C-2 1 Impact of Acid Deposition on Plant-Environment Systems

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The disturbance and degradation of natural ecosystems by human activities have drastically increased since the Industrial Revolution in the 18th century and have produced our present dat global environmental problems.

"Acid Rain" or more properly "acid deposition" caused by combustion of fossil fuels is one serious global environmental problem. In Europe, Northern America and Asia acid deposition has caused direct and indirect impacts to natural ecosystems.

From April 1993 to March 1996 following studies were carried out to clarify the impacts of acid deposition on the trees (*Cryptomeria japonica*), microorganisms(*Armillaria mellea*) and soils on Yakushima Island and mountain areas of the northern Kanto area.

Studies were also carried out on the biological and chemical processes in the laboratory and on naturally acidic environments to understand the tolerance and impact of acidic substances on the organisms and acidification processes within natural ecosystems.

(1)pH Distribution in radial sections of the stem and root of *Cryptomeria japonica*
(2)Effect of acids on the growth and germination of *Armillaria mellea*
(3)Potential factors responsible for the decline of Japanese cedar (*Cryptomeria japonica*) in urban areas
(4)Behavior of mineral elements in soil-plant system under the impact of acidification
(5)Estimation of the influence of plant growth on the soil acidification
(6)On the possibility of calcium deficiency in granite bedrock areas on Yakushima Island
(7)Surface alteration of mica during acid dissolution
(8)Studies on behavior of aluminum in soil and its chemical speciation
(9)Estimation of short and long term effects of acid rain by observation of mountainous river waters in Yakushima Island
(10)Degradation of chlorophyll by nitrogen dioxide generated from nitrite by the peroxidase reaction
(11)Evaluation of acidic deposition onto the forest canopies
– Study on the method of estimating dry deposition and leaching rate dividedly –

(1) pH distribution in radial sections of the stem and root of *Cryptomeria japonica*

The pH distribution in the outer bark, inner bark, cambium and xylem layers of a conifer, *Cryptomeria japonica*, was measured using an iridium oxide electrode and a flat-type glass electrode. The outer bark of *C. japonica* was strongly acidic and there was a difference in pH between the outer and inner bark. The outer bark pH was about 3 through the surface to the inside. The inner bark pH was about 4 at the outer bark side and about 6 near the cambium side. The pH was maximal (about 6.3) in the cambium, and slightly lower in the outer xylem. pH was determined in sub-sections of the outer bark afterchemical washing with pure water,

hydrochloric acid, barium chloride and formic acid suggested that water-insoluble carboxyl (-COOH) groups bound to organic polymers contribute to the strong acidity of the outer bark.

(2) Effect of acids on the growth and germination of *Armillaria mellea*

The effect of acids on the germination and growth of the fungus Armillaria mellea was investigated to clarify the relationship between acid deposition and damage to forest by the fungus. The optimum pH for the growth of the fungal hyphae was 6-7. The fungus did not grow at a pH of less than 2.5, but it was relatively acid tolerant and grew within a pH range of 3-4. The optimum pH for germination of the spores was 6-6.5, but no germination was observed at a pH of less than 3. On the other hand, the pH range for germination of the fungus in the presence of soil microorganisms was 4-4.5, and no spores germinated at a pH of more than 5 as a result of bacterial growth.
These findings suggest that the increase of *Armillaria mellea* is favored by acid conditions in the natural environment where the fungus competes with other microorganisms.

(3) Potential factors responsible for the decline of Japanese cedar (*Cryptomeria japonica*) in urban areas

Amounts and elemental compositions of epicuticular wax were analyzed in Japanese ceders (*Cryptomeria japonica*) collected from Saitama Prefecture, where the trees are in significant decline, and compared to those in the samples from the plains and mountainous areas of other prefectures in the Kanto District around Tokyo. Although the wax amounts of current leaves in all samples showed a gradual decrease by erosion after the leaf expansion from May to August, the rates of decrease were clearly faster in Saitama Prefecture than the other areas. A decrease in C/O ratios of the wax, which may lower the hydophobicity and consequently raise the wettability of the leaf surface, was also observed in the ceders from Saitama Prefecture. Such increased erosion and deterioration of the wax accelerates the transpiration and the leaching of nutrients from the leaf surface. In addition, a large quantity of aerosol was found to be deposited on the leaf surface in Saitama Prefecture, a part of which had invaded the stomata. This phenomenon may have further accelerated water and nutrients loss through incomplete closing of the stomata. Since aerosols on the leaf surface also hinder the transmission of solar light, a light-deficiency may have exerted another negative effect on the Saitama ceders. Japanese ceders are very sensitive to water stress, and control cuticular transpiration by increasing the wax under water-deficient conditions. Therefore, accelerated water loss due to wax deterioration and interference with stomatal functioning may have dealt the ceders a fatal blow under the higher temperature and lower rainfall conditions recently experienced in the Kanto Plain, especially in urban areas such as Saitama Prefecture. Deficiency of nutrients and light, and exposure to air pollutants may impart an additional stress to the ceders.

(4) Behavior of mineral elements in soil-plant system under the impact of acidification

Changing mineral status of soil under acidification impact will affect the growth and elemental composition of plants. In order to characterize elemental composition of plants under acidic environment, 3 types of laboratory and field experiments were designed; 1) long term changes in chemical quality of soil and plants under continuous acid load, 2) chemical changes of a soil during acidification, and 3) mineral composition of tea grown on various acid soils.
1. Effect of continuous acid load on soil and plant. Effects of impulsive and/or continuous load of H_2SO_4 on soil environment were investigated by use of indoor lysimeters filled with Tsukuba light-colored andosol to which limed sewage sludge had been applied at a rate of 40 kg m^{-2}. One lysimeter, called 'LC' plot, received 31 mm equivalent of 0.1 mol L^{-1} H_2SO_4. Then the plot was irrigated with dil. H_2SO_4 (pH 3.5) for 4 years at a rate of 31 mm $week^{-1}$. To another lysimeter, 'SW' plot, 13 mm of 0.1 mol L^{-1} H_2SO_4 was applied at first and the same volume of distilled water as LC plot was irrigated thereafter. Rape plants were cultivated on both plots for 17 times during the experimental period. Level of pH at the surface soil

declined to 7.0 - 7.4 by the acid impulse, but recovered to around 8.4 within 4 months. The yield and Mn concentration of the crop became higher in the LC plot relative to the SW plot. Potassium level was pararelled with the crop yield in the LC plot. The load of 0.1 mol L^{-1} H_2SO_4 caused leaching of S, Ca and Mg by the depth of 30 cm (SW) or 80 cm (LC). Continuous irrigation of pH 3.5 water slightly elevated the levels of these elements in the soil solution at the depths of 30 and 55 cm. In accordance with the cultivation, S, Ca, Mg and especially NO_3-N concentrations in soil solution showed oscillationat the upper 10 -55 cm layers. At the 30 cm depth of the LC plot, Zn and Cd levels were increased during 300 - 500th day with simultaneous increase of TOC and pH values, suggesting mobilization of these metals as chelated forms.

2. Chemical changes of a soil during acidification process. A light-colored andosol previously applied with limed sewage sludge was used as a test soil. Columns filled with the soil were continuously eluted with either 1) constant (pH 3) or 2) stepwise increase (pH 3 → 2.5 → 2) of H_2SO_4 strength, and changing levels of pH and chemical elements were monitored. The acid load of the first 72 ~ 76 cmol (+)kg^{-1} did not alter the pH (7 ~ 8) of eluted water while alkalinity, Ca and Mg concentrations gradually decreased. Increase in the strength of the loaded acid resulted in the increase in the levels of these constituents without changing the pH values of the eluted water, indicating the contribution of carbonated dissolution and cation exchange reactions to acid buffering capacity of the soil. This buffering region was followed by the second one of pH 4.1 ~ 4.2, which was characterized by disappearance or decreased levels of alkalinity, Ca and Mg, and simultaneous increase of Al level in eluted water. The third buffering region around pH 3.2 was observed only in the 2nd series of the experiment where Fe level was increased. Ratio of Mg/Ca and Si level in eluted water and input-S/output-S ratio became higher with progressing load of acid,suggesting the destruction of clay minerals. Spiked elution of Zn and Cd was found at around 4 cmol(+)kg^{-1} of the acid load. These metals in addition to Mn appeared again in the 2nd and 3rd buffering regions.

3. Mineral composition of tea grown on various acid soils. Tea (*Camellia sinensis* L.) leaves and the soils were sampled from 11 sites of Shizuoka Prefecture, Japan. The soils showed low bulk density and low pH (H_2O) (4.17 ± 0.85). Levels of Al, Fe and Mn in ammonium nitrate extracts decreased with increasing soil pH. In contrast, Zn and P levels were correlated with levels of C and N and bulk density, suggesting the concomitant incorporation of these elements with organic amendments. Lead and As were suspected as insecticide origin because of significant correlation with each other. A serpentine soil contained large amount of Ni, Cr, Mg, Co and Cd, which was reflected to the leaf concentrations. As compared to ordinary plants, tea leaves were characterized with high content of Al, Mn. P, As, Ni, Cr and Co, and with low Zn level. Most of elements were accumulated in mature leaves while K, P, Zn and Ni levels were higher in younger ones. Calcium, Sr and B contents in leaves increased with the soil pH, and simultaneously an inverted relationship was observed for Mn. Positive correlation coefficient was obtained for Ca, Mg, Mn, Cu, Ni, Cd, Co and Cr between leaf and soil. However, leaf Al behaved independently from pH and Al in soil. In spite of such limitation, these results suggest a possibility to use tea leaves for monitoring mineral status of acid tea soil.

(5) Estimation of the influence of plant growth on the soil acidification

There have been many studies for the influence of the load of acidifier on the soil acidification, however, few studies about the influence of plant growth on it has been performed. In this study it isreported to evaluate the influence of plant growth on the soil pH changein soil-plant system. Three types of nitrogen compounds (ammonium-N, nitrate-N and organic-N) were used as acidifiers and winter barley was cultivated in aclosed pot to avoid leaching of mineral elements and H^+ form soil-plant system. Nitrification of nitrogen compounds was performed as follows. Before cultivation of winter barley, nitrogen compounds were added separately to the soil (Gray lowland soil) and they were incubated to nitrify enough for 42 d. At 25°C. Winter barley was cultivated by two series, one was ordinary cultivation and the other was the cultivation after nitrification of nitrogen compounds. After cultivation, soil pH, the contents of inorganic nitrogen in the soil and the contents of some macro nutrients absorbed by the plant were measured. After cultivation there was slight difference between the soil pH values of the

two cultivation series. The total load of proton during cultivation was estimated from the summation of the proton load in accordance with mineral absorption during the plant growth and the proton load in accordance with the nitrification of N compounds. The soil pH was calculated from the estimated proton load by using the soil pH buffer curve. The calculated pH value well accorded with the soil pH value by water extract method.
These result suggests that it is possible to evaluate the influence of the plant growth on the soil pH change by examining the quantities ofsome minerals absorbed by plants.

(6) On the possibility of calcium deficiency in granitic bedrock areas on Yakushima Island

Sr isotopes were applied as a natural tracer to understand and evaluate the effect of the atmospheric input to the soil-vegetation system on Yakushima in Southern Kyushu. Riverwater has a distinct $^{87}Sr/^{86}Sr$ ratio dependent on the bedrock in the basin (granitic rocks of Mioceneage and sedimentary rocks of Paleogene age), while its Na/Cl ratio and Mg/Na ratio are close to that of seawater. This indicates that dissolved ions in Yakushima riverwaters are derived largely from the selective dissolution of Ca minerals, such as plagioclase and carbonate in the bedrock, and that the dominant contribution of Na, Mg, and Cl is from seasalts in rainwater. On the other hand, the $^{87}Sr/^{86}Sr$ ratio of landplants is almost identical to that of seawater and is basically independent of the underlying substrate. This suggests that rainwater exerts a significant contribution to the of transport material in plants and soilwater, as well as to the chemical composition of riverwater. In the soil-column at Yotsuse site of the northwestern part of the island, the Ca content is ten times lower than the substrate granitic rock, while the concentration of Al and Ti increase uniformly toward the surface. This is due to the high accumulation of Kosa aerosol in the upper soil which is mainly composed of clay minerals (illite, kaolinite, etc.) with a high Al content and $^{87}Sr/^{86}Sr$ ratio, and low Ca content. The deficiency of Ca in the soil due to weathering of granitic rocks and the large amount of wet and dry deposition with low Ca content lend support to the view that the soil-vegetation condition in Yakushima is sensitive to the behavior of ca which is indispensable for the growth of plants.

(7) Surface alteration of mica during acid dissolution

As a model of the chemical weathering of silicate minerals, surface alteration of acid-leached mica (biotite) was examined by secondary ion mass spectrometry (SIMS), X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). This work shows that Fe, Mg, K and Al ions are selectively leached during acid dissolution, resultig I the formation of an altered layer (depthe of alteration = ~ 100nm) on mica surface.

(8) Studies on behavior of aluminum in soil and its chemical speciation.

Aluminum in soil samples was extracted into 1:1 distilled water (soluble Al) and 1 M KCl (exchangeable Al). The soil samples tested were granitoid soil (GS) and sedimentary soil (SS) collected in Yakuisland and Gunma Prefecture. The concentration of soluble Al was relatively low for both GS and SS, and decreased along with the increase in sampling depth (0, 50 cm, 1 m). On the other hand, higher concentration of exchangeable Al was observed in GS than in SS. Chemical species of soluble and exchangeable Al was investigated by HPLC with fluorometric detection. Considerable parts of the soluble Al were found to be $AlL_x^{<+1}$, or AlL_x^{2+}, that is, complexed Al with organic or inorganic ligands, whose percentage in the soluble Al decreased along with the increase in sampling depth. Moreover, the most part of soluble Al for SS in Gunma Prefecture is very inert against ligand exchange reaction and exists in the fraction of > m.w. 10,000. Exchangeable Al, on the other hand, exists mostly as hydrated Al^{3+}.

(9) Estimation of short and long term effects of acid rain by observation of mountainous river waters in Yakushima Island

Yakushima Island, one of the Japanese National Parks, is located in the East China Sea, 800 km east of Shanghai. Yakushima Island has been exposed to acid rain of which the annual mean pH is 4.65 and the annual mean precipitation is about 4,300 mm. Acid rain falls frequently and intensely in short duration. Because of its steep landform and thin soil layer, the effect of acid rain on plants, soil layer and freshwater should be estimated by classifying it into the direct one in a short term and the other in the long term. Especially, the long term effect is important in terms of the critical load of acid rain to a watershed. The observations of mountainous river water quality in the island were made lower reach points in all major rivers and from the upper reach to lower reach in two large rivers during the period of 1992-95. Most river waters were low pH (5.5 to 6.5), alkalinity (less than 0.1 meq/l) and electric conductivity (less than 4 mS/m). The linear regression relations among these three indices were obtained with high correlation coefficients. The storm runoff observation of a large mountainous river in early spring 1994 indicated that the low values in pH (min. 5.56), alkalinity (min. 0.0194 meq/l) and ion concentrations were mainly cased by surface runoff during the short time at peak flow.

(10) Degradation of chlorophyll by nitrogen dioxide generated from nitrite by the peroxidase reaction

Nitrite, but not nitrate, added to a mixture containing horseradish peroxidase (HRP) and H_2O2, bleachedchlorophyll (Chl). The optimum pH for Chl bleaching was 4.0. Ascorbate, *p*-hydroxyphenyl acetate (HPAA), glycyltyrosine (Gly-Tyr) and amines such as morpholine and diethylamine inhibited Chl bleaching. The reaction products from HPAA and Gly-Tyr showed an absorption peak at 438 nm in an alkaline solution. The laser-Raman spectrum of the product from HPAA showed a band at 1336 cm^{-1} identical to that of authentic 2-nitrotyrosine. These results indicated the formation of nitrogen dioxide, the one-electron oxidation product of nitrite, which caused Chl bleaching in the nitrite/H_2O_2/HRP system. Nitrite caused neither inhibition of the ascorbate peroxidase reaction nor Chl bleaching with the H_2O_2/ascorbate peroxidase system.

(11) Evaluation of acidic deposition onto the forest canopies
-Study on the method of estimating dry deposition and leaching rate dividedly-

In order to estimate dry deposition and leaching rate dividedly ontothe forest canopies, model calculation using material balance method (SO_4^{2-} was used as model substances) based on throughfall measurementswas investigated at the four forests of Cryptomeria japonica, Quercus serrata, Q.myrsinaefolia and Chamaecyparis obtusa in Kobe city. Dry deposition rate on the four forest canopies was higher than thaton open field, and the rate onto C. japonica forest was much higher than those of other forests. In this forest, total flux was about three times larger than the incident deposition.

K^+, Mn, Mg^{2+} and Ca^{2+} were found to be leached from the four forest canopies, whereas HÅ$^+$, $NH4^+$, Zn, Cu and Pb were adsorbed in these cano-pies. And the orders of the leaching velocity were K^+> Mn, Mg^{2+}> Ca^{2+},and *Q. serrata> C. japonica> C. obtusa.* These results were agreed to those of the leaching experiments using leaves of above trees.

The fact that a significant correlation found between the leaching rates of Mn, Mg^{2+} and Ca^{2+} and the dry deposition rate of H^+ indicate that the input of acidic deposition onto forest canopies had effects on nutrient cycling in the forest ecosystems.

Above results show that our method based on both throughfall measur-ements and model calculation is available to the estimation of dry deposition and leaching rate onto the forest canopies.