}}}{A_{\text{control}}} \tag{1}$$

Where, r_{metal} is dry matter growth of metal treatment for a soil; A_{control} is the average dry matter growth of control for that soil.

Significant relationships were found between available Pb and RDMG of ryegrass (r=0.82, p<0.01) and millet (r=0.80, p<0.03) (Fig.2).

2.5 Available metals used to assess EC_{20} and EC_{50} values

EC_{20} and EC_{50} values (Table 4) in different soils were calculated based on the dose-response curves (GraphPad, 3.0). In general, EC_{20} and EC_{50} values decreased with increasing soil pH, clay and OC content. As EC_{20} and EC_{50} values differed greatly in different soils, it is very hard to assess a plant yield reduction according to total metals concentrations in soil without knowing the soil properties.

Available Pb used to assess the EC_{20} and EC_{50} of plants were calculated when available Pb and RDMG of a plant was significantly related. The calculation procedure was as follows: For example, the linear regression between available Pb and RDMG of millet was significant (p<0.03) and the equation was y = -0.0016x + 0.96 (Fig.2). In the clean control soil, x (available Pb) was accepted as zero to calculate the RDMG, which was 0.96 in this case. Therefore, calculated RDMG of millet at EC_{20} and EC_{50} were (1–20%) ×0.96=0.77 and (1–50%) ×0.96=0.48, respectively. Accordingly, x (available Pb) was calculated as 119 mg/kg at EC_{20} and 300 mg/kg at EC_{50} of millet grown on Pb spiked soils. Therefore, available Pb used to assess EC_{20} and EC_{50} of millet was 119 and 300 mg/kg, respectively. Similarly, available Pb used to assess EC_{20} and EC_{50} of ryegrass was 63 and 157 mg/kg, respectively.

2.6 Effect of soil types on bioaccumulation of Pb to plants

Bioconcentration factors (BCF) were calculated to reflect the bioaccumulation of metals in the plants grown in spiked soils. The BCF value is equal to the

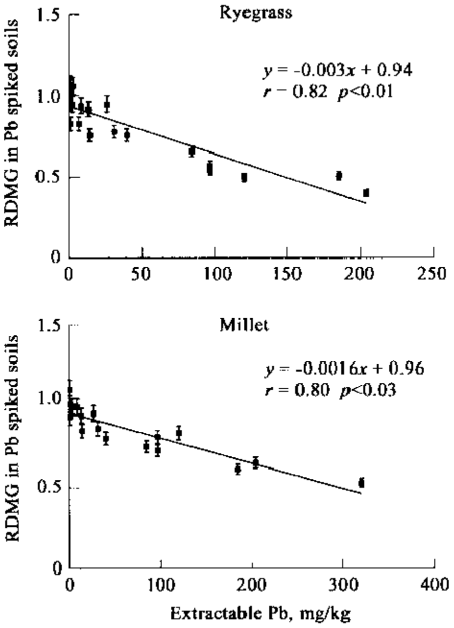

Fig.2 Relationships between extractable Pb and RDMG of ryegrass and millet

Table 4 EC_{20} and EC_{50} values of ryegrass and millet (mg/kg)

Soil	Ryegrass		Millet	
	EC_{20}	EC_{50}	EC_{20}	EC_{50}
Teller	380±19.0	693±34.7	605±30.3	1102±55.1
Sassafras	492±24.6	896±44.8	603±30.2	1099±55.0
Webster	2482±124	4523±226	2338±117	4259±213
Kirkland	549±27.5	1001±50.1	3395±170	6186±309
Richfield	1620±81.0	2951±148	2505±125	4564±228
Available Pb across all soils	70.0±3.50	174±8.70	145±7.30	362±18.1

metal concentration in plant biomass/spiked metal concentration in soil as described by Equation (2):

$$\mathrm{BCF}=\frac{C_{\mathrm{plant}}}{C_{\mathrm{soil}}} \qquad (2)$$

Where C_{plant} is metal concentration in plant biomass; C_{soil} is spiked metal concentration in soil.

BCF values (Table 5) of Pb were inversely related to soil pH, OC and clay content. BCF values of the two acidic soils (Teller, Sassafras) were much greater than those of the soils with higher pH (Webster, Richfield, Kirkland). BCF values of the two sandy soils (Teller, Sassafras) were much greater than the higher clay content soils (Webster, Richfield, Kirkland). Meanwhile, BCF values of the Kirkland soil were much greater than those of the Webster soils, which suggested that the high organic content in Webster soil decreased the uptake rate of the metals. The results are comparable with other research reports. Lagerwerff *et al.* (1977) found that the Pb content in the plant was reduced by 9%—21% when the soil pH was raised from 5.2 to 7.2. According to

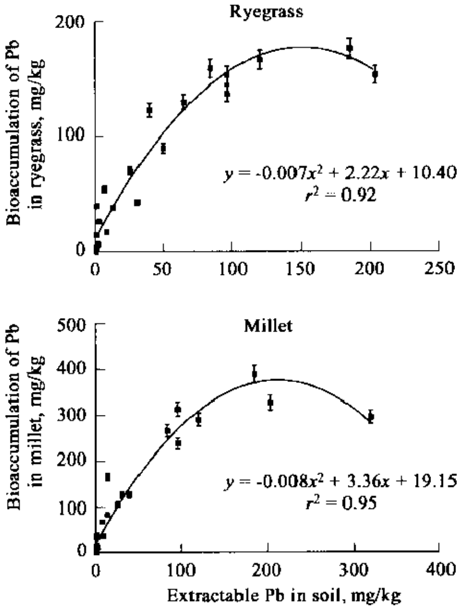

Fig.3 Relationships between extractable Pb and plants uptake

Table 5 Bioconcentration factors (BCF) of Pb in two studied plants in all studied soils

soils	Spiked Pb concentration, mg/kg					
	250	500	1000	3000	5000	Average
			BCF of Pb in ryegrass			
Teller	0.17±0.004	0.18±0.003	0.14±0.0031	NG	NG	0.17±0.0043
Sassafras	0.44±0.0098	0.36±0.009	0.23±0.0064	NG	NG	0.34±0.0074
Webster	0.02±0.0003	0.01±0.0001	0.02±0.0002	0.04±0.0009	0.03±0.0003	0.02±0.0005
Kirkland	0.04±0.001	0.04±0.0009	0.07±0.0018	0.06±0.0013	NG	0.05±0.0013
Richfield	0.02±0.0005	0.03±0.0004	0.05±0.0011	0.04±0.0009	0.03±0.0006	0.03±0.0008
			BCF of Pb in millet			
Teller	0.51±0.012	0.63±0.0106	0.33±0.0074	NG	NG	0.49±0.012
Sassafras	0.42±0.009	0.53±0.0141	0.39±0.0097	NG	NG	0.45±0.012
Webster	0.02±0.0006	0.02±0.0006	0.04±0.0007	0.04±0.0004	0.05±0.0011	0.03±0.0008
Kirkland	0.07±0.0015	0.07±0.0016	0.08±0.0019	0.1±0.0023	0.06±0.0014	0.08±0.0018
Richfield	0.05±0.0009	0.07±0.0017	0.04±0.0009	0.02±0.0004	0.03±0.0004	0.04±0.0005

Note: NG, no growth

Aten and Gupta (1996), the BCF value of Pb to ryegrass on five neutral soils was 0.06. However, the BCF value decreased to 0.02 in seven soils with pH around 7.

2.7 Relationships between available Pb in soil and plant uptake

Non-linear regressions between available Pb in soil and Pb uptake by plants are shown in Fig.3. Very strong positive relationships were found between available Pb and Pb uptake by the two plants ($r^2 = 0.92$ and $r^2=0.95$ for ryegrass and millet, respectively). Therefore, the uptake of Pb from soil by ryegrass and millet could be predicted based on the 0.1 mol/L $Ca(NO_3)_2$ available Pb regardless of the soil properties. The assessment of plant uptake by available Pb was much better than total Pb concentration in soil. Our results are comparable with other studies. Deng *et al.* (2004) demonstrated Pb accumulation by plants was significantly and positively related to DTPA-available fraction. In Switzerland, the extraction of metals from soils using 0.1 mol/L $NaNO_3$ was used to assess risks due to metal contamination and appeared to be a much better predictor of metal availability than total meal concentration (Gupta and Allen, 1993).

3 Conclusions

In summary, germination rate was not a sensitive enough indicator of the metal phytotoxicity. EC_{20} and EC_{50} of Pb had positive relationships with soil pH, OC and clay content. Available Pb used to assess EC_{20} and EC_{50} of millet was 119 and 300 mg/kg, respectively. Available Pb used to assess EC_{20} and EC_{50} of ryegrass was 63 and 157 mg/kg, respectively. BCF of Pb was inversely related to soil pH, soil OC and clay content. Relationships between 0.1 mol/L $Ca(NO_3)_2$ available Pb and plants uptake were strong across the two studied plants (r^2 ranged from 0.92 to 0.95). Therefore, 0.1 mol/L $Ca(NO_3)_2$ available Pb may be used to assess the availability, phytotoxicity and bioaccumulation to the two plants tested.

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