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Enhanced Accumulation of Lead in *Brassica pekinensis* by Soil-Applied Chloride Salts

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Lead is one of the most important heavy metal pollutants that pose serious environmental and human health hazards. Due to a high affinity for soils, lead is estimated to have a long retention time, in some cases, for as long as 5000 years (Friedland, 1990). Therefore, remediation of Pb-contaminated soils has become a task associated with protecting human beings from long term of Pb hazard. Recently, phytoremediation defined as the use of green plants to remove pollutants from the environment is emerging as a promising strategy for cleanup of the metal contaminated sites (Cunningham and Ow, 1996; Salt et al. 1998). For phytoremediation to be applied successfully, the plants are required to hyperaccumulate heavy metals in their shoots. To do this, two ways have been suggested. One is to use wild hyperaccumulators that have the ability to accumulate dramatically high concentration of heavy metals under natural conditions (Baker et al., 2000). However, these plants usually have low biomass, thus their applications are apparently limited. Another way is to use soil amendments to induce crops with high biomass to hyperaccumulate heavy metals (Cunningham and Ow, 1996; Salt et al., 1998).

One of the important chemical functions of soil is cation exchange reaction. It is known that such cations as Na^+ and Ca^{2+} can release soil adsorbed heavy metals by cation exchange, thus making the metals available to plants (Dai et al., 1996). Heavy metal solubility can also be increased through combination of the metal with inorganic and organic ligands in soil solution to form complexes. EDTA, a synthetic organic ligand that is able to chelate metals, for example, has been shown to enhance lead uptake and translocation in plants (Blaylock et al., 1997; Huang et al., 1997). Chloride is an inorganic ligand that may selectively combine with heavy metals to form complexes (Hahne and Kroontje, 1973; Dai et al., 1996). Previous studies have shown that heavy metal concentrations in plants are positively related to soil salinity (Mclaughlin et al., 1994; Smolders et al., 1998; Grieve et al., 1999). Therefore, it would be possible to increase heavy metal solubility, and thus to enhance plant uptake and translocation by means of soil-applied amendments which provide both cations capable of exchanging soil-adsorbed heavy metals and ligands combining with the metals to form soluble complexes.

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In this study, we focus our attention on effects of several chloride salts on the solubility of soil-adsorbed Pb and the plant uptake and translocation of the metal. The results may have implications on phytoremediation of Pb-contaminated soils. They may also have interest with our understanding the influence of chloride salts on the fate and ecotoxicological effects of Pb in the environment.

MATERIALS AND METHODS

Pot experiments were conducted to evaluate the effects of amendments on solubility of soil-adsorbed Pb. Some parameters of the soil were measured as : pH 5.6, cation exchange capacity (CEC) 51.6 m equiv/100g dry soil, organic matter content 3.0%, and total soil Pb 25.3 mg kg^{-1}. The air-dried and mixed soil was screened to pass through a1-mm sieve, and the water content was determined. The screened soil was placed in 11-cm diameter round plastic pots (200g dry soil per pot). Lead as Pb $(NO_3)_2$ was added in the pots, each having 100mg Pb (about 2.5m M Pb kg^{-1} dry soil). Three days after adding Pb, the pots were amended with NaCl, KCl, NH_4Cl, $CaCl_2$, $FeCl_3$ or EDTA. The former five amendments each had three levels of treatment, i.e., 10, 30 and 90 mM kg^{-1} dry soil, while EDTA had a 2.5 mM kg^{-1} dry soil treatment. Each treatment replicated three times. The soils were watered to field capacity and kept at room temperature (15-25℃). Three days after addition of the amendments, one-tenth fraction of the soil was taken from each pot (equal to 20g dry soil) and mixed with redistilled water (soil: water =1:2.5). The soil samples were agitated on an orbital shaker for 1 h, and then precipitated for 3 h. The supernatant was filtered and measured for Pb concentration.

Pot experiments were also performed to test effects of the amendments on Pb uptake and translocation in plant. Soil for plant culture was prepared as the same for soil experiments mentioned above. *Brassica pekinensis* Rupr, a common vegetable, was selected for experiments. Seeds of cultivar Xiayangbei were purchased from vegetable seed market in Wuhan. Seeds were sterilized in 3% formalin for 5 min, washed with redistilled water for three changes, and soaked in water over night. The soaked seeds were evenly sown in 11-cm diameter round plastic pots filled in 200g (DW) soil, each pot with 15 seeds. The pots were watered daily till seed germination. Then the young seedlings were grown in a cultural facility at temperature 15-25 ℃ and equipped with supplementary lighting (14 h photoperiod, 4000 lux). Every 3 days the seedlings were fertilized with full strength Hoagland's solution containing 5 mM L^{-1} KNO_3, 5 mM L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 2mM L^{-1} $MgSO_4$, 1mM L^{-1} KH_2PO_4, mixture of 0.02 mM L^{-1} $FeSO_4 \cdot 7H_2O$ and 0.02 mM L^{-1} Na-EDTA, 0.045 mM L^{-1} H_3BO_3, 0.01 mM L^{-1} $MnCl_2 \cdot H_2O$, 0.8 μ M L^{-1} $ZnSO_4$, 0.3 μ M L^{-1} $CuSO_4 \cdot 5H_2O$ and 0.1 μ M L^{-1} $NaMoO_4 \cdot 2H_2O$.

When the seedlings were developing 5 or 6 leaves, they were thinned out to retain 7 uniform ones per pot. A total of 36 pots were randomly divided into 12 groups, each group with 3 pots (replicates). Each pot was added with 100mg Pb as Pb $(NO_3)_2$. Three days after Pb addition, ten groups of pot were amended with 10mM and 30mM kg^{-1} NaCl, KCL, NH_4Cl, $CaCl_2$ and $FeCl_3$ respectively, one group with

2.5 mM kg^{-1} EDTA, and one group as control without amendment added. During the treatment period, the seedlings were fertilized with Hoagland's solution every three days. The plants were harvested two weeks after Pb treatment. Plant roots were washed with 5 mM L^{-1} Ca $(NO_3)_2$ for 10 min to desorb the Pb adsorbed on root surface, and further washed with redistilled water. The plants were separated into roots and shoots, and dried in an oven at 60℃ for 24h. Then biomass (DW) was determined.

Lead concentration in soil solution was directly analyzed by flame atomic absorption spectrophotometer (AAS) (WF-5). Dried plant tissues were cut into small pieces with stainless steel scissors, digested in concentrated HNO_3 first at room temperature over night, then under a heating condition till the tissue particles dissolved. The samples were further digested with a mixture of HNO_3 and $HClO_4$ (5:3,V/V) heated over an oven. After cooling, the extracts were diluted and made up to 25 mL with 1 M HNO_3. Lead concentrations of the extracts were determined by AAS.

Two level-nested ANOVA was performed to examine effects of amendments and amendment concentrations on Pb concentration in soil solution. One-way ANOVA was used to test amendment effects on biomass, Pb concentration and Pb amount in plant. If the F-value showed significant differences ($P<0.05$), means were compared with Duncan's multiple range test (LSR).

RESULTS AND DISCUSSION

Addition of amendments significantly increases soluble Pb concentration in the soil (Table 1). The influences are reflected in two aspects. First, different amendments demonstrate different effects on increasing soluble Pb concentration ($P<0.01$). At all the three treatment levels, the same ranking of Pb desorption effect is observed as fellows: $FeCl_3>CaCl_2>NH_4Cl>KCl>NaCl$. Second, amendment amount added in the soil also plays a marked role in increasing soluble Pb concentration ($P<0.001$). Lead in the soil solution increases with increasing amendment amount for all the five salts.

Table 1. Lead concentration in soil solution (μg Pb g^{-1} soil) in relation to addition of five chloride salt amendments

Amendment Concentration (mM kg^{-1} soil)	Amendment				
	NaCl	KCl	NH_4Cl	$CaCl_2$	$FeCl_3$
0	7.5±0.8	7.5±0.8	7.5±0.8	7.5±0.8	7.5±0.8
10	11.3±1.7	12.8±3.1	22.1±7.2	34.7±3.5	160.9±16.8
30	14.3±0.9	28.2±9.9	65.6±6.1	107.8±32.7	312.5±16.9
90	39.3±2.7	127.8±40.2	132.2±41.7	158.4±23.1	352.4±19.9

Results are mean±SD (n=3)

Addition of amendments (chloride salts and EDTA) in the soil significantly influences plant biomass (Table 2). Amending 2.5 mM EDTA, 10 mM FeCl3 and 30 mM NH4Cl in the soil markedly reduces the shoot biomass. Amending 10 mM

FeCl3 also significantly reduces the root biomass. It is interesting to note that amending NaCl increases the root biomass.

Table 2. Biomass (mg plant^{-1}, DW) of *Brassica pekinensis* after two-weeks of culture in Pb-enriched and amendment-added Soil

Treatment (mM kg^{-1} soil)	Biomass		
	Shoot	Root	Total
Control	82.2 ± 11.4^{ab}	11.9 ± 1.6^{c}	94.1 ± 10.0^{ab}
EDTA(2.5)	62.2 ± 7.0^{cd}	12.2 ± 2.0^{bc}	77.4 ± 5.2^{cd}
NaCl(10)	82.6 ± 10.4^{ab}	17.6 ± 1.5^{a}	100.2 ± 9.6^{ab}
NaCl(30)	76.2 ± 2.3^{bc}	15.8 ± 1.7^{ab}	92.0 ± 2.7^{bc}
KCl(10)	97.7 ± 8.6^{a}	12.9 ± 2.6^{bc}	110.7 ± 7.3^{a}
KCl(30)	71.4 ± 8.6^{bc}	12.2 ± 0.8^{bc}	83.6 ± 7.9^{bc}
NH_4Cl(10)	71.4 ± 5.6^{bc}	12.3 ± 2.1^{bc}	83.8 ± 7.4^{bc}
NH_4Cl(30)	53.5 ± 13.9^{d}	12.2 ± 2.6^{bc}	65.7 ± 15.6^{de}
$CaCl_2$(10)	76.5 ± 6.1^{bc}	13.4 ± 2.9^{bc}	89.9 ± 4.3^{bc}
$CaCl_2$(30)	71.6 ± 7.0^{bc}	13.7 ± 0.7^{abc}	85.4 ± 7.3^{bc}
$FeCl_3$(10)	54.9 ± 11.2^{d}	4.9 ± 0.7^{d}	59.9 ± 12.0^{e}

Results are mean$\pm$SD(n=3).Means with different letters are significantly different from one another ($P<0.05$) according to Duncan's multiple range test (LSR).

Lead concentrations in shoot and roots are significantly affected by addition of EDTA and chloride salts (Table 3). Amending 2.5 mM EDTA, 10 mM FeCl3 and 30 mM NH4Cl markedly increases Pb concentration in shoot. Of these amendments, 10 mM $FeCl_3$ shows the strongest effect on increasing shoot Pb concentration, and it is followed by 30 mM NH_4Cl. In the case of roots, amending 10 mM $FeCl_3$ also dramatically increases Pb concentration. On the contrary, Pb concentration in roots by amending EDTA is relatively lower than the control. Pb concentration in roots is not significantly affected by the other treatments.

Pb amounts in shoot, root, total (shoot + root) and shoot/root ratio are also significantly affected by addition of amendments in the soil (Table 3). Compared to the control, Pb amount in shoot is significantly increased through amending 2.5 mM EDTA,30 mM NH_4Cl and 10 mM $FeCl_3$ by more than 2-,3- and 4- fold respectively. The changes of Pb amount in shoot and in roots lead to different shoot/root ratios of Pb amount among the treatments. The highest ratio is found in the case of amending EDTA, and it is followed by 10 mM $FeCl_3$ and then by 30 mM NH_4Cl. The ratios of the other treatments are not significantly different from the control. It should be noted here that although the shoot/root ratio of Pb amount for EDTA treatment is the highest, the corresponding total Pb amount is however the lowest.

The present results clearly indicate that soil-applied NH_4Cl and $FeCl_3$ enhance Pb accumulation in *B. pekinensis*. Several mechanisms may be concerned with the enhancement of NH_4Cl and $FeCl_3$ on Pb accumulation. First, it has been well established that NH^+_4 and Fe^{3+} can exchange soil-adsorbed heavy metals, consequently leading to increase heavy metal solubility and bioavailability (Dai et al, 1996). In this study, soluble Pb in soil is significantly increased in 30mM NH_4Cl and 10mM $FeCl_3$ treatment by 9- and 21- fold respectively as compared to

Table 3. Lead concentration (μ g Pb g^{-1} tissue) and amount (Pb concentration x tissue biomass, μ g Pb $plant^{-1}$) in plant tissue of *Brassica pekinensis* after two-weeks of culture in Pb-enriched and amendment-added soil

Treatment	Concentration		Amount			
(mM kg^{-1}soil)	Shoot	Root	Shoot	Root	Shoot + Root	Shoot/Root
Control	236.3±90.4^{d}	8002.7±182.8^{b}	16.4±1.8^{d}	94.0±14.9^{a}	110.4±16.4^{cd}	0.176±0.019^{d}
EDTA (2.5)	574.0±175.1^{c}	3513.1±833.5^{c}	36.9±9.4^{bc}	41.9±3.1^{b}	78.8±10.8^{d}	0.881±0.218^{a}
NaCl (10)	181.9±64.9^{d}	5852.8±550.9^{bc}	15.0±5.7^{d}	103.8±18.5^{a}	118.8±21.8^{bcd}	0.144±0.052^{d}
NaCl (30)	359.9±180.0^{cd}	7272.3±1804.9^{b}	27.7±14.8^{cd}	113.9±24.4^{a}	141.6±10.1^{abc}	0.150±0.020^{d}
KCl (10)	250.9±197.7^{d}	7716.3±3708.9^{b}	22.0±14.7^{cd}	93.8±35.0^{a}	118.4±47.5^{bcd}	0.161±0.010^{d}
KCl (30)	407.5±153.5^{cd}	7926.6±991.2^{b}	28.2±7.3^{cd}	96.7±9.3^{a}	124.9±7.8^{bcd}	0.295±0.089^{cd}
NH_4Cl (10)	417.1±36.9^{cd}	7668.8±216.0^{b}	29.8±4.1^{cd}	99.7±22.5^{a}	129.6±26.4^{bc}	0.304±0.031^{cd}
NH_4Cl (30)	893.3±161.0^{b}	9405.2±2122.0^{b}	47.2±11.7^{b}	117.4±45.4^{a}	164.6±50.4^{ab}	0.451±0.208^{bc}
$CaCl_2$ (10)	218.3±25.1^{d}	7793.3±1582.0^{b}	15.9±2.8^{d}	103.5±25.9^{a}	119.4±23.3^{bcd}	0.165±0.080^{d}
$CaCl_2$ (30)	290.4±13.3^{d}	9453.0±1266.6^{b}	20.8±2.1^{cd}	128.3±17.7^{a}	149.1±19.6^{abc}	0.163±0.010^{d}
$FeCl_3$ (10)	1366.4±277.5^{a}	24171.2±3841.6^{a}	76.6±30.5^{a}	117.4±1.3^{a}	194.0±29.3^{a}	0.654±0.267^{ab}

Results are mean±SD (n=3).Means with different letters are significantly different from one another ($P<0.05$) according to Duncan's multiple range test (LSR).

the control (Table 1). The elevated soluble Pb in soil would possibly increase Pb bioavailability, thus enhancing Pb uptake by roots. This explanation is consistent with previous reports by other researchers (Kabata-Pendias and Pendias, 1984; Chlopecka, 1996). Second, chloride might have played a role in Pb bioaccumulation, particularly in Pb translocation from root to shoot. Chloride has been shown to be a ligand capable of combining with Pb to form inorganic complexes such as $PbCl^{+}$,$PbCl_2^{0}$,$PbCl_3^{-}$ and $PbCl_4^{2-}$ (Dean,1992).In $CaCl_2$ extracts having a pH lower than 7.5, for example, inorganic complexes are mainly due to chloride, accounting for 16-25% of the total Pb concentration in the solution (Lebourg et al., 1998). In the present study, soil pH is always lower than 5.6 (data not shown). It has been suggested that the upward movement of metal cations through xylem may be retarded due to high cation exchange capacity of the xylem cell walls (Salt et al., 1998). Apparently, because of their less cation exchangeability, Pb-chloride complexes should be more readily transported though the xylem to the shoots, rather than be retained in the roots. As a result, higher shoot/root ratios of Pb amount have been observed (Table 3)

Above explanations may also apply to the Pb bioaccumulation in amending NaCl and KCl. The soluble Pb increase in NaCl and KCl treatment (Table 1) corresponds to the Pb elevation in the plants (Table 3). This fact indicates that NaCl and KCl could increase Pb bioaccumulation by changing soluble Pb in the soil, though simultaneously salt stress induced transpiration depression (Eshel and Waisel, 1984; Hagemeyer and Waisel, 1989; Carvajal et al., 1999) could on the other hand reduce the Pb translocation in the plants. For the same reason, it could be suggested that the less effect of NaCl and KCl on stimulation of Pb accumulation in the plants compared with NH_4Cl and $FeCl_3$ (Table 3) might be mainly attributed to their lower capacity to produce soluble Pb (Table 1).

As for the $CaCl_2$ amendment, a pronounced discrepancy occurs between the Pb accumulation in the plants and the Pb solubility in the soil. Addition of $CaCl_2$ significantly increases Pb solubility (Table 1). However, $CaCl_2$ shows the weakest effect, if any, on stimulating Pb translocation in plants (Table 3). It suggests therefore that there may be antagonistic interactions between Ca and Pb in bioaccumulation. Similar observations have been reported in the literature (Kabata-Pendias and Pendias, 1984), but the mechanisms for this antagonism remain to be shown. A possible explanation might lie in the competition between Ca and Pb in the processes of metal uptake and translocation in plants. It was reported that Pb^{2+} could permeate readily through Ca^{2+} channels in the membrane of animal cells (Tomsig and Suszkiw, 1991). If this process is also true for plant root cells, it can be assumed that Pb uptake via intracellular (symplastic) pathway would be reduced due to Ca competition with Pb for the transmembrane carriers. In this connection, a considerable proportion of Pb would be expectantly retained in the apoplastic space of the roots, accordingly unable to be transported to the shoots. Furthermore, Pb translocation might be influenced by the competition between cations in xylem fluid as well. As illustrated in a model study (White et al., 1981), Ca competes with Cd for organic ligands such as citric acid in the xylem fluid. This would apparently influence heavy metal translocation in plants.

Previous studies have shown that EDTA can enhance Pb accumulation in plant

shoots, and it is regarded as the best amendment so far for Pb phytoextration (Blaylock et al., 1997; Huang et al., 1997; Wu et al., 1999). Our results confirm above investigations in that EDTA significantly elevates Pb concentration in shoot (Table 3). On the other hand, however, the present results do not indicate EDTA being the most efficient amendment in respect to the Pb accumulation in the shoots. Either Pb concentration or amount in the shoots by amending 2.5 mM EDTA is significantly lower than amending 30mM NH_4Cl and 10 mM $FeCl_3$ (Table 3). Of cause, the shoot Pb concentration might be higher if high levels of EDTA have been added. But EDTA is toxic to plants (Cooper et al., 1999). In the case of Pb contaminated soils, addition of high concentration of EDTA (>1 mM kg^{-1}) usually reduces plant biomass significantly. For example, 1mM kg^{-1} EDTA reduced *B. juncea* dry weight by 33% (Blaylock et al., 1997), 3 mM kg^{-1} HEDTA reduced more than 50% of the shoot dry weight of ragweed, redtop and sunflower (Cooper et al., 1999). As a result, when EDTA added to the soil reaches a certain level, the Pb amount in plants would not increase further due to the biomass decrease. Huang et al. (1997) showed that Pb translocation in pea reached a steady state with 0.5g kg^{-1} HEDTA added to the soil, and Pb translocation in corn only increased 12% (from 72% to 84%) when HEDTA added to the soil increasing from 0.5 to 2.0g kg^{-1}. In this study, shoot biomass in 2.5 mM (=0.73g kg^{-1}) EDTA treatment is significantly lower than the control, and it falls at the same level with those of 30 mM NH_4Cl and 10 mM $FeCl_3$ treatment (Table 2). Morever, the total Pb amount in EDTA treatment is the lowest, even lower than the control (Table 3). Therefore, our results demonstrate that NH_4Cl and $FeCl_3$ amendments could be comparable, if not superior, to EDTA in enhancing Pb accumulation in the plants. This raises the suggestion that these chloride salts could be considered as alternatives in applying soil amendments for phytoremediation. As a fertilizer, NH_4Cl amendment may show its advantage particularly in phytoremediation of sterile Pb-contaminated soils.

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