Jour. Korean For. Soc. Vol. 98, No. 5, pp. 602~608 (2009)

JOURNAL OF KOREAN FOREST SOCIETY

Cadmium Accumulation, Phosphorus Concentration and Growth Response of Cd-treated Ectomycorrhizal Poplar Cuttings

Sim-Hee Han[1]*, Du-Hyun Kim[1], Nelly S. Aggangan[1], Pan-Gi Kim[2] and Kyung Joon Lee[3]

[1]*Department of Forest Resources Development, Korea Forest Research Institute, Suwon, 441-350, Republic of Korea*
[2]*Division of Forest Environment and Resources, Kyungpook National University, Sangju, 742-711, Korea*
[3]*Department of Forest Resources, Seoul National University, Seoul, 151-921, Korea*

Abstract : We investigated whether cadmium (Cd) toxicity affects phosphorus (P) concentration and growth of poplar, which might be related to the ectomycorrhizal associations. *Populus ×tomentoglandulosa* cuttings were treated with 0.1 mM and 0.4 mM $CdSO_4$ and inoculated with ectomycorrhizal fungus, *Pisolithus tinctorius* (Pt) and grown in autoclaved peat vermiculite mixture for five months under greenhouse conditions. Ectomycorrhizal plants showed significantly higher Cd concentration in leaves, stems and roots than in non-mycorrhizal plants. Likewise, P contents in leaves and roots of ectomycorrhizal plants were higher than those of non-mycorrhizal plants. Acid phosphatase activity in leaves of ectomycorrhizal plants, however, was significantly lower than that of non-mycorrhizal plants. 0.1 mM Cd significantly increased P content in leaves and stems of non-mycorrhizal plants. In spite of high P concentration, which is accompanied by lower acid phosphatase activity, plant growth was not improved by inoculation with *P. tinctorius*. Total plant dry weight was lower than the non-mycorrhizal counterpart. The results imply that this might be caused by the large amount of energy consumption to alleviate Cd toxicity resulted from high Cd accumulation in their tissues.

Key words **:** *Populus ×tomentoglandulosa, Pisolithus tinctorius, P content, acid phosphatase, growth*

Introduction

Poplar (*Populus*) trees have been reported as good candidates for use in phytoremediation because they root deeply, cycle large amount of water and grow rapidly (Newman *et al*., 1997; Palmroth *et al*., 2002; Witting *et al*., 2003). Poplar trees are known to be associated with the ectomycorrhizal fungus *Pisolithus tinctorius* (Cripps and Miller, 1995; Cairney and Chambers, 1999). Infection by ectomycorrhizal (ECM) fungi may benefit hybrid poplar growing in contaminated soils by providing greater access to water and nutrients and protecting the trees from direct contact with the toxic contaminants (Gunderson *et al*., 2007).

Cadmium (Cd) is invariably concentrated in the organic surface horizon of soils, where seedlings are rooted, together with any associated mycorrhizas (Smith and Read, 1997). Great interest has been raised in the ability of ectomycorrhizal (EM) fungi to regulate uptake of heavy metals. In a number of cases, growth and survival of several species of pine growing on strip mine soil with high levels of metals and extreme pH values were improved by inoculation with *Pisolithus tinctorius* (Pers.) Coker and Couch (Berry, 1982).

Phosphorus (P) is present in limiting amounts in many soils and is consequently a very important factor affecting plant growth (Bielski, 1973). The role of P in limiting forest growth has been identified in many differing forest ecosystems throughout the world. Soil P is largely in the form of myoinositol hexaphosphate (phytic acid salts), a form unavailable for direct plant uptake (Bieleski, 1973). This organically bound P may constitute 50-67% of the total soil P supply and cannot be utilized without the solubilization of orthophosphate (McLaren and Skujins, 1971).

Ho (1979) demonstrated an increase in acid phosphatase activity (acid phosphomonoesterase, EC 3.1.3.2) in low pH forest soils and suggested this as a mechanism for increased levels of available P for plant uptake. Acid phosphatase consists of a broad group of enzymes that share the ability to hydrolyze various phosphate esters with the release of phosphate ions (Hall and Hawes, 1991). Acid phosphatase activity in the plant

*Corresponding author
E-mail: simhee02@forest.go.kr

increases with phosphorus deficiency (Reid and Bieleski, 1970; McLachlan, 1976; Besford, 1979).

Mycorrhizal associations have been demonstrated to potentially improve plant health and growth through increased uptake of required nutrients particularly P (Harley and Smith, 1983). The pool of available P to plants can be increased by colonization with mycorrhizal fungi through an increase in absorptive surface area, greater uptake efficiency, or less available to the non-mycorrhizal tree. Ectomycorrhizal fungi, which significantly enhance phosphate uptake by host roots (Bowen, 1973), are believed important in catalyzing the hydrolysis of organic phosphates and thus influencing the phosphorus cycle. However, these positive mycorrhizal effects could be reduced by various stresses such as drought and high level of heavy metals. The objective of the present study was to investigate whether Cd toxicity affects P concentration and growth of *Populus ×tomentoglandulosa* cuttings, which might be related to the ectomycorrhizal associations.

Materials and Methods

1. Plant and inoculum preparation

The clones used in this study were mainly hybrid poplar (*P. ×tomentoglandulosa*). The stem cuttings were taken from one-year old micropropagated stock plants that were maintained in the nursery of the Department of Forest Genetic Resources, Korea Forest Research Institute in Suwon, Korea.

Culture of *P. tinctorius* was obtained from Chonnam National University in Korea and the fungus was maintained as described previously (Marx, 1969). Fungal inoculum was prepared using the method of Marx and Bryan (1975). An 1-L flask was filled with a mixture of 60 mL peat and 840 mL vermiculite. Liquid MMN solution (400 mL) with 5 g glucose was added into the 900 mL peat-vermiculite mixture, mixed thoroughly and then autoclaved for 1h at 121°C. The sterilized MMN -peat-vermiculite mixture was inoculated with 10 MMN agar plugs (7 mm diameter) removed from the edge of actively growing colonies of *P. tinctorius*. For the control treatment, 10 MMN agar plugs without mycelium were added to the mixture. The flasks were kept at 20°C in the dark. After the 3-month incubation, the *P. tinctorius* colonized substrate was removed from the flasks, washed with running water to remove non-assimilated nutrients, and kept in a plastic bag at 5°C for 3 days until used for inoculation. Mycelial growth of *P. tinctorius* in the substrate can be seen through the naked eye.

2. Production of mycorrhizal *P. ×tomentoglandulosa*

Ectomycorrhizal associations between *P. ×tomentoglandulosa* and *P. tinctorius* were synthesized aseptically in plastic pots filled with peat-vermiculite (1:1 v/v) mixture. The growth medium contained peat that passed through a 2 mm sieve and vermiculite that was retained in a 3 mm sieve. The mixture was autoclaved for 1h at 121°C. The fungal inoculum (*P. tinctorius* colonized substrate) was mixed with the autoclaved clean peat-vermiculite mixture at a ratio of 1:6 by volume and then placed into a plastic pot. Hybrid poplar cuttings (approximately length 10 cm ± 1) stored in the refrigerator for one month were inserted into rooting plastic pots filled with a mixture of fungal inoculum and growth mixture.

3. Cd treatment

Cd treatment was applied for thirty days after inserting the stem cuttings. Cd was applied every three days over a five-month period. At each Cd application, approximately 200 mL of 0.1 mM or 0.4 mM $CdSO_4$ solutions were applied to the containerized plants. The pots were placed in plastic dishes to retain leached nutrients and $CdSO_4$ solution. Pots were randomized in the greenhouse and rearranged every two or three weeks throughout a five-month experimental period to minimize positional effects. During the experimental period (May-October), daily mean temperature and relative humidity were 23.1 ± 2.1°C and 74.3 ± 10.9%, respectively.

4. Mycorrhizal infection

Ectomycorrhizae were detected easily after soaking in cool tap water for 5 minutes. Counting of mycorrhizal root tips (7 mm and shorter belonging to the orders of the secondary and smaller roots) was done under a dissecting microscope. Percentage of mycorrhiza infection was expressed as the number of infected lateral root tips divided by the total number of such root tips multiplied by 100.

4. Biomass and Cd determination

At harvest, shoots and roots were carefully separated, rinsed thoroughly with distilled water twice and later partitioned into leaves, stem and roots. After oven drying the tissues at 70°C to constant weight (three days), the dry weights were recorded. Dried leaves, stems and roots (0.5 g each) were ground and Cd concentration was measured by inductively coupled plasma spectrometer (ICPS-1000IV, Shimadzu, Japan). Cd content is the product of Cd concentration and plant dry weight.

6. P content and acid phosphatase activity

At the end of the 5 month trial, leaves and roots of the freshly harvested plants were used for the determination of acid phosphatase activity following the technique of Macfall *et al*. (1991). P concentration in the ground tis-

sue was measured by inductively coupled plasma spectrometer (ICPS-1000IV, Shimadzu, Japan). P content is the product of P concentration and plant dry weight.

7. Experimental design and statistics

The experiment was conducted following two factors in Randomized Complete Block Design with 25 replicates per treatment. Factor A was the Cd treatment (0, 0.1 and 0.4 mM) and Factor B was the mycorrhiza inoculation treatment (non-mycorrhizal and mycorrhizal with *P. tinctorius*). Data were statistically analyzed using SAS System for Windows, Version 8.01 (SAS Institute, USA). Mean values per treatment were compared by GLM. When significant differences ($P \leq 0.05$) were indicated Duncan's multiple range tests (Duncan, 1955) were performed.

Results

Figure 1 represents the monthly changes of Cd concentration in the leaves, stems and roots of one-year-old poplar cuttings treated with 0.4 mM Cd solution. Ectomycorrhizal plants consistently gave higher Cd concentrations in the leaves, stems and roots than those in the non-mycorrhizal plants throughout the growing season (Figure 1). Cd concentration in the leaves and stems of mycorrhizal plants were higher in June, August and September. Cd concentration was highest in September which significantly dropped in October.

In October, leaf Cd concentration in mycorrhizal plants was significantly lower than non-mycorrhizal plants. Cd concentrations in leaves, stems and roots of non-mycorrhizal plants increased sharply in September and

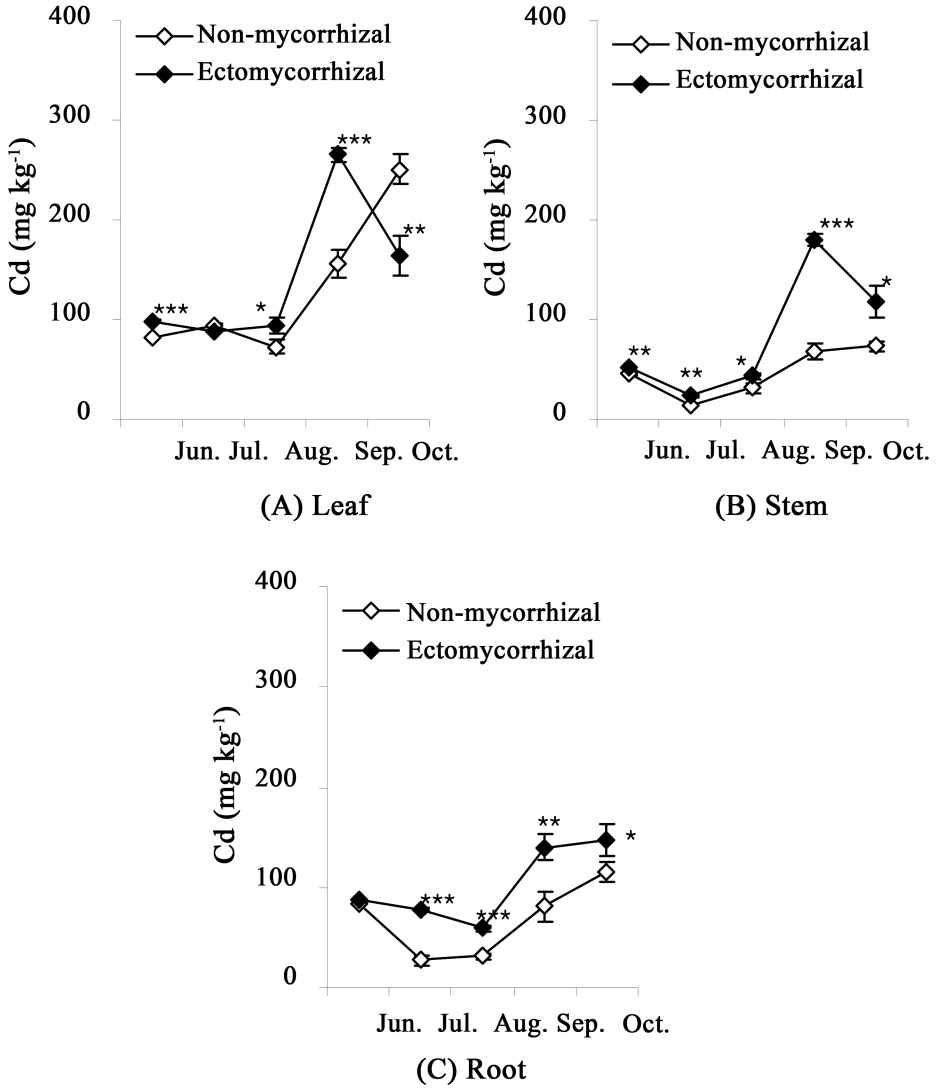

Figure 1. Monthly Cd concentration in the leaves, stems and roots of one-year-old non-mycorrhizal and mycorrhizal *P.* ×*tomentoglandulosa* cuttings grown in an autoclaved 1:1 peat vermiculite mixture with 0.4 mM $CdSO_4$. All values are means of three replicates±SD; *, ** and * indicate significant difference between non-mycorrhizal and ectomycorrhizal plant at P<0.05, 0.01 and 0.001, respectively.**

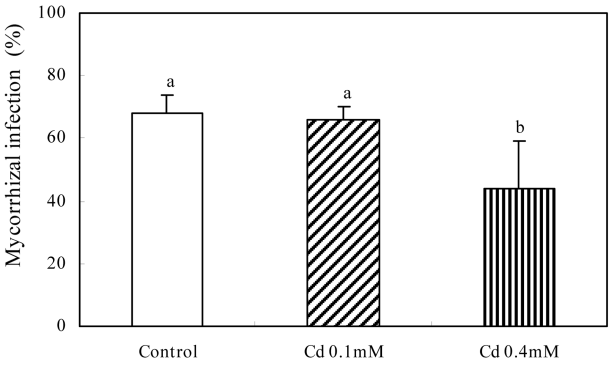

Figure 2. Mycorrhizal infection rate of 1-year-old *P.* ×*tomentoglandulosa* cuttings inoculated with ectomycorrhizal fungus, *P. tinctorius* grown in an autoclaved 1:1 peat vermiculite mixture with three levels of $CdSO_4$. All non-mycorrhizal plants did not have mycorrhiza infected roots. Means with the same letter are not significantly different at 5% level using Duncan's multiple range test.

were highest in October.

Ectomycorrhiza infected root tips were predominantly monopodial and the mycelium associated with *P. tinctorius* treatment was dark brown in color. Ectomycorrhizal root infection ranged from 44 to 68%. Control seedlings were free of ectomycorrhizae. Cd level of 0.1 mM did not affect root colonization by *P. tinctorius*. However, increasing the Cd level to 0.4 mM decreased the rate of root colonization by 65% relative to the Cd-untreated cuttings (Figure 2).

Acid phosphatase (AP) activity in leaves of cuttings showed significant ($P \leq 0.05$) difference between non-mycorrhizal and ectomycorrhizal plants but there were no significant differences among Cd levels and the interaction between mycorrhizal status and Cd treatment. AP activity in the leaves of ectomycorrhizal plants was significantly lower than that in non-mycorrhizal plants (Figure 3). AP activity in the roots was not affected by mycorrhizal inoculation and Cd treatment. However,

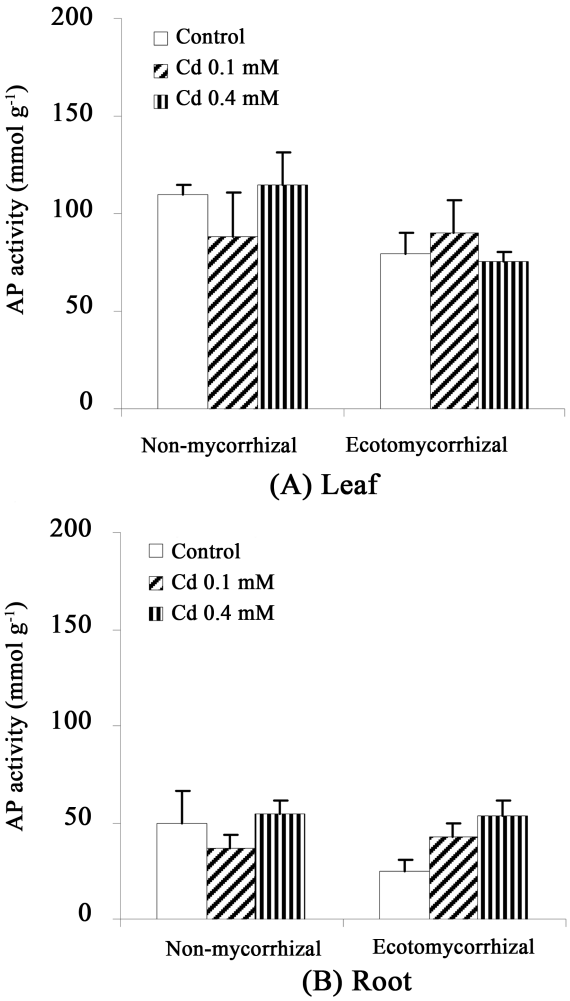

Figure 3. Acid phosphatase activity in the leaves and roots of one-year-old *P.* ×*tomentoglandulosa* cuttings inoculated with ectomycorrhizal fungus, *P. tinctorius* grown in an autoclaved 1:1 peat vermiculite mixture with three levels of $CdSO_4$. All values are means of three replicates ± SD. Leaf AP activity: Pt, $Cd^{n.s.}$, Pt×$Cd^{n.s.}$; Root AP activity: $Pt^{n.s.}$, Cd*, Pt×Cd*; *, ** and *** indicate significant at P < 0.05, 0.01 and 0.001, respectively, and n.s.= not significant.**

Table 1. Phosphorus concentration in leaves, stems and roots of one-year-old *P.* ×*tomentoglandulosa* cuttings inoculated with ectomycorrhizal fungus, *P. tinctorius* grown in an autoclaved 1:1 peat vermiculite mixture with three levels of $CdSO_4$.

Factor	Treatment	Leaf	Stem	Root
		P (mg kg^{-1})		
Non-mycorrhizal	Control	1400±114	1013±129	3284±300
	0.1 mM	2792±263	1271±236	2759±154
	0.4 mM	1499±513	1182±43	3061±420
Ectomycorrhizal	Control	2388±116	1346±455	3857±316
	0.1 mM	2784±370	1448±130	3831±561
	0.4 mM	2979±290	1570±486	4728±350
Pr > F	Pt	***	n.s.	***
	Cd	***	n.s.	*
	Pt × Cd	**	n.s.	n.s.

All values are means of three replicates ± SD; * P < 0.05, ** P < 0.01, *** P < 0.001, and n.s.=not significant.

Table 2. Dry weight of one-year-old *P. ×tomentoglandulosa* cuttings inoculated with ectomycorrhizal fungus, *P. tinctorius* grown in an autoclaved 1:1 peat vermiculite mixture with three levels of $CdSO_4$.

Factor	Treatment	Leaf	Stem	Root	Total	SR ratio
		Dry weight (g)				
Non-mycorrhizal	Control	2.36±0.24	1.01±0.15	1.60±0.07	4.97±0.28	2.11±0.23
	0.1 mM	2.09±0.10	1.01±0.15	1.64±0.36	4.74±0.55	1.95±0.41
	0.4 mM	2.22±0.38	0.99±0.05	1.37±0.27	4.58±0.64	2.37±0.20
Ectomycorrhizal	Control	1.60±0.21	0.53±0.06	0.86±0.31	2.98±0.13	2.85±1.59
	0.1 mM	1.76±0.16	0.58±0.17	0.85±0.04	3.19±0.36	2.75±0.32
	0.4 mM	1.74±0.21	0.65±0.21	0.83±0.15	3.21±0.33	2.93±0.39
Pr > F	Pt	***	***	***	***	n.s.
	Cd	n.s.	n.s.	n.s.	n.s.	n.s.
	Pt × Cd	n.s.	n.s.	n.s.	n.s.	n.s.

All values are means of three replicates ± SD; *** P <0.001, and n.s.= not significant.

there was a significant ($P \leq 0.05$) interaction between mycorrhizal and Cd treatments. Cd treatment did not show a clear influence on AP activity in non-mycorrhizal plants, whereas AP activity in ectomycorrhizal plants increased linearly with the increasing levels of Cd treatment.

Table 1 shows the P content in leaves, stems and roots of 1-year-old poplar cuttings inoculated with ectomycorrhizal fungus, *P. tinctorius* at three levels of Cd added into the soil. Leaf and root P contents were significantly affected by inoculation with *P. tinctorius* and Cd treatments. Moreover, there was a significant interaction between mycorrrhizal inoculation and Cd treatments on leaf P content. On the other hand, stem P content was not affected by any of the treatments. Leaf and stem P contents of mycorrhizal plants were higher than those in non-mycorrhizal plants (Table 1). P contents in the leaves and stems of non-mycorrhizal plants were significantly increased with 0.1 mM Cd solution. 0.4 mM Cd treatment significantly increased P contents in the leaves, stem and roots of mycorrhizal plants.

Dry weights of leaves, stems and roots were significantly different between non-mycorrhizal and ectomycorrhizal plants (Table 2). Leaf, stem and root dry weights of ectomycorrhizal plants were significantly lower than those of non-mycorrhizal plants. There was no effect of Cd treatments and the interaction between mycorrhizal inoculation and Cd treatments on plant dry weight.

Discussion

Ectomycorrhizal fungi play an important role in nutrient uptake under natural conditions, suggesting that mycorrhizal fungi might influence the uptake and accumulation of these toxic ions including heavy metals. A comparison of heavy metal concentrations in shoots of mycorrhizal and non-mycorrhizal plants has been taken in many studies. These studies have shown that the concentration in the non-mycorrhizal plants was higher than in the mycorrhizal plants, in the needles/leaves as well as in the stem (Colpaert and van Assche, 1993). Three reasons were suggested to explain these results. First, reduced concentrations in the shoots of mycorrhizal plants could be attributed by the dilution of the metal due to improved shoot growth (Jentschke and Godbold, 2000; Han *et al*., 2006). Second, in some cases, some ectomycorrhizal fungi have been shown to restrict heavy metal uptake and transport by binding the heavy metals onto fungal cell walls, by sequestration in vacuoles and/or by chelation by organic acids (Leyval *et al*., 1997; Jentschke and Godbold, 2000). Lastly, toxic metals may impair transpiration of plants by either interfering with stomatal regulation or reducing water uptake by the root system, or both (Barceló and Poschenrieder, 1990). In this case, portion of the absorbed heavy metals may be transported to the shoot via the transpiration stream, thus, reduced transpiration may result in decreased translocation of metals into the shoots.

Interestingly, however, in this study, Cd concentration in the shoot of mycorrhizal plants was higher than in the non-mycorrhizal counterpart (Figure 1) similar to what has been shown in our previous study (Han *et al*., 2001). In addition, many studies have reported that mycorrhiza enhanced the uptake of Cu (Gildon and Tinker, 1983), Zn (Davies, 1987), Ni (Killham and Firestone, 1983), Cd (Guo *et al*., 1996; Joner and Leyval, 1997), Pb (Díaz *et al*., 1996) and other metals (Galli *et al*., 1994). Mycorrhizal plants had higher Cd than the non-mycorrhizal ones because the former had lower biomass than the latter (Tables 1 and 2).

Mycorrhizal fungi may alter metal sensitivity of their hosts by either directly affecting metal availability and speciation or indirectly modifying plant physiological

processes, for example, by phytohormone action (Gogala, 1991; Vodnik *et al*., 1999; Jentschke and Godbold, 2000). Mycorrhizal fungi might even increase the metal translocation into the shoots.

The above two results create a complicated situation for the interpretation of mycorrhizal effects on shoot contents, as transpiration- driven metal translocation may differ between non-mycorrhizal and mycorrhizal plants because of differences in metal intoxication (Jentschke and Godbold, 2000).

Mycorrhizal formation was strongly suppressed by high Cd concentration in poplar cutting (Figure 2) like some forest tree seedlings inoculated with *Suillus lutes* (Dixon, 1988; Dixon and Buschena, 1988).

Nevertheless, several ectomycorrhizal fungi can protect their host plants against toxicity of heavy metals present at elevated concentrations in soil (Jones and Hutchinson, 1988; Colpaert and Van Asshe, 1992). In our study, although it wasn't significantly different, the growth of non-mycorrhizal plants was depressed lightly at 0.4 mM Cd treatment. On the contrary, the growth of ectomycorrhizal plants did not show significant changes by Cd treatment (Table 2).

Acid phosphatase activity in the leaves of ectomycorrhizal plant was lower than that of non-mycorrhizal plants (Figure 3). This means that the leaves of ectomycorrhizal plants retained higher P concentration in comparison with non-mycorrhizal plants (Table 2) but this is not true in the root of Cd-treated cuttings, except for control cuttings.

Acid phosphatase activity in the plant increases with phosphorus deficiency (Reid and Bieleski, 1970; McLachlan, 1976; Besford, 1979). Besford (1979) indicated that out of the 15 nutrient imbalances, only phosphorus deficiency markedly increased acid phosphatase activity in leaves. That work suggested that it may well be possible to use this enzyme as an indicator of phosphorus deficiency.

In another investigation comparing leaf and root phosphatase activities in wheat grown in nutrient solution, McLachlan and De Marco (1982) showed that leaf phosphatase was not necessarily a better measure of the phosphorus status of the growing plant than root phosphatase. However, as leaves are more accessible than roots, leaf activity was suggested as a potentially useful measure of the growing plant's phosphorus status.

In this present study, in spite of high P concentration in the ectomycorrhizal plants, the growth was not improved in comparison with non-mycorrhizal plants. In fact, growth was reduced significantly in relation to the non-mycorrhizal plants (Table 2). This plant reaction can be considered as a series of compensation effects as a defense mechanism against Cd toxicity which results from high Cd accumulation in mycorrhizal plants.

Conclusion

Ectomycorrhizal plants maintained high P concentration in their tissues in comparison with non-mycorrhizal plants under both control and Cd treatments. It was considered as the ectomycorrhizal positive effects. However, in spite of having high P concentration, which was accompanied by lower acid phosphatase activity, their growth was not improved by inoculation with *Pisolithus tinctorius*. Total plant dry weight was lower than the non-mycorrhizal counterpart. The results imply that this might be caused by the large amount of energy consumption to alleviate Cd toxicity resulted from high Cd accumulation in their tissues.

References

1. Barceló, J. and Poschenrieder, C. 1990. Plant water relations as affected by heavy metal stress: a review. Journal of Plant Nutrition 13: 1-37.
2. Berry, C.R. 1982. Survival and growth of pine hybrid seedlings with *Pisolithus* ectomycorrhizae on coal spoils in Alabama and Tennessee. Journal of Environmental Quality 11: 709-715.
3. Besford, R.T. 1979. Nutrient imbalances in tomato plants and acid phosphatase activity in the leaves. Journal of the Science of Food and Agriculture 30: 275-280.
4. Bieleski, R.L. 1973. Phosphate pools, phosphate transport and phosphate availability. Annual Review of Plant Physiology 24: 225-252.
5. Cairney, J.W.G. and Chambers, S.M. 1999. *Pisolithus*. pp. 1-31. In: Cairney, J.W.G and Chambers, S.M. (Ed.), Ectomycorrhizal fungi: Key genera in profile. Springer, Berlin.
6. Colpaert, J.V. and Van Assche, J.A. 1992. Zinc toxicity in ectomycorrhizal *Pinus sylvestris*. Plant and Soil 143: 201-211.
7. Colpaert, J.V. and Van Assche, J.A. 1993. The effects of cadmium on ectomycorrhizal *Pinus sylvestris* L. New Phytologist 123: 325-333.
8. Cripps, C.L. and Miller, O.K. 1995. Ectomycorrhizae formed in vitro by quaking aspen: Including *Inocybe lacera* and *Amanita pantherina*. Mycorrhiza 5:357-370.
9. Davies, F.T. Jr. 1987. Mycorrhizal fungi, fertility and media effects on growth and nutrition of *Rosa multiflora*. Plant and Soil 104: 31-35.
10. Díaz, G, Azcón-Aguilar, C. and Honrubia, M. 1996. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. Plant and Soil 180: 2451-2459.
11. Dixon, R.K. 1988. Response of ectomycorrhizal *Quer-*

cus rubra to soil cadmium, nickel and lead. Soil Biology and Biochemistry 20: 555-559.
12. Dixon, R.K. and Buschena, C.A. 1988. Response of ectomycorrhizal *Pinus banksiana* and *Picea glauca* to heavy metals in soil. Plant and Soil 105: 265-271.
13. Galli, U., Schuepp, H. and Brunold, C. 1994. Heavy metal binding by mycorrhizal fungi. Physiologia Plantarum 92: 364-368.
14. Gildon, A. and Tinker, P. 1983. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. II. The effect of infection on uptake of copper. New Phytologist 95: 263-268.
15. Gogala, N. 1991. Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi. Experientia 47: 331-340.
16. Gunderson, J.J., Knight, J.D. and Van Rees, K.C.J. 2007. Impact of ectomycorrhizal colonization of hybrid poplar on the remediation of diesel-contaminated soil. Journal of Environmental Quality 36: 927-934.
17. Guo, Y., George, E. and Marschner, H. 1996. Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean and maize plants. Plant and Soil 184: 195-205.
18. Hall, J.L. and Hawes, C. 1991. Electron microscopy of plant cells. Academic Press, London, p 466.
19. Han, S.H., Lee, J.C. Oh, C.Y. and Kim, P.G. 2006. Alleviation of Cd toxicity by composted sewage sludge in Cd-treated Schmidt birch (*Betula schmidtii*) seedlings. Chemosphere 65: 5421-546.
20. Han, S.H., Lee, K.J. and Hyun, J.O. 2001. The Cd and Pb accumulation in various tissues of rooted cuttings of four Populus species inoculated with ectomycorrhizal fungi, *Pisolithus tinctorius*. Journal of Korean Forestry Society 90: 495-504 (in Korean with English abstract).
21. Harley, J.L. and Smith, S.E. 1983. Mycorrhizal symbiosis. Academic Press, New York.
22. Ho, I. 1979. Acid phosphatase activity in forest soil. Forest Science 25: 567-568.
23. Jentschke, G. and Godbold, D.L. 2000. Metal toxicity and ectomycorrhizas. Physiologia Plantarum 109: 107-116.
24. Joner, E.J. and Leyval, C. 1997. Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae/ Trifolium subterraneum* mycorrhiza from soil amended with high and low concentration of cadmium. New Phytologist 135: 353-360.
25. Jones, M.D. and Hutchinson, T.C. 1988. Nickel toxicity in mycorrhizal birch seedlings infected with *Lactartius rufus* or *Scleroderma flavidum*. I. Effects on growth, photosynthesis, respiration and transpiration. New Phytologist 108: 451-459.
26. Killham, K. and Firestone, M.K. 1983. Vesicular-arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. Plant and Soil 72: 39-48.
27. Leyval, C., Turnau, K. and Haselwandter, K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7: 139-153.
28. Macfall, J., Slack, S.A. and Iyer, J. 1991. Effects of *Hebeloma arenosa* and phosphorus fertility on root acid phosphatase activity of red pine (*Pinus resinosa*) seedlings. Canadian Journal of Botany 69: 380-383.
29. Marx, D.H. 1969. The influence of ectomycorrhizal fungi on the resistance of pine roots to pathogenic infections: I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59: 153-163.
30. Marx, D.H. and Bryan, W.C. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. Forest Science 21: 245-254.
31. McLachlan, K.D. 1976. Comparative phosphorus responses in plants to a range of available phosphorus situations. Australian Journal of Agricultural Research 27: 323-341.
32. McLachlan, K.D. and De Marco, D.G. 1982. Acid phosphatase activity of intact roots and phosphorus nutrition in Plants. III. Its relation to phosphorus garnering by wheat and a comparison with leaf activity as a measure of phosphorus status. Australian Journal of Agricultural Research 33: 1-11.
33. McLaren, A.D. and Skujins, J. 1971. Soil biochemistry. Marcel Dekker, New York.
34. Newman, L.A., Strand, S.E., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M., Shurtleff, B.B., Wilmoth, J., Heilman, P. and Gordon, M.P. 1997. Uptake and biotransformation of trichloroethylene by hybrid poplars. Environmental Science and Technology 31: 1062-1067.
35. Palmroth, M.R.T., Pichtel, J. and Puhakka, J.A. 2002. Phytoremediation of subarctic soil contaminated with diesel fuel. Bioresour. Technol. 84: 221-228.
36. Reid, M.S. and Bieleski, R.L. 1970. Changes in phosphatase activity in phosphorus deficient *Spirodela*. Planta 94: 273-281.
37. Smith, S.E. and Read, D.J. 1997. Mycorrhizal Symbiosis, second ed. Academic Press, San Diego.
38. Vodnik, D., Jentschke, G., Fritz, E., Gogala, N. and Godbold, D.L. 1999. Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. Physiologia Plantarum 106: 75-81.
39. Wittig, R., Ballach, H.J. and Kuhn, A. 2003. Exposure of the roots of *Populus nigra* L., cv. Loenen to PAHs and its effect on growth and water balance. Environ. Sci. Pollut. Res. Int. 10: 235-244.

(Received August 27, 2009; Accepted September 25, 2009)