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# The Effects of Heavy Metals on Microbial Biomass and Activity in Contaminated Urban Park Soils

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# 도시 공원의 토양에서 중금속이 미생물의 생체량과 활성에 미치는 영향

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## ABSTRACT

The relationship between Pb, Zn, Cd and the microbial biomass and activity were investigated in three public park soils of central and outer London. Variability with distance from the roadside and profile were studied. The heavy metal concentrations were the highest in Hampstead Heath and Hyde Park with high traffic density and the lowest in Hainault. The highest concentrations of heavy metals were found adjacent to the roadside in the upper parts of the soil profile. Dehydrogenase activity, adenosine tri-phosphate and ergosterol contents used as indices of microbial biomass and activity were generally higher in Hainault, and also higher in the upper parts of the soil profile. Simple regression analysis indicated that the microbial biomass and activity were affected significantly by moisture content, water holding capacity, total organic carbon, total nitrogen, and organic matter rather than heavy metal concentration. Highest inputs of nitrogen and carbon were associated with high inputs of heavy metals, all three being derived from vehicle emissions adjacent to the road. The Hyde Park and Hampstead Heath microbial populations were able to respond to the C and N input positively by increase in biomass and activity, whereas the Hainault populations could not. This result suggests adaptation in the former to heavy metals, but not in the latter.

## INTRODUCTION

Heavy metals are essential micronutrients for microorganism, plant-animal and human to grow, reproduce and/or survive, but they are toxic in excessive amounts. Environmental pollution with heavy metals is concomitant with development of human industrial history. Soil pollution with heavy metals is a serious problem in industralized countries.

The effects of heavy metals on both environment and organisms has been increasingly studied recently. These studies include the metals concentrations of soil(Culbard *et al.*, 1988; Moir and Thornton, 1989; Tong, 1990), the effect of heavy metals on plant-animal or microorganisms(Duxbury, 1986; Harris and Birch, 1987), interactions with enzymes or other elements in soil(Tate, 1987; Doelman and Haanstra, 1989), and metal tolerance of microorganisms(Olson and Thornton, 1982; Tyler *et al.*, 1989).

But ecophysical studies of heavy metal effects on microbial activity are limited especially in urban soils. Heavy metal released through human activities may become available to microorganisms more readily than those occuring naturally as a result of chemical weathering. Culbard *et al.* (1988) reported heavy metal contamination in urban soils from various parts of UK. Thornton(1990) also investigated soil contamination in urban area with definition of contamination source of urban soil. The main source of metals in urban soils are reported to include vehicular and industrial emissions, flaking paint and fossil fuel, and tyre rubber(Moir and Thornton, 1989; Hewitt and Candy, 1990). According to a report of the Department of Environment(1989), a reduction of lead emissions from petrol was noted in 1986 when unleaded petrol was first sold in the UK.

In addition to heavy metal concentration in urban soils, microbial biomass and activities are important to soil biological parameters in any assessment of the impact of stress and disturbance.

The aim of this study was to determine heavy metal concentrations in urban park soils and to investigate whether these parameters can be used as indices of soil stress by measuring microbial biomass and activities.

## STUDY SITE

The soil samples were collected from Hampstead Heath(HM) and Hyde Park(HY) in Cental London and Hainault Country Park(HA) outside of London. HM and HY are located near the busiest main road in Central London and HA is at the boundary of northeast Greater London(Fig. 1). Most soils of London area are clay loams. The traffic densities of the study area are 83,000, 57,000, and 8,400 vehicles per day for HM, HY, and HA, respectively(Department of Transport, Computer Data Base, 1991). The study was conducted from November 1990 to January 1991 as higher amount of airborne lead was usually found in winter(Ludwig *et al.*, 1965). Weather conditions are relatively stable. The monthly rainfall was usually 65~97mm. During this period, the weather in London is cold, with mean monthly temperatures of 3.9~7.4℃(Monthly Digest of Statistics, 1991) and most houses requre space heating.

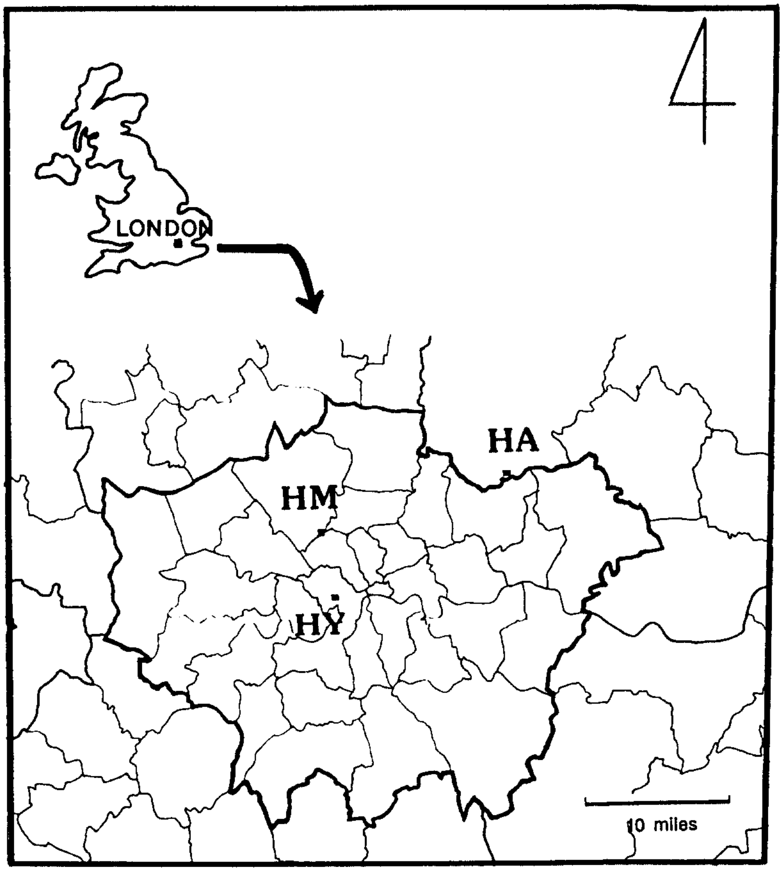


**Fig. 1.** Location of sampling site in London. HM;Hampstead heath, HY:Hyde park, HA;Hainault country park.

## MATERIALS AND METHODS

### Soil sampling and analysis

The soil samples were collected along a transect perpendicular to the roadside at distances of 0.5, 4, 16, and 64m and soil depths of 0~5, 5~10, 10~15, and 15~30cm from the soil surface with 30cm length soil corer. The samples were placed in a sterile plastic bags and in the laboratory were unpacked and chopped under aseptic conditions and stored at 5℃ until needed for analysis. Subsamples of soil were taken for pH(Corning ion analyzer 150), moisture content, water holding capacity determinations. The remainder of the sample was air-dried, lightly ground with mortar, passed through a 5mm stainless steel mesh sieve and used for the total organic carbon(C), total nitrogen(N), organic matter

(OM), and extractable Pb, Zn, and Cd. Total organic C and total N were measured by Perkin-Elmer 2400 CHN Elemental Analyzer. OM was calculated by using total organic C value by Schollenberger's method(1945). The extractable heavy metal concentrations were measured with Instrumentation Laboratory AA/AE Spectrophotometer 357 by digestion twice in conc. $HNO_3$ and conc. HCl.

### Measurement of microbial biomass and activity

Microbial biomass and activity were estimated by dehydrogenase activity(DHA), adenosine 5'-triphosphate(ATP), and ergosterol contents of the soil samples. DHA indicates the activity of the soil microbial population because dehydrogenation is the oxidation of carbon compound and is used as an index of respiration in the soil. The method used was the colorimetric determination of 2, 3, 5-triphenyl formazan(TPF) produced by reduction of 2, 3, 5-triphenyl tetrazolium chloride by soil microorganisms(Tabatabai, 1982). ATP as a means of estimating the microbial biomass was measured by Inubushi, Brooks, and Jenkinson's method(1989). The soil samples were kept at 50% water holding capacity for 5 days at 24℃. ATP in 1.5g soil was extracted by a trichloroacetic acid phosphate-paraquat reagent. The 25ml extractants were sonicated and filtered on ice. Tris-EDTA buffer was added to neutralize extractants. The soil extract/buffer mixture was allowed to react with LUMIT luciferin-luciferase enzyme(Sonco Ltd) and light output was determined by LKB 1251 luminometer. Ergosterol, the predominant fungal sterol was used as a measure of fungal biomass. The procedure consists of saponification, partition, and evaporation of the soil sample followed by HPLC determination against a standard (Zelles *et al.*, 1987; Zill *et al.*, 1988). Three replicates were measured for each sample.

### Statistical analysis

The physiochemical and microbiological soil characteristics were used for simple regression analysis of variance using the Statgraphics Package(Statistical Graphics Corporation, 1987).

## RESULTS AND DISCUSSION

### Soil Chemistry

The ranges, mean values and significance levels of the physicochemical characteristics of the soil samples are shown in Table 1 with averaging all distances and all depths per site. The pH ranged from 5.0~8.53 and was significant with distances($P$=0.001). There was a decreasing in pH with increasing distance from the roadside. On the other hand, moisture content, WHC, total organic C, total N, and OM were significant with depth, with the highest values in 0~5cm layer, and decreasing with soil depth. There were ranges of 1.48~10.23% in total organic C, 0.12~0.69% in total N, and 2.57~17.81% in

**Table 1.** Range and mean of physicochemical characteristics of three site soils.

| | | Range | Mean | S.D. | Distance | Depth |
|---|---|---|---|---|---|---|
| | pH | 5.00~8.53 | 6.17 | 1.16 | *** | N.S. |
| | ,MC (%) | 5.44~19.96 | 10.32 | 5.19 | N.S. | *** |
| HM | WHC (g/g) | 0.25~0.78 | 0.43 | 0.15 | N.S. | *** |
| | C (%) | 1.48~10.23 | 3.51 | 2.20 | N.S. | * |
| | N (%) | 0.12~0.59 | 0.23 | 0.13 | N.S. | ** |
| | OM (%) | 2.57~17.81 | 6.10 | 4.53 | N.S. | ** |
| | pH | 5.23~7.92 | 6.57 | 0.91 | *** | N.S. |
| | MC (%) | 8.84~26.27 | 16.54 | 5.24 | N.S. | ** |
| HY | WHC (g/g) | 0.36~0.90 | 0.56 | 0.18 | N.S. | * |
| | C (%) | 2.45~7.86 | 4.76 | 1.87 | N.S. | * |
| | N (%) | 0.13~0.50 | 0.29 | 0.11 | N.S. | * |
| | OM (%) | 4.26~13.68 | 8.27 | 3.25 | * | * |
| | pH | 5.13~8.16 | 6.29 | 1.04 | *** | N.S. |
| | MC (%) | 8.17~27.95 | 16.40 | 4.82 | N.S. | * |
| HA | WHC (g/g) | 0.33~0.92 | 0.49 | 0.17 | N.S. | N.S. |
| | C (%) | 2.03~8.07 | 4.58 | 20.2 | N.S. | *** |
| | N (%) | 0.212~0.69 | 0.41 | 0.15 | N.S. | *** |
| | OM (%) | 3.54~14.03 | 7.98 | 3.52 | N.S. | *** |

HM ; Hampstead heath, Hy ; Hyde park, HA ; Hainault, MC;moisture content, WHC ; water holding capacity, C;total organic carbodn, N ; total nitrogen, OM ; organic matter, S.D. ; standard deviation, *$P<0.05$, **$P<0.01$, ***$P<0.001$, N.S.:not significant(n=48).

OM.

### Heavy metals

Fig. 2 shows Pb, Zn, and Cd concentrations in three sites with soil distance and depth. The highest mean Pb concentration of 1,071.45μg/g air dried soil has been reported in HM for the top soil(0~5cm) away 0.5m from the road. HY and HA sites had mean Pb concentration of 922.70μg/g and 309.70μg/g in the same soil depth and distance, respectively. Also HM site had the highest mean concentrations of 387.65μg/g Zn and 6.75μg/g Cd for the top soil away 0.5m from the roadside, consistent with the corresponding traffic density. As shown in summary of ANOVA of Table 2, three kinds of heavy metals were very significant with site, distance, and depth except the site for Cd. There were similar decreasing tendencies with soil depth and distance in Pb and Zn concentration, but in case of Cd, such tendency was not clear.

There are several reports about metal concentrations in British urban soils(Culbard *et al.*, 1988; Moir and Thornton, 1989; Thornton, 1991). These metal concentrations compare with values of a range of 28-1, 260μg/g and a geometric mean of 294μg/g for Pb, a range of 34~482μg/g and a geometric mean of 183μg/g for Zn, and a range of <1~2μg/g and

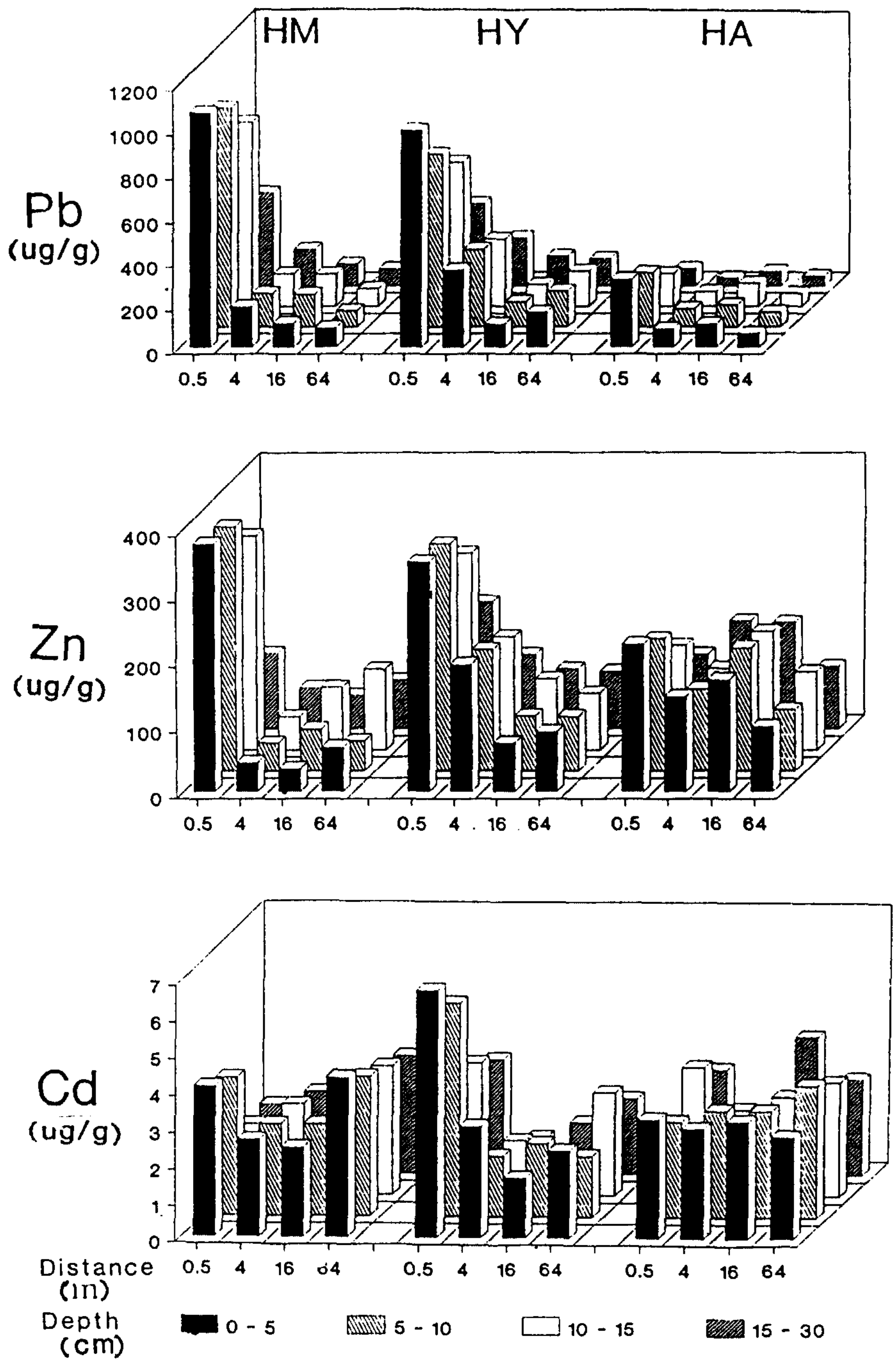


**Fig. 2.** Lead, zinc, and cadmium concentration along the distance and depth of soil samples in investigated sites. HM;Hampstead heath, HY;Hyde park, HA;Hainault

a geometric mean of 1.0μg /g for Cd found in London public garden soil(Culbard *et al.*, 1988; Hewitt and Candy, 1990). Warren and Birch(1987) reported the mean concentration of heavy metals in soil along the A13 Newham way which is a major arterial roadway in East London carrying approximately 70,000 vehicles per day. These mean concentrations of metal at roadside were 1,769.1μg /g, 971,9μg /g, and 6.0μg /g for Pb, Zn, and Cd, respectively. These results with distance from the road agreed with present data decreasing with distance.

**Table 2.** Analysis of variance for lead, zinc, and cadmium concentration along the site, distance, and depth in investigated sites.

| | Pb | Zn | Cd |
|---|---|---|---|
| Site | *** | ** | N.S. |
| Distance | *** | *** | *** |
| Depth | *** | ** | * |
| Site × Distance | *** | *** | *** |
| Site × Depth | N.S. | N.S. | N.S. |
| Distance × Depth | *** | *** | N.S. |

The dominant source of Pb in urban soil is the emission of Pb aerosol from the gasoline vehicles and industry, flaking paint and fossil fuel, and bonfire residue. The primary source of Zn in urban street is probably the attribution of vehicle tyre rubber. Sources of Cd in urban environment are much less well characterized than are those of Pb but tyre rubber and smelter emission, waste incineration, the use of sewage sludge, phosphate fertilizer and metal plating may make contributions. It seems possible that relatively low concentration of Zn and Cd is due to good road surface maintenance in London. When the highest metal concentrations in the present results were compared with Guidelines for Contaminated Soils(Inter-departmental Committee on the Redevelopment of Contaminated Land, 1987), HM site can be considered as the soils contaminated with Pb and slightly contaminated with Zn, in HY site slightly contaminated with Pb and Zn, and in HA site uncontaminated with Pb and Zn. For Cd, HM and HY sites were contaminated and slightly contaminated at the HA site. But mostly metal concentration from >4m distnace samples from the roadside had typical values for uncontaminated soils.

**Microbial biomass and activity**

Fig. 3 shows microbiological characteristics of DHA, ATP, and ergosterol contents in three sites with soil distance and depth. The HA site for DHA, ATP, and ergosterol contents had the highest in all distances and depths and the HM site had the lowest. The topsoil showed the highest in all three characteristics. Pancholy *et al.* (1976) reported DHA was much higher in the control soil than in bare Zn smelter site. According to Brookes and McGrath(1984), ATP concentrations in soils were smaller in high than in low metal soils. Soil ATP measurements provide an independent measure of soil total microbial biomass instead of the unreliability of counting microbial numbers. These above results showed similar tendency with the present data. Ergosterol content of soil is also a good index of fungal biomass. These parameters indicate well the disturbance level of any

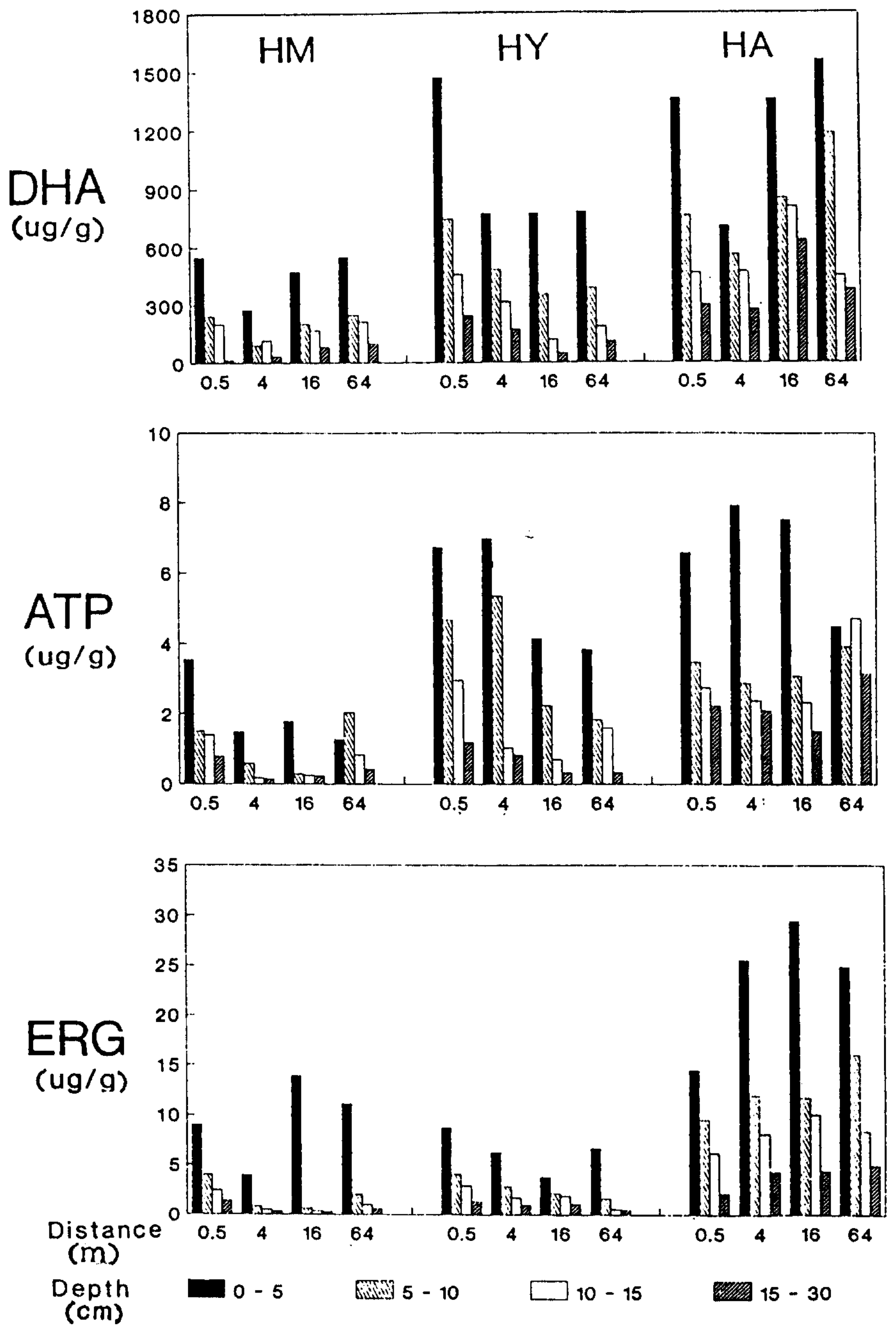


**Fig. 3.** Microbiological characteristics of DHA, ATP, and ergosterol contents along distance and depth in investigated sites. HM;Hampstead heath, HY;Hyde park, HA;Hainault, DHA;dehydrogenase activity, ATP;adenosine tri-phosphate, ERG;ergosterol.

**Table 3.** Analysis of variance for DHA, ATP, ergosterol contents along the site, distance, and depth in investigated sites. DHA : dehydrogenase activity, ATP : adenosine tri-phosphate, ERG : ergosterol.

| | Pb | Zn | Cd |
|---|---|---|---|
| Site | *** | *** | *** |
| Distance | ** | * | N.S. |
| Depth | *** | *** | *** |
| Site × Distance | *** | N.S. | * |
| Site × Depth | ** | ** | *** |
| Distance × Depth * | N.S. | N.S. | |

*$P<0.05$, **$P<0.01$, ***$P<0.001$, N.S. ; not significant

soil sample(Harris and Birch, 1990). As shown in Fig. 3, ergosterol of all of the other parameters appears to be the most sensitive. But unexpectedly, the contents of three parameters even in 0.5m soil from the roadside were not relatively low in spite of high metal concentrations. This is considered to be due to car oil, pedestrian litter and animal excretes providing an organic input. DHA, ATP, and ergosterol contents in three sites were significant with site and depth($P$=0.001) as shown in Table 3. They were also significant with distance but in case of ergosterol not significant.

**Effects of heavy metals and other characteristics on microbial biomass and activity**

Correlation coefficients obtained by simple regression analysis indicate the extent and direction of the effects of physiochemical characteristics, in addition to the heavy metal content, on microbiological characteristics of investigated urban soils(Table 4). DHA, ATP, and ergosterol content in three sites were positively related to moisture content, WHC, total organic C, total N, and OM significantly. And three parameters for microbial biomass and activity were highly correlated with each other($P$=0.001).

But DHA, ATP, and ergosterol content were not significantly correlated with heavy metal contents across the sites. These results suggest that the inhibitory effects of heavy metal cannot be simply evaluated in terms of microbial activity in urban soils, which agrees with the results of other studies(Olson and Thornton, 1982; Ohya *et al*, 1988). It would appear that, in this study, the microbial populations in the central London Parks (HM and HY) had developed tolerance to a level such that they could respond positively to the inputs of carbon and nitrogen associated with vehicle emissions and other roadside characteristics, despite this being associated with concomitant higher inputs of heavy metals.

The populations found in the rural park(HA), however, exhibit no such adaptation. Olson and Thornton(1982) suggested that the bacterial populations could withstand a small input of Cd into the environment without showing a significant change in numbers and bacteria from the highly polluted soil would be less sensitive to Cd additions than isolates from other soils with a relatively low level of contaminating Cd. And Ohya *et al.* (1988) found that the microbial biomass was not affected significantly by easily soluble Zn+Pb(extractable with 0.1N) and the biomass was accounted for as a function of cation exchange capacity, total organic C, and the numbers of fungal colonies present($R^2$=0.

**Table 4.** Correlation coefficients by simple regression analysis of microbiological characteristics in three soil sites.

| | | HM | HY | HA |
|---|---|---|---|---|
| DHA | pH | 0.07 | 0.11 | −0.07 |
| | MC | 0.84*** | 0.82*** | 0.66*** |
| | WHC | 0.71*** | 0.85*** | 0.57*** |
| | C | 0.54*** | 0.77*** | 0.74*** |
| | N | 0.62*** | 0.71*** | 0.74*** |
| | OM | 0.54*** | 0.76*** | 0.74*** |
| | Pb | 0.18 | 0.64*** | 0.35* |
| | Zn | 0.27 | 0.60*** | 0.18 |
| | Cd | 0.44** | 0.71*** | 0.30* |
| | DHA | – | – | – |
| | ATP | 0.57*** | 0.72*** | 0.50*** |
| | ERG | 0.75*** | 0.92*** | 0.74*** |
| ATP | pH | 0.22 | 0.02 | 0.01 |
| | MC | 0.56*** | 0.70*** | 0.34** |
| | WHC | 0.50*** | 0.67*** | 0.30* |
| | C | 0.69*** | 0.74*** | 0.45** |
| | N | 0.64*** | 0.75*** | 0.44*** |
| | OM | 0.69*** | 0.72*** | 0.45*** |
| | Pb | 0.49*** | 0.49*** | 0.27 |
| | Zn | 0.52*** | 0.43** | 0.12 |
| | Cd | 0.30* | 0.49*** | 0.12 |
| | DHA | 0.57*** | 0.72** | 0.50*** |
| | ATP | – | – | – |
| | ERG | 0.62*** | 0.71*** | 0.57*** |
| ERG | pH | 0.00 | 0.08 | −0.26 |
| | MC | 0.77*** | 0.80*** | 0.68*** |
| | WHC | 0.76*** | 0.86*** | 0.38** |
| | C | 0.76*** | 0.77*** | 0.83*** |
| | N | 0.83** | 0.72*** | 0.88*** |
| | OM | 0.76*** | 0.76*** | 0.83*** |
| | Pb | 0.18 | 0.56*** | 0.06 |
| | Zn | 0.17 | 0.52*** | 0.01 |
| | Cd | 0.28 | 0.62*** | 0.18 |
| | DHA | 0.75*** | 0.92** | 0.74*** |
| | ATP | 0.62*** | 0.71*** | 0.57*** |
| | ERG | – | – | – |

HM : Hampstead heath, HY : Hyde park, HA : Hainault, MC : moisture content, WHC : water holding capacity, C : total organic carbon, N : total nitrogen, OM : organic matter, DHA : dehydrogenase activity, ATP : adenosine tri-phosphate, ERG : ergosterol, *$P<0.05$, **$P<0.01$, ***$P<0.001$, N.S. :not significant.

692). Nevertheless, the proportions of metal tolerant to the total population are quantitatively related to the soil metal content(Olson and Thornton, 1982). According to Yamamoto *et al.* (1985), the numbers of fungal colonies are positively related to the soil metal content, and the metal-tolerant proportion of the population increase with the metal content. But in contrast, Pancholy *et al.* (1975) reported a significantly larger fungal population in a control compared with a metal-polluted site. And Brookes and McGrath(1984) found that amounts of soil microbial biomass in soils receiving sewage sludge or sludge-containing composts were much smaller than in soils which received farm yard manure over the same period. These conflicting results probably arise because the metal-tolerant microbial populations measured were developed to different extents.

Many environmental factors influence soil characteristics including microbial activity in urban soil because of the diversity of pollution sources(Thornton, 1990). Of course, total microbial population or activity may be used as an index of the inhibitory effects of metals when compared with a noncontaminated sample of the same soil. Because heavy metals may also influence the abundance and diversity of organisms comprising them as well as affecting the activity of microbial populations. Occasionally, the addition of particular heavy metals may result in an increase in a particular part of a community at the apparent expense of another. What is more, there are some striking examples in which particular species appear to require the presence of high metal concentrations, as in the case of *Paecilomyces farinisus*, which was only isolated from soils contaminated with $>1,000\mu g/g$ Cu $g^{-1}$ dry weight(Nordgren *et al.* 1983). In such situations, microorganisms are able to increase their abundance because of the reduction in competition from organisms which are more metal sensitive. But there are many other ecological interactions involving microorganisms, the outcome of which must be affected significantly by metal tolerance. Metal tolerance is not easy to define because a metal concentration requiring tolerance for one particular group of organisms may fall within the normal physiological range of another in mixed microbial populations. Also the observed difference are not due to the impact of heavy metals once tolerance has developed. When considering the ecophysiology of a system, microbial biomass consist of metabolic activity, and under field conditions, most of the total biomass is thought to be dormant for most of the year. The dormant population is less sensitive to inhibition by the metals than the metabolically activated one and when dormancy is released, e.g. during litterfall, it is possible that the heavy metal pollution is alleviated by a 'super-abundance' of chelating and absorbing against compounds, thus obviating the need for true tolerance on this part of the now active populations.


## 적 요

런던 중심부에 위치한 Hampstead Heath(HM), Hyde Park(HY)와 런던 외곽의 Hainault

Country Park(HA)의 토양에서 중금속(Pb, Zn, Cd)과 미생물의 생체량과 활성과의 관계를 조사하였다. 중금속 농도는 도로의 교통량에 비례하여 HM과 HY에서 높았고 HA에서 낮게 나타났으며, 특히 도로에서 0.5m 떨어진 0~5cm 깊이의 토양층에서 높았다. 미생물의 생체량과 활성의 지수로서 사용된 DHA(dehydrogenase activity), ATP와 Ergosterol 함량은 HA에서 높았고 일반적으로 토표층에 가까울수록 높게 나타났다. 단일 회귀분석을 한 결과 미생물의 생체량과 활성은 중금속 농도보다는 수분함량, 수분보유능(WHC), 총 유기탄소, 총 질소, 유기물 함량과 유의적인 상관관계가 있었다. 높은 질소와 탄소량은 자동차의 배기분출물에서 나온 중금속의 높은 유입량과 관계가 있으며, HM과 HY의 미생물 개체군은 탄소와 질소의 유입에 반응하여 그 생체량과 활성이 증가하였으나, HA개체군은 그렇지 못하였다. 이것은 HM과 HY 개체군의 중금속에 대한 적응을 보여주고 있음을 나타낸다.

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