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Studies on the Enzyme Activities and Heavy Metals of Forest Soil in Mt. Nam, Seoul

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남산 삼림 토양에서의 효소 활성도와 중금속 함량에 관한 연구

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ABSTRACT

This study was carried out to investigate to determine seasonal variation of dehydrogenase activity, phosphatase activity, adenosine tri-phosphate content and some physicochemical properties, such as soil pH, moisture content, organic matter and several heavy metal concentrations from Apr. 1997 to Jan. 1998 in *Pinus densiflora* and *Quercus mongolica* forest in Mt. Nam, to explain a relationship between enzyme activity and the soil factors. There were ranges of 4.03~4.65 in soil pH, 18.65~51.09% in moisture content and 6.69~95.95% in organic matter. The organic matter content decreased with soil horizon, showing the higher values in *Q. mongolica* forest. In comparison to the results of Kawngneung site as control area, there were slightly differences due to a development level of forest ecosystem and microbial degradation of organic matter. The heavy metal concentrations showed 32.50~75.55 μg/g in Cu, 69.33~134.84 μg/g in Zn, 57.02~150.32 μg/g in Pb, and 0.36~1.00 μg/g in Cd in Mt. Nam. These values are higher than in kwangneung site because of long-term exposure to air pollutants from central city. On the other hand, ATP contents in Mt. Nam were lower than in Kawngneung site in relation to soil organic matter, moisture content and relatively high heavy metal concentrations. ATP contents per soil weight was largest in F+H layer and in spring time of other seasons. Dehydrogenase activity as an index of soil microbial activity had a ranges of 170.67~1,221.66 μg TPF/g that showed lower values than in Kawngneung site. However, phophatase activity had a contray tendency due to P fertilization for a continuous management. Those values increased through spring to a maximum in the summer and fall in autumn. This is basically caused by metabolic state of soil on the biological activity and several and several factors, such as aeration, soil temperature,vegetation and microflora.

Key words: Enzyme activities, Heavy metals, Forest soil. ATP.

INTRODUCTION

Microorganisms in soil influence on cycling of inorganic and organic materials through the ecological niches of decomposers and affect on plant growth and soil fertility in the process (Brendecke *et al.* 1993).

The action of microorganisms in rhizosphere in affected on materials excreted by self-decomposition of litter, branch, and plant root in their habitat. The soils composed of different forest type have different microbes and their enzyme activities.

Much researches had been carried out that soil microbial biomass depends on vegetation type of higher plant and soil conditions. Cobb (1932) studied the annual variation in microbial population in broad leaf and conifer leaf forest soil in New York Botanical Garden. The results showed that bacterial biomass in broad leaf forest soil was higher two times in upper layer soil and six times in lower layer soil than in conifer forest soil. Insam and Haselwandter (1989) suggested that a rate of microbial metabolism, biomass, soil respiration varied with successional process of higher plant. And Pancholy and Rice (1973) reported that a kind and activity of enzyme changed with a step of succession in old field.

Many studies about microbial distribution and soil enzyme activities in particular area have been reported in Korea. They involved that studies on the dehydrogenase activity in accordance with heavy metal concentration (Kim and Lee 1993), on the relationship between microbial distribution and enzyme activities (Lee *et al.* 1994), on the microbial distribution in forest soil (Cho *et al.* 1992, Choi *et al.* 1994, Song *et al.* 1995), and on the microbial distribution related to distinct vegetation types (Lee and Shim 1994). Numerous investigations into the activity of enzyme in several site (pasture land, meadow, arable land, and forest) have been carried out through the world (Rastin *et al.* 1988, Stott *et al.* 1985, Beyer *et al.* 1993, Bolton *et al.* 1985). However, there is little information available about enzyme activity in forest soil especially in relation to forest type, soil layers and season.

Our objectives were to determine seasonal variation of dehydrogenase, acid phosphatase, adenosine tri-phosphate content as measures of total microbial activity and biomass and some of the more important soil factors, such as soil pH, moisture content,organic matter, several heavy metal concentrations over a period of 1 year in conifer and deciduous forest, furthermore, to verify whether there is a relationship between enzyme activity and the soil factors determined.

METHODS

Study sites

The study sites were set in *Pinus densiflora* stand and *Quercus mongolica* stand of Mt. Nam (265 m) located in the center of Seoul city area. In the past the dominant vegetations were oak trees but present are *Pinus densiflora* (25.5%), *Quercus mongolica* (26.6%), *Robinia pseudo-acacia* (32.9%) and *Prunus sargentii* (5.9%) (Lee 1986). *Pinus densiflora* forest is found in the southern slope, *Quercus mongolica* in northern slope of the mountain and *Robinia pseudo-acacia* in the border of the street and town. To compare the status of biochemical properties of study site with those of natural forests, the natural reserve forest site was set in Kwangneung Forest Experiment Station (37°45'N and 127°10'E) in Kyunggi Province.

Sampling and analysis

Samples were collected from two sites, one was coniferous forest growing with *Pinus densiflora* and the other was deciduous forest growing with *Quercus mongolica*. The control samples in Kwangneung were collected in *Abies holophylla* plantation forest and the other was a mixed deciduous forest growing with *Quercus mongolica, Zelkova serrata, Carpinus laxiflora, Carpinus cordata*. Samples were seasonally sampled in litter layer, mixed layers of fermentation and humus (F+H), A_1 and A_2 layers in Apr. Jul. Oct. in 1997 and Jan. in 1998. Randomly sampled soil at each site was mixed and carried to the lab for analysis. Stones were removed from the sample which was then homogenized by hand and sieved (<2 mm). For the duration of the analysis the samples were kept cool at 4℃.

Subsamples of soil were taken for pH (ORION

Model 520A) and moisture content determinations. The remainder of the sample was air-dried, lightly ground with mortar, passed through a 5 mm stainless steel mesh sieve, and used for the analysis of Pb, Zn, Cd and Cu. The heavy metal concentrations were measured with atomic absorption spectrophotometer (Perkin Elmer, AAnalyst 100) by microwave digestion (CEM MDS-2000) twice in conc. HNO_3 and conc. HCl.

Enzyme activities

1. Dehydrogenase

Dehydrogenase activity (DHA) was determined by the method of Tabatabai (1982). Moist soil was treated with 3.5 ml of 1% TTC (2,3,5-triphenyltetra zolium chloride). That solution was mixed on a vortex and incubated for 24 h at 37℃ in the dark. After incubation, TPF (triphenyl formazan) formed by the reduction of TTC was extracted with 100 ml methanol by shaking for 4hr. Reactive products were measured at 485 nm with a spectrophotometer.

2. Phosphatase

Phosphatase activity was measured using p-nitrophenyl phosphate as a substrate (Tabatabai 1982). After an 1 hour incubation of substrate-buffer (MUB 6.5) and soil, 0.5 M $CaCl_2$ and 0.5 M NaOH were usually added to stop the reaction and extract the product (p-nitrophenol). The products were determined photometrically at 410 nm.

ATP determination

ATP was extracted by the method of Inubushi *et al.* (1989). The moist soil was kept at 50% water holding capacity for 5 days at 24℃. That soil was ultrasonicated with an extracting solution containing trichloroacetic acid phosphate-paraquat reagent on ice and filtered. The filtrates were mixed with tris-EDTA buffer (pH 7.75) and reacted with Lumit luciferin-luciferase enzyme (FL-AA, Sigma Co). The light emitted is measured by Luminometer (Auto Lumit LB 953).

Analysis of variance among physicochemical properties, microbiological characteristics and heavy metal concentrations was carried out by SAS Package.

RESULTS AND DISCUSSION

Environmental factors

The mean values of physicochemical characteristics of soil samples are shown in Table 1 with forest type and soil horizon. The pH of soil ranged about 4.03~4.24 in *P. densiflora* forest soils and 4.38~4.65 in *Q. mongolica* forest soils. The pH had slightly higher values in *Q. mongolica* forest soils.

The percentage of organic matter decreased with soil depth remarkedly ($P<0.001$).

Moisture content showed from 18.65% to 51.09 % with forest type and soil horizon. Average moisture content was higher in the upper layer (F+H) than in lower layer ($P<0.01$). Moisture contents of soil at Kwangneung sites were 23.73~59.46% in coniferous forest and 28.45~61.96% in deciduous forest. This result of moisture content was affected due to a development level of forest ecosystem and microbial degradation of organic matter. Generally, it is known that the optimum level of moisture content for the activities of aerobic bacteria is about 50~75% at any soil texture (Alexander 1977). It is supposed that moiture contents at Kwangneung sites increased by development of vegetation, activities of microbial degradation and accumulation of organic matter, comparing to those results of Mt. Nam sites. The moisture content was higher in *Q. mongolica* than in *P. densiflora* forest soil. It is considered that soil moiture contents increased by the result of adsorption effect of accumulated organic matter ($r=0.722$) according to vegetation types. Annual mean litterfall was 280.61 g/m^2 in *P. densiflora* forest and 706.06 g/m^2 in *Q. mongolica*. Litter production of *Q. mongolica* was higher two times than *P. densiflora*.

Table 1. Some physicochemical properties of each soil horizon in *Pinus densiflora* and *Quercus mongolica* forest

Forest horizon	pH (H_2O, 1:5)	Moisture content (%)	Dry weight (g/m²)	Organic matter	
				(%)	(g/m²)
P. densiflora					
L	–	19.22±1.31	280.61±29.15	95.95±6.49	269.59±20.74
FH	4.20±0.09	49.62±6.90	2717.03±21.13	47.76±8.75	1297.65±98.17
A_1	4.03±0.17	30.86±5.54	1816.77±201.89	17.38±3.68	315.75±27.23
A_2	4.24±0.30	18.65±0.63	1242.75±156.36	6.69±0.18	83.14±2.24
Q. mongolica					
L	–	23.97±1.77	706.06±69.14	92.38±0.46	652.26±3.25
FH	4.65±0.35	51.09±6.99	7510.91±835.54	39.95±4.34	3000.61±325.97
A_1	4.38±0.48	37.94±2.08	3036.23±313.69	17.58±2.20	533.77±66.80
A_2	4.39±0.27	26.56±1.09	2381.34±341.19	8.63±0.37	205.51±8.81

Values are means with SE.
(L:Litter layer, FH:Fermentation and Humified layer, A_1:A_1 layer, A_2:A_2 layer)

Table 2. Heavy metal concentration and amount in the *P. densiflora* and *Q. mongolica* forest with each soil horizon

Forest horizon	Cd		Cu		Pb		Zn	
	Conc. (ug/g)	Amot. (mg/m²)	Conc. (ug/g)	Amot. (mg/m²)	Conc. (ug/g)	Amot. (mg/m²)	Conc. (ug/g)	Amot. (mg/m²)
P. densiflora								
L	0.78±0.18	0.22±0.00	54.59±2.75	15.32±0.08	143.48±11.86	40.26±0.35	93.22±2.15	26.16±1.52
FH	0.87±0.24	2.36±0.11	70.86±5.68	192.53±5.61	120.72±10.88	328.00±9.10	134.84±9.85	366.36±14.28
A_1	0.56±0.14	1.02±0.03	48.14±1.27	87.56±0.27	94.30± 8.55	171.32±1.73	87.55±3.14	159.06±4.67
A_2	0.54±0.10	0.67±0.02	32.50±3.54	40.39±0.55	82.71± 9.57	102.79±5.08	79.59±8.69	98.91±3.24
Q. mongolica								
L	1.00±0.03	0.71±0.00	33.47± 3.90	23.63±0.46	150.32± 5.69	106.13±11.67	104.80±1.34	74.00±5.11
FH	0.73±0.11	5.48±0.09	75.55±24.55	567.45±20.51	114.08±10.52	856.84±25.73	112.68±8.63	846.33±24.70
A_1	0.59±0.06	1.79±0.02	46.18±13.10	40.21±4.11	117.96± 9.38	358.15±15.49	108.87±5.96	330.55±14.88
A_2	0.36±0.25	0.86±0.09	35.30±16.25	84.06±5.54	57.02±13.80	135.78± 4.71	69.33±2.69	165.10±10.49

Values are means with SE.

Table 2 shows mean values of Pb, Zn, Cd, and Cu concentrations in investigated sites with forest type and soil horizon ($P<0.05$). The higher mean heavy metal concentration was found in upper layer than in lower layer ($P<0.05$) and *Q. mongolica* forest soil than in *P. densiflora* forest soil except of Cu concentration. The results of heavy metal concentrations at Kwangneung sites had similar tendencies with those of Mt. Nam site. But mean values of Pb, Zn, Cd and Cu concentrations were much lower than in Mt. Nam site. It ranged 33.37~88.28 μg/g in Pb, 59.91~114.27 μg/g in Zn, 0.15~0.60 μg/g in Cd and 33~12.62 μg/g in Cu.

ATP determinations

ATP content has been shown to be a good estimates of the micobial biomass in soil, since the amount of ATP correlates with other estimates of soil microbial biomass. ATP plays a central role in metabolism and it is maintained at a fairly constant intracellular concentration. Intercellular nucleotides such as ATP is used as an index of metabolic activity and biomass in environment (Pangburn *et al.* 1994) instead of the unreliability of counting microbial numbers.

Fig. 1 shows seasonal changes of ATP contents in the investigated sites. ATP content per soil weight was largest in the F+H layer. The content in the A_1 and A_2 layer was relatively low. The value showed

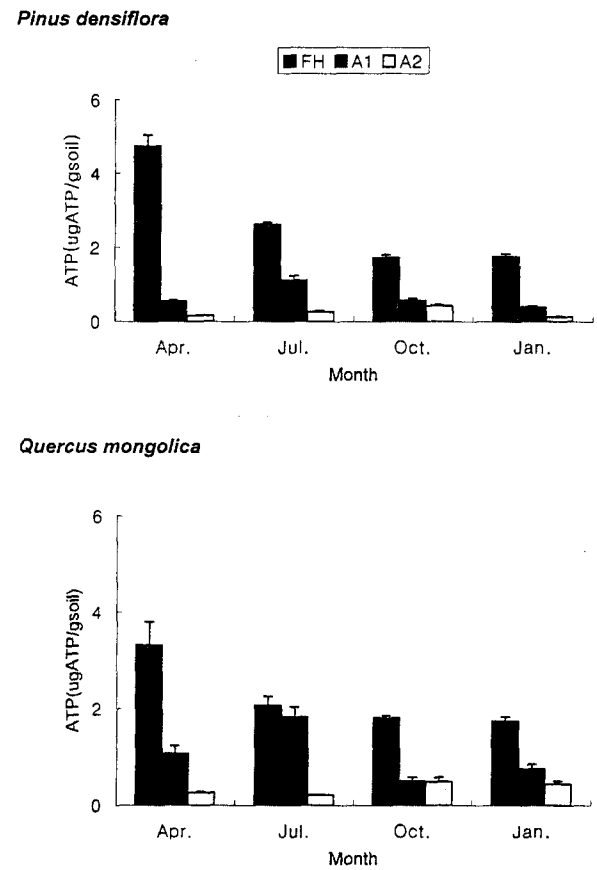

Fig. 1. ATP content of soils in *P. densiflora* and *Q. mongolica* forest with season and soil horizon.
Apr.: April, Jul.: July, Oct.: October, Jan.: January

the highest in spring time ($P<0.05$) of other seasons. In contrary with these results, ATP content at Kwangneung site had higher values with respect to both forest type and soil horizon than in Mt. Nam. It ranged about 2.32~10.13 μg/g in coniferous forest soil and 1.06~8.12 μg/g in deciduous forest soil. It was believed that this results were related to soil organic matter, moisture content and heavy metal concentrations.

Enzyme activities

DHA indicates the activity of the soil microbial popualation because dehydrogenation if the oxidation of carbon compound and is used as an index of respiration in the soil. And the phosphatase are involved in trasformation of organic and inorganic phosphorus compounds in soil. Their activities may play a significant role in P availability to plants from native soil organic P compounds. It is generally accepted that plants utilize only inorganic P and since in a large proportion of soil P is organically bound, the mineralization of this organic fraction can be an important factor in plant nutrition (Rastin *et al.* 1988).

Fig. 2 shows seasonal variations in DHA and acid phosphatase activity in the investigated sites. F+H layer soils were higher in the values of DHA than in other layers. The rate of decline was much greater in the upper part of the profile than in lower part. Generally metabolically depressed microorganisms during winter were reactivated in spring. Other studies record that soil phosphatase activity can increase through spring to a maximum in the summer months and then fall in autumn (Rastin *et al.* 1988 and Song *et al.* 1995). These results were coincident with the other studies. Ross (1970) stated that DHA appeared to be more dependent on the metabolic state of soil or on the biological activity if the microbial population that any other free enzymes present. And so seasonal pattern seems to depend upon many factors, such as aeration, soil moisture content, vegetation and microflora.

The value of DHA showed closer relationship with soil organisms measured by ATP ($r=0.782$) and phosphatase activity ($r=0.811$). It can be concluded that soil microorganism plays a significant role in secretion of the enzyme and enzyme activities in soil environment.

In comparison with DHA of soil in Mt. Nam, that in Kwangneung was remarkedly high due to organic matter and relatively low heavy metal concentration. On the other hand, phosphatase activity in Mt. Nam was rather higher than in Kwangneung because of P fertilization for a continuous management. The values of DHA ranged about 238.77~1,345.18 μg/g in coniferous and 382.79~1,596.94 μg/g in deciduous forest soil.

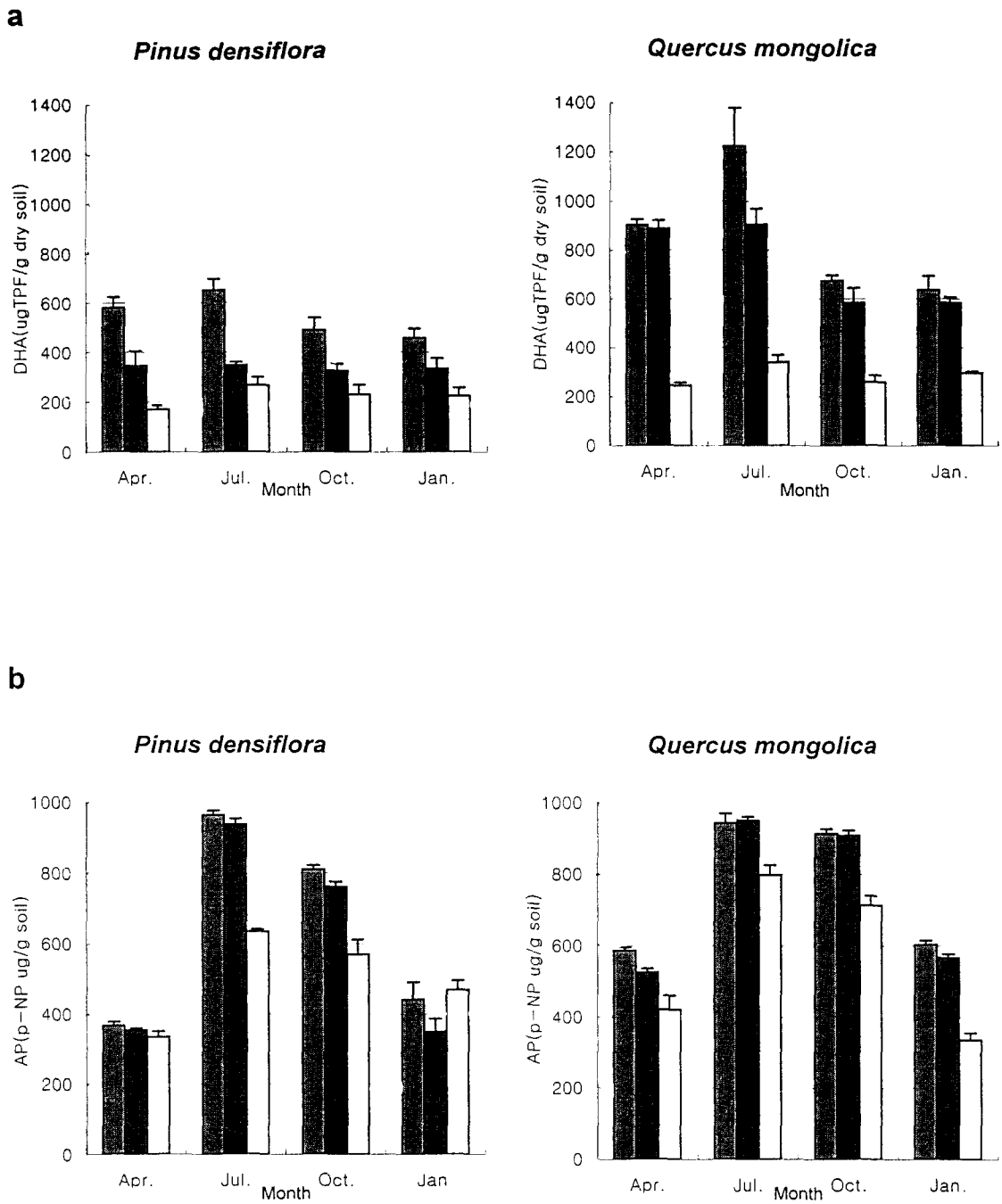

Fig. 2. Soil enzymes in *P. densiflora* and *Q. mongolica* forest with season and soil horizon.
a. Dehydrogenase activity b. Acid phosphatase activity

적 요

서울 남산의 소나무림과 신갈나무림의 임상에서 토양 효소 활성도의 계절적인 변화를 조사하기 위하여 탈수소효소 활성과 인산분해효소 및 ATP 함량을 분석하여 토양의 중금속 함량 및 물리 화학적인 특성과 비교하였다. 남산토양의 중금속 함량은 Cu 32.50~75.55 μg/g, Zn 69.33~134.84 μg/g, Pb 57.02~150.32 μg/g, Cd 0.36~1.00 μg/g으로 나타나 서울외곽에 위치한 광릉토양

의 함량과 비교하였을 때 비교적 높은 수준을 보였는데 이는 오염물질에 장기적으로 노출되었음을 시사한다. TPF값으로 나타낸 토양의 탈수소효소 활성은 170.67~1,221.66 μg/g으로 광릉지역보다 약간 낮은 수준으로 나타났고 표층이 하층에 비해 높았고 계절적으로는 여름에, 신갈나무림에서 더 높게 나타났다. 인산분해 효소는 광릉지역이 오히려 더 높게 나타났는데 이는 남산의 지속적인 인 시비 효과인 것으로 생각되며 다른 요소는 탈수소효소와 유사한 경향을 나타냈다. ATP함량은 0.13~4.74 μg/g의 범위로 광릉지역과 비교하여 낮게 나타났으며 표층토에서 높았고 봄에 크기가 증가하여 겨울까지 계속 감소하는 경향을 보였다. 이같은 결과는 토양의 대사적인 상태, 통기성, 온도, 수분, 유기물 및 중금속 함량 등에 의존함을 알 수 있다. 본 연구의 결과는 만성적으로 오염물질에 노출된 도시림 토양의 건강도를 평가하는데 토양효소 활성과 ATP 함량을 지수로 사용할 수 있다는 가능성을 제시하고 아울러 오염물질이 토양생태계에 미치는 영향을 자세히 규명하기 위해서는 좀 더 장기적인 연구가 필요하다고 할 수 있다.

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