Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

ISSN: 0972-6268 | Vol. 13 | No. 1 | pp. 31-36 | 2014

Original Research Paper

Effects of Lead and Water Stress on Soil Enzyme Activities from Two Plant Species

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com
Received: 22-12-2013
Accepted: 25-1-2014

Key Words:
Lead
Water stress
Soil enzyme activity
Sophora japonica
Platycladus orientalis

ABSTRACT

Through simulative methods, the influence on soil pH, the soil enzyme activities (urease, invertase and alkaline phosphatase) by the single and combined stress of water and lead were studied in a pot experiment. Five levels of Pb (0, 300, 500, 1000 and 2000 mg/kg soil) and four levels of water stress (soil relative water content (SRW), 100%, 80%, 60%, 40%) were applied. *Sophora japonica* and *Platycladus orientalis* were grown in soil-filled pots for pH and soil enzyme activities assays. The results showed that soil pH was affected by Pb and water deficiency. The addition of Pb and water stress caused soil alkalization in *Platycladus orientalis*, while acidification in *Sophora japonica* under water stress. Pb could stimulate the soil enzyme activities to some extent. The degree of influence on enzyme activities was related to plant species. Urease and invertase activities increased at 80% and 60% soil relative water content in *P. orientalis* and *S. japonica*. Interactions between Pb concentrations and water stress levels significantly impacted the three soil enzyme activities, synergism (ΔU<0) dominated the interaction in *P. orientalis*, while antagonism (ΔU>0) dominated the dominance for invertase and alkaline phosphatase in *S. japonica*.

INTRODUCTION

Soil contamination with heavy metals has become a serious problem throughout the world. Heavy metals could have long-term hazardous impacts on soil ecosystem after they enter the soil and are accumulated (Khan et al. 2007, Majer et al. 2002). Lead is one of the most common heavy metals, and it has been receiving much attention for its widespread distribution and toxicity to the environment (Zeng et al. 2007). Soil enzymes are the main participants in all of the biochemical processes in soil, they activate complex organic compounds change into simple inorganic compounds (Kang & Freeman 1999), and act as biological catalysts of important reactions to produce essential compounds for both soil microorganisms and plants (Moreno et al. 2003). The activity of soil enzymes is easily affected by natural and anthropogenic factors (Gianfreda et al. 1996, Gianfreda et al. 2002), and some enzyme activities involved in transformation of C, N, P and S (Belyaeva et al. 2005). Heavy metal pollution is known to have adverse effects on soil biological functions, including the size, activity and diversity of the soil microbial community and impair specific pathways of nutrient cycling (Chander et al. 2001, Kandeler et al. 1996). There is strong evidence that lead affects soil microbes and enzyme activities (Marzadori et al. 1996, Yang et al. 2006). But the soil enzyme activity depends on the concentration and oxidation state of the heavy metal, and on the soil characteristics (Speir et al. 1999).

Plants could affect the soil biota, microorganisms, and also influence the temperature and water content of the soil (Grierson & Adams 2000, Viketoft et al. 2005). Different plant species can associate with microbial communities with unique characteristics (Yang et al. 2007). There are many studies on plant species affecting soil microorganisms. Wardle et al. (1998) suggested that plant traits were important in determining plant species effects on soils. Bardgett (1999) also suggested that the abundance and activity of soil microorganisms were more regulated by plant species traits. However, very little is known about the effects of plant existence on the impacts of lead on soil enzyme activities.

Water is an important factor for plant, especially in arid and semi-arid areas. Water deficit, especially in Loess Plateau of China significantly restricts plant growth. Therefore, the present study was undertaken with the objectives: the impacts of elevated Pb; water stress and their interactions on soil enzyme activities; and the effects of plant on activities of soil enzymes under different Pb concentration soils.

MATERIALS AND METHODS

Soil and plants for the experiment: Soil for the experiment was collected from the top layer of a typical Lou soil at the Northwest A&F University, Yangling, Shaanxi province, China (34°16'N, 108°4' E). Soil pH was 7.90±0.12 in distilled water (1:2.5 vol/vol soil:water). The soil had 13.70 g/kg organic matter, 0.73 g/kg total N, 35.91 mg/kg available P, and 96.52 mg/kg available K. Lead concentration in the soil was 18.4 mg/kg.

Two plant species, *Sophora japonica* and *Platycladus orientalis* were selected. *P. orientalis* has strong resistance to cold, drought and saline-alkaline soil, and also has a certain resistance to lead. It is usually used as main afforestation tree species for fragile ecological environment reconstruction and mining area ecological environment restoration in western China. *S. japonica* is widely used as street greening tree species, meanwhile, it has a strong resistance to smoke poison. One-year-old *P. orientalis* and *S. japonica* saplings of approximately the same height and basal diameter were selected from the nursery garden.

Experimental design: The experiment was a factorial design with five Pb levels (0, 300, 500, 1000 and 2000 mg/kg soil), and four levels of water stress (soil relative water content (SRW), 100%, 80%, 60%, 40%) (Table 1). Pb was applied as lead acetate $[Pb(CH_3COO)_2 \cdot 3H_2O]$ and equilibrated completely for one month. After that, the prepared soil samples were filled into plastic pots (31 cm height × 27 cm diameter), and then flooded with tap water. Similar size two saplings of *P. orientalis* and one sapling of *S. japonica* were transplanted to each pot. The soil surface was covered with 2 kg grit to reduce evaporation of water. The experiment was carried out in the artificial shed of the College of Resources and Environment, Northwest A&F University, to prevent rainwater. In the whole culture process, natural illumination was used, and the soil samples always kept with natural fertility. The treatments were randomly assigned to pots and replicated three times. The soil samples were collected to a depth of 20 cm, air dried, and then sieved through a 1 mm nylon screen.

Soil enzyme assays: Urease activity was estimated by colorimetric determination of the ammonium released during the soil sample incubation with urea at 37° for 24 h, expressed as mg NH_3-N per g soil per 24 h (Guan et al. 1986). Alkaline phosphatase activity was determined with the method of Zeng (2007), shown as mg C_6H_5OH per g soil per 24 h. Invertase activity was determined by Guan (1986), expressed as mg glucose per g soil per 24 h. Control tests without soils or substrates were carried out to evaluate the spontaneous or abiotic transformation of substrates.

Table 1: Experimental scheme for drought and lead interaction.

Pb concentration (mg/kg)	Soil Relative Water Content			
	CK (100%)	T_1 (80%)	T_2 (60%)	T_3 (40%)
CK	CK	T_1	T_2	T_3
A (300)	A	AT_1	AT_2	AT_3
B (500)	B	BT_1	BT_2	BT_3
C (1000)	C	CT_1	CT_2	CT_3
D (2000)	D	DT_1	DT_2	DT_3

Net change of soil enzyme activity (ΔU) was calculated to assess the interaction between Pb and water stress:

$$\Delta U = (U_{water+Pb} - U_{ck}) - (U_{water} - U_{ck}) - (U_{Pb} - U_{ck})$$

Statistical analysis: All analyses were done on a completely randomized design. All data were statistically processed using Excel XP, SPSS 17.0, and analysed by one-way ANOVA with the lowest standard deviations (LSD) test. Comparisons with $P< 0.05$ were considered significantly different.

RESULTS

After growing in pots for six months, the presence of Pb in the soil caused visible changes in the process of development of the two species (Fig. 1). Soil Pb increased biomass significantly, and reached the maximum at 2000 mg/kg and 500 mg/kg Pb treatments, 1.66 and 1.56 times as much as that of control, respectively for *P. orientalis* and *S. japonica*. Water stress reduced the biomass of the two species significantly, with more obvious effect on *S. japonica* than on *P. orientalis*. High soil Pb reduced biomass under 40%. The biomass was lower than that under single Pb treatment, but higher than that under single water stress under interaction of water and Pb. However, the effect of interaction for *S. japonica* was not as obvious as *P. orientalis*.

As shown in Fig. 2, difference existed between the soil of the two species for the influence of Pb and water on the soil pH. The soil pH increased initially until the Pb concentration reached 1000 mg/kg, and then decreased after the peak with increase in Pb levels in *P. orientalis*, while reached the peak at 2000 mg/kg in *S. japonica*. Water stress increased the soil pH in *P. orientalis* at all Pb concentrations, except 300 mg/kg; while decreased in *S. japonica* in all Pb concentrations, except at 500 mg/kg. Moreover, the soil pH in *S. japonica* was higher than that in *P. orientalis*.

Urease activity in *P. orientalis* and *S. japonica* responded in different ways when increasing concentrations of Pb were added to the soil. In *S. japonica*, Pb increased urease activity at 300-1000 mg/kg, and then decreased at 2000 mg/kg, but still higher than the control. However, urease activity in *P. orientalis* reached the highest at 300 mg/kg, and lower than the control at 1000 mg/kg, but not significant. Low Pb concentration caused a clear increase in invertase and alkaline phosphatase activities compared to the

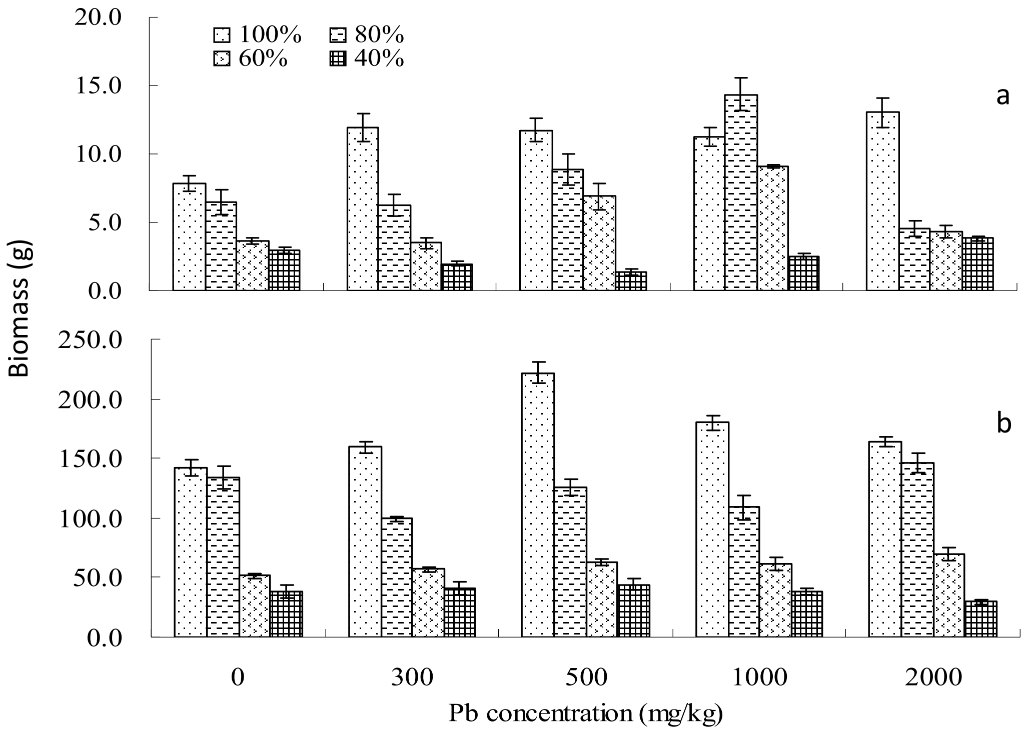

Fig. 1: Biomass of *Platycladus orientalis* (a) and *Sophora japonica* (b) under different Pb and water stress treatments.

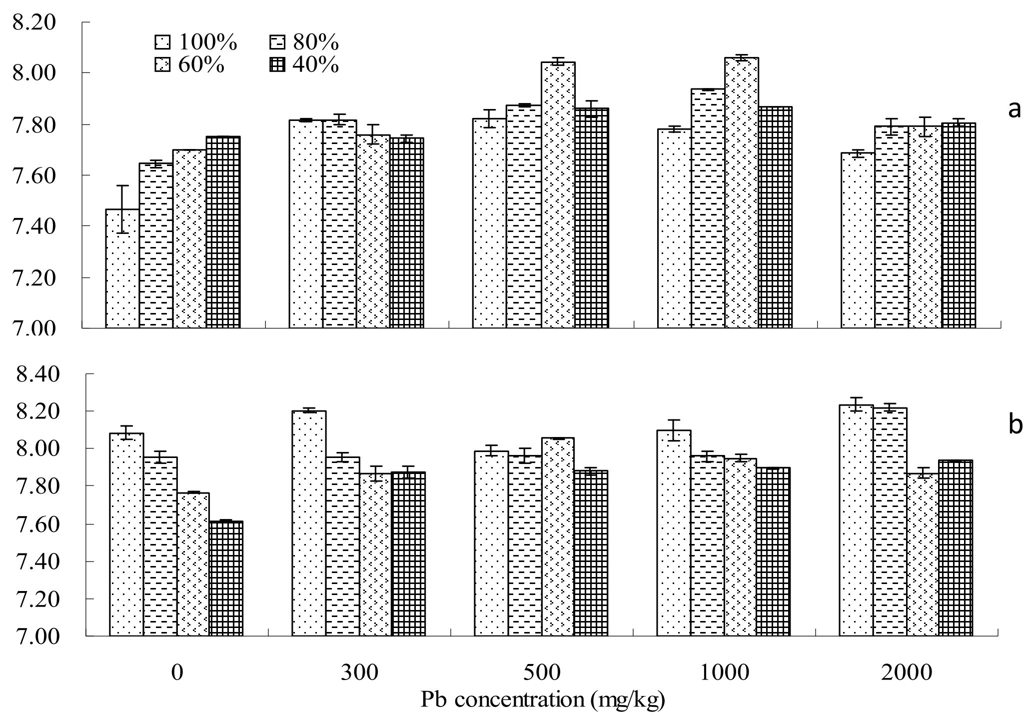

Fig. 2: Changes in soil pH in pots with *Platycladus orientalis* (a) and *Sophora japonica* (b) exposed to Pb and water stress.

Table 2: Soil enzyme activity in tested soil (mg/(g.d)).

Treatment	Urease		Invertase		Alkaline phosphatase	
	a	b	a	b	a	b
CK	1.11	1.16	7.10	9.02	4.01	4.07
T_1	1.16	1.16	8.45	9.12	5.50	3.53
T_2	1.16	1.28	8.69	9.20	5.55	3.50
T_3	1.13	1.37	7.44	8.81	4.88	3.50
A	1.37	1.17	8.13	9.27	4.55	4.62
AT_1	1.32	1.30	8.35	9.85	5.83	4.97
AT2	1.34	1.35	8.77	9.31	4.63	5.49
AT_3	1.20	1.29	8.83	9.05	3.56	4.40
B	1.23	1.32	7.45	8.46	4.13	3.96
BT_1	1.30	1.25	8.21	9.12	4.54	4.07
BT_2	1.30	1.23	8.78	9.31	4.29	3.73
BT_3	1.09	1.19	8.70	8.33	3.03	2.71
C	1.10	1.37	6.40	7.99	3.04	3.35
CT_1	1.12	1.31	7.50	8.01	3.47	4.02
CT_2	1.23	1.23	8.22	8.84	3.54	3.49
CT_3	1.05	1.14	7.11	6.92	4.34	2.98
D	1.22	1.22	5.69	7.18	2.38	2.22
DT_1	1.27	1.28	6.83	9.17	2.98	2.92
DT_2	1.07	1.04	7.32	7.53	3.24	2.49
DT_3	1.07	1.01	7.46	6.30	3.53	2.24

Table 3: Net change in different soil enzyme (mg/g.d.

Treatment	Urease		Invertase		Alkaline phosphatase	
	a	b	a	b	a	b
AT_1	-0.10	0.12	-1.14	0.48	-0.21	0.89
AT2	-0.08	0.06	-0.96	-0.14	-1.46	1.44
AT_3	-0.19	-0.09	0.36	0.00	-1.86	0.35
BT_1	0.03	-0.07	-0.59	0.56	-1.08	0.65
BT_2	0.03	-0.21	-0.26	0.67	-1.38	0.34
BT_3	-0.15	-0.34	0.91	0.07	-1.97	-0.68
CT_1	-0.02	-0.06	-0.26	-0.08	-1.06	1.21
CT_2	0.09	-0.26	0.23	0.66	-1.04	0.71
CT_3	-0.07	-0.30	0.36	-0.87	0.43	0.20
DT_1	0.00	0.05	-0.21	1.89	-0.89	1.24
DT_2	-0.20	-0.31	0.04	0.16	-0.68	0.84
DT_3	-0.17	-0.43	1.43	-0.67	0.28	0.59

control at 300 mg/kg, then decreased lower than the control at 1000 mg/kg in *P. orientalis* and *S. japonica* (Table 2).

Water stress changed the soil enzyme activities significantly. Urease and invertase activities increased at 80%, 60% treatments in *P. orientalis* and *S. japonica*. Alkaline phosphatase activity in *S. japonica* decreased with the increasing of water stress levels.

Interactions between Pb stress and water stress levels significantly impacted the three soil enzyme activities (Tables 2 and 3). However, the types of interactions were dependent on the concentrations of Pb and levels of water stress. Pb and water stress showed synergism on urease at high levels in *P. orientalis* and *S. japonica*. The combined effects of Pb and water stress on invertase showed synergism at low levels and antagonism at high water stress levels, while on alkaline phosphatase were antagonistic at high Pb concentrations and high water stress level in *P. orientalis*. In *S. japonica*, the Pb and water stress on invertase and alkaline phosphatase were mainly antagonism.

DISCUSSION

Effect of soil enzyme activities under elevated Pb: It is well know that any element, if the concentration is higher than a certain range, it will bring adverse effect to plants and microorganisms (Zeng et al. 2007). Urease activity was considered to be sensitive to heavy metals (Aoyama et al. 1996). Phosphatase activity can be a good indicator of biological activity of soils (Dick et al. 1993). Results from our experiment indicated that Pb impacted soil enzyme activities differently. The activities of urease, invertase and alkaline phosphatase all increased initially, but decreased gradually after the peak with increase of Pb concentration in *P. orientalis* and *S. japonica*, indicating that Pb could stimulate the soil enzyme activities to some extent. This might be owing to the environmental selection of strains resistant to the toxicity of Pb (Fliessbach et al. 1994). Change of urease activity in *P. orientalis* and *S. japonica* were greater than the other two enzymes. Moreover, all the three enzyme activities increased greater in *P. orientalis* than in *S. japonica*, suggesting that *P. orientalis* and *S. japonica* may alleviate Pb impacting on the soil enzyme activities. However, *P. orientalis* was superior to *S. japonica.*

Water stress on soil enzyme activities: The water content of the soil prior to sampling is an important consideration when using enzyme activity as an indicator of soil pollution, furthermore, soil moisture response is enzyme-specific (Hinojosa et al. 2004). Soil enzyme activity showed a considerable sensitivity to slight decrease in water availability (Sardans & Peñuelas 2005). This study showed that enzyme activities were affected by water stress. The *P. orientalis* had lower activities of urease and invertase but higher activity of alkaline phosphatase than *S. japonica*. The high enzyme activities in *S. japonica* soil might be the large roots and fibrous root. Wang (2006) pointed out that the activity of alkaline phosphatase decreased with a decrease in pH. Our results also showed that the decrease in pH under water stress resulted in the decreased alkaline phosphatase in *S. japonica.* Our results were in agreement with many others reported in the literature, showing that alkaline phosphatase activity was well correlated with soil water availability (Sardans & Peñuelas 2005).

Interaction of Pb and water stress on soil enzyme activities: There existed generally mutual action between soil water content and Pb. For urease, synergism (ΔU<0) dominated the interaction in *P. orientalis* and *S. japonica*. For invertase and alkaline phosphatase, antagonism (ΔU>0) dominated the dominance in *S. japonica* while synergism (ΔU<0) in *P. orientalis*. The interaction was also related with the levels of Pb and water stress. The interaction of Pb and water stress on invertase showed synergism at low levels and antagonism at high water stress levels in *P. orientalis*. At the same time, the interaction was different as a result of the plant species cultivated in the pots. Soil is an extremely complex system, the interaction of water and Pb, along with the different plant species contains many physical and chemistry reactions, and the effects for the soil enzyme activities were the result of combination of all the factors.

CONCLUSION

Low Pb concentrations (≤500 mg/kg) could stimulate the soil enzyme activities, while high Pb concentrations restrained the soil enzyme activities. Urease and invertase activities increased at 80% and 60% treatments. Alkaline phosphatase activity in *S. japonica* decreased with the increase of water stress levels.

The types of interaction were not only related with different stress, but also with the levels of stress. At high levels of Pb and water stress, the interaction showed synergism on urease. The interaction on invertase showed synergism at low levels and antagonism at high water stress levels. The plant species could impact the soil enzyme activities under Pb and water stress. In *S. japonica*, the Pb and water stress on invertase and alkaline phosphatase were mainly antagonism, while synergism in *P. orientalis*

ACKNOWLEDGEMENTS

This research was supported by National Natural Science Foundation of China (31170579) and by the Specialized Research Fund for the Doctoral Program of Higher Education (20110204110009). Thanks are due to the Department of Environmental Science, College of Resources and Environment, Northwest A&F University for technical guidance and help for this study.

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