Effects of Airborne Particulates on Nitrogen Fixation in Legumes and Algae

Richard P. Sheridan

Department of Botany, University of Montana, Missoula 59801.

Data reported in this symposium represent the combined research effort by the Environmental Laboratory and credit is extended to William Olsen, John Porter, Tony Jewett, and Cyndi Williams for important contributions.

In 1974, 40×10^6 metric tons of fertilizer were used worldwide. In contrast, biological nitrogen fixation has been estimated to be 174 $\times 10^6$ tons per year with 90×10^6 tons of this total occurring in agricultural soils (20). Assuming that the use of nitrogen fertilizer continues to increase at the current rates, by the year 2000 approximately 200×10^6 metric tons will be required to meet agricultural demands. Table 1 compares the amounts of nitrogen fixed by the abiological Haber-Bosch process to biological fixation by diazotrophs. The recent scarcity of petroleum for the synthesis of nitrogen fertilizers, the scarcity of the product, and the high energy input required have produced an intense interest in alternative sources of agricultural nitrogen.

DIAZOTROPHIC ORGANISMS

Nitrogen fixation occurs in free-living diazotrophs which include some nonphotosynthetic bacteria such as *Clostridium, Azotobacter, Klebsiella, Enterobacter,* and *Bacillus polymyxa* (62). Photosynthetic bacteria are active N_2 fixers—*Rhodospirillum, Chlorobium thiosulfatophilum, Chromatium vinosum,* and *Ectothiorhodospira* (69). The blue-green algae form a group of nitrogen fixing organisms of major importance. Genera including *Gleocapsa, Anabaena, Lyngbya, Trichodesmium, Plectonema, Phormidium,* and *Oscillatoria* have been shown to be active diazotrophs. These genera occupy either soil or aquatic ecosystems. Certain blue-green algae (eg, *Anabaena azolla*) live within leaf pockets of an aquatic fern forming an associative symbiotic relationship while others exist helotistically with lichens. *Collema* and *Peltigera* are genera of lichens having the N_2-fixing blue-green algae *Nostoc* as phycobiont (41). The obligatory symbiotic nitrogen fixation by *Rhizobium* spp. bacteria in association with the leguminous angiosperms such as alfalfa, clover, and soybeans is well known. Less known, but important, are the obligatory symbiotic N_2 relationships between bacteria and conifers (Douglas fir), grasses (*Digitaria, Paspalum*), and maize. Some confusion exists concerning the classification of the N_2 fixing organism that is associated with alder and *Ceanothus* and which may be an actinomycete (62).

Nitrogenase.—Nitrogenase acts on diatomic nitrogen as substrate and produces ammonia as the product (Fig. 1). Tests on all purified enzyme preparations thus far indicate that the enzyme has two major protein components. Each component is distinguished by its molecular weight and nonprotein inclusions. The largest protein has a molecular weight between 200,000 and 270,000, contains 1–2 Mb atoms, 17–36 Fe atoms, and 14–28 acid-labile S atoms per molecule (30). The smaller of the two protein subunits has a molecular weight of 55,000–67,000 with four atoms of iron-sulfur per protein molecule (73). There is some evidence that nitrogenase enzymes isolated from organisms of different phyla have a common structure (12). Often recombination of the protein subunits was more successful when both protein fractions were obtained from either aerobic or anaerobic organisms than when aerobic protein fractions are combined with anaerobic fractions (10). Both protein fractions share extreme sensitivity to oxygen and are instantaneously inactivated by air. The reduction of N_2 to ammonia requires 12–15 moles of ATP. In free-living photosynthetic diazotrophs the source of ATP is derived from photosynthesis and the resultant photosynthate. Sucrose is the photosynthetic product which funcitons as the energy source for *Rhizobium* sp. bacteria living within root nodules of legumes.

TABLE 1. Sources, uses, and production of reduced nitrogen

Industrial N_2 Haber Process	40×10^6
Lightning	10×10^6
Combustion	20×10^6
Ozonation	15×10^6
Biological N_2 - Fixation	
All Ecosystems	175×10^6
Agricultural	90×10^6
Agriculture in 1975	40×10^6
Agriculture by 2000	200×10^6

00031-949X/79000184$03.00/0

Increased rates of photosynthesis result in a corresponding increase in N_2 fixation rates (19,20). This relationship becomes important in air pollution studies because the phytotoxicant may inhibit N_2 fixation by direct action on nitrogenase or indirectly by inhibiting photosynthesis and thereby limiting ATP or photosynthate.

THE STUDY SITE

Coal development.—The Fort Union Basin coal reserve includes portions of Montana, Wyoming, both South and North Dakota, and associated lignite formations. Within the area it has been estimated that there are 1,230.6 quads (1 quad = 10^{15} BTU) of strippable coal. This is equivalent to 94 times the amount of coal burned in 1974 (R. Curry, *personal communication*). This does not imply that this represents the amount of coal that will eventually be burned in the area, but the size of the area provides background information concerning the impact of both combustion and surface mining with off-site combustion. The study site identified in this proposal is Colstrip, Montana, located in Rosebud County in southeastern Montana. The site presently has active coal strip-mining, and two 350 MW coal-fired electricity generating plants owned by a consortium of Northwest Utilities. Two additional generating units, each having a 750 MW capacity, are planned. Table 2 gives data for the stack emissions of generating units 1 and 2, and Table 3 shows the projected emissions from generating units 1–4.

A second study area located near Anaconda, Montana, was chosen because the Anaconda Company operates a copper ore smelter in the area. The emissions include SO_2 and the heavy metals copper, zinc, cadmium, lead, and arsenic.

Although both study areas are located in the state of Montana, they represent emission types having both national and international counterparts.

Flora.—The flora of the Colstrip area is a cool season-short grass ecosystem containing several diazotrophic organisms (Fig. 2). The soil has a surface flora of cyanophytes with five major species represented. The lichens *Collema tenax* and *Lecidea* sp. are ubiquitous and function as nitrogen fixers and soil stabilizers owing to their extensive system of rhizines. The major nonagricultural diazotroph is sweet clover with two species present—*Melilotus officinalis* (yellow sweet clover) and *M. alba* (white sweet clover). Vast areas of the Crow Indian Reservation and most of the draws are extensively occupied by this legume.

TABLE 2. Estimated atmospheric emissions of Colstrip, Montana, electric generating units 1 and 2[a]

	Unit 1 capacity			Unit 2 at 1/3 capacity	Units 1&2 at 1/3 capacity
	1/3	1/2	Full		
SO_2 (tons/yr)	3,023	4,535	9,071	3.023	6,046
NO_x (tons/yr)	3,046	5,190	10,380	3,460	6,920
Particulates (tons/yr)	268	403	806	268	536
H_2O (tons/yr)	353,320	529,980	1,059,960	353,320	706,640
F (lbs/yr)	2,333	3,500	7,000	2,333	4,666
Pb (lbs/yr)	400	661	1,322	440	880

[a] Source: Montana Department of Health and Environmental Sciences, 1974 (18). Prior to the completion of Units 1 and 2 the airshed was determined to be pristine (18).

TABLE 3. Calculated stack emission from Colstrip, Montana, electric generating units 1–4[a]

	Number of units		
	1 or 2	3 or 4	1–4
SO_2 (tons/yr)	9,071	20,292	58,726
NO_x (tons/yr)	10,380	23,218	67,196
Particulates (tons/yr)	806	1,787	5,186
F (lbs/yr)	7,000	15,800	38,600
Pb (lbs/yr)	1,322	2,938	8,520
Be (lbs/yr)	65.7	146	423

[a] Gordon (*personal communication*).

Alfalfa cultivars Ranger and Vernal were included as study plants because both are important to agriculture in northwest Montana, Wyoming, and the Dakotas. Soybean (*Glycine max* 'Kent') was added to broaden the study results to include an agricultural species important to the central and eastern United States.

Nitrogen-fixation assay.—Biological nitrogen fixation proceeds via the following pathway:

$$\underset{\text{diatomic nitrogen}}{N \equiv N} \xrightarrow[6H^+]{6e^-} \underset{\text{ammonia}}{2\ NH_3}$$

The assay technique employed in the results reported employed the reduction of acetylene (C_2H_2) to ethylene (C_2H_4) with gas chromatographic detection of the product ethylene. The system takes advantage of the fact that the nitrogenase nitrogen reducing system is capable of reducing compounds other than N_2—specifically acetylene and ethylene. These easily separate on a gas chromatography column and the hydrogen flame ionization detector system is extremely sensitive. Ethylene concentrations as low as 1 $\mu\mu$ mole can be detected.

The assay technique has been employed using free-living nitrogen fixers like *Clostridium pasteurianum* and *Azotobactor vinelandii* and symbiotic fixers like *Rhizobium* in soybean nodules (33).

The acetylene assays were performed according to Stewart et al (66–68).

Rhizobium-containing nodules were removed from soybean, but whole roots plus nodules of alfalfa or sweet clover were added to rubber-stoppered vials, acetylene was injected and the closed systems were incubated at 24 C for a time period sufficient to give three-quarter scale ethylene spikes for the controls. The reaction was stopped with either cold (0 C) or 0.2 ml of 50% (w/w) trichloroacetic acid, and 1 ml of gas was injected into a PYE gas

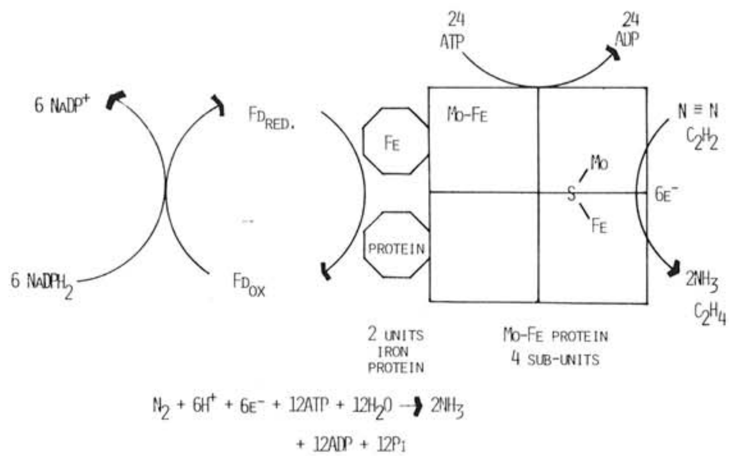

Fig. 1. Components and stoichiometry of nitrogenase enzyme.

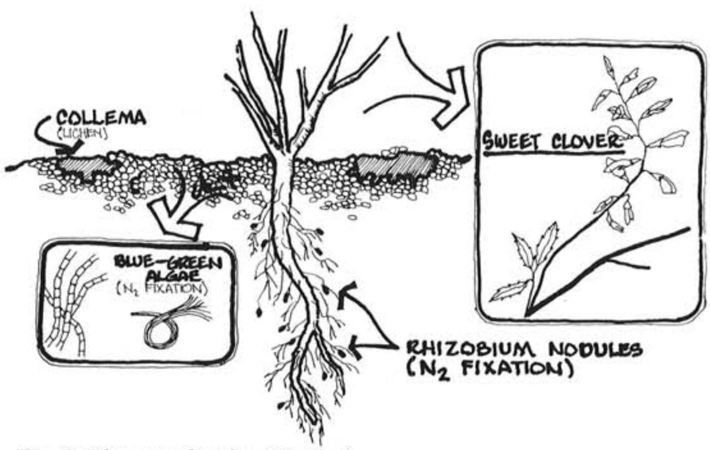

Fig. 2. Diagram of native Colstrip flora.

chromatograph fitted with a stainless steel column (Poropak R) with the injector and detector at 40 C and the column at 50 C. The nitrogen carrier delivered the C_2H_2-C_2H_4 mixture to the flame ionization chamber for quantification. The lichen *Collema tenax* was added intact to sample vials whereas the soil cyanophytes were analyzed in aqueous medium. Rates of acetylene reduction by angiosperms were based on dry weight and those by the lichens and cyanophytes on total cell protein (38).

DIAZOTROPHS IN THE COLSTRIP AREA

Soil cyanophytes.—Blue-green algae (Phylum Cyanophyta) are capable of reducing molecular nitrogen to ammonia and they are present worldwide in most soil systems (24). Heterocystous blue-green algae have been studied most extensively with regard to N_2 fixation although at least one nonheterocystous blue-green (*Gleocapsa*) alga definitely has been shown to fix nitrogen (51). The presence of the heterocyst in N_2-fixing cyanophytes appears to be a morphological adaptation which functions to protect the nitrogenase enzyme from photosynthetically produced oxygen. First, it has been shown that the photosystem-2 inhibitor, DCMU, has little effect on nitrogenase activity (4,7,8,38). Secondly, the action spectrum of nitrogenase activity is similar to that of photosystem-1, not photosystem-2 (13). Thirdly, that the absence of photosystem-2 light wavelengths has no effct on nitrogenase activity agrees with DCMU results (5) and lastly, there is no demonstrable Emerson enhancement of N_2 fixation in heterocysts (39) and ribulose 1,5-diphosphate activity could not be demonstrated (70). These data indicate that the heterocyst is not exposed to the O_2 evolved by photosynthesis. ATP required for N_2 fixation appears to be provided by photophosphorylation and oxidative phosphorylation. The ATP derived from cyclic photophosphorylation probably regulated nitrogenase activity in photosynthetic diazotrophic heterocystous cyanophytes (9).

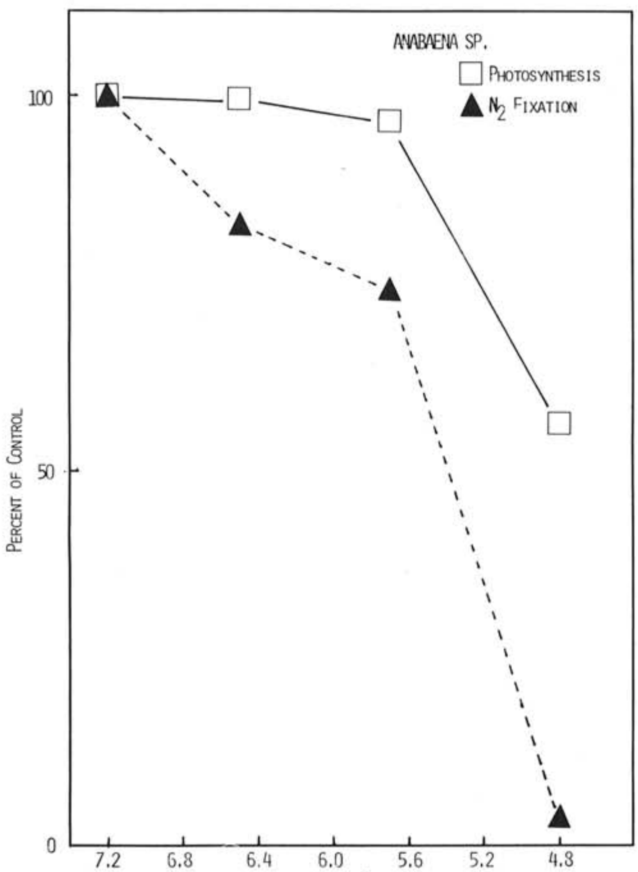

Fig. 3. Photosynthesis (O_2) and nitrogen fixation rates (as C_2H_4) are shown for *Anabaena* treated with various pH solutions of H_2SO_4 for 24 hr.

Heterocyst formation in heterocystous cyanophytes and subsequent nitrogenase activity appears to be controlled by two linked operons. Combined nitrogen represses and the absence of combined nitrogen derepresses these operons (59–61).

The nitrogen fixing blue-green algae are distributed worldwide. It is only in the marine environment that they are scarce yet occasionally form plankton blooms. Most reports concerning N_2 fixing cyanophytes have centered on mesotrophic freshwater systems. In general, these have shown that the group has a remarkable ability to grow rapidly in waters with low levels of nitrogen and phosphorus (68). Phosphate is stored as polyphosphate bodies within the cell (26,27) whereas nitrogen is stored as phycocyanin and in granules composed of aspartic acid and arginine (57).

The blue-green algae usually occupy soils having a pH range of 7.5–8.5 (15); however, Stewart (65) has reported nitrogen fixing *Haplosiphon* in acid boglands. In general, no nitrogen fixing algae are found in soils below pH 5.4. This ecological characteristic becomes very important when assessing the impact of SO_2 and associated acid rains that occur near coal-fired electrical plants. The contribution of soil cyanophytes to reclaimable soil has been extensively studied by Singh (58). He reported soil pH decreases of pH 9.5 to 7.5 with the total available phosphate increasing by 200%. the growth yield was approximately double and the nitrogen content was threefold higher for grasses growing in soils having the natural blue-green algal flora. Other contributions to soil by cyanophytes were increases in the soil organic fraction and permeability to water. The presence of *Schizothrix, Porphyrosiphon,* and *Scytonema* spp. were significant in restoration of saline and semi-arid soils to agricultural productivity. A survey of Swedish soils showed that aerobic nitrogen fixation does not occur below the soil surface (24). The nitrogen contribution by blue-green algae to the tundra biome ranged between 4.3 and 9,560 mg $N/m^2/yr$ with a mean value of approximately 130 mg N/m^2. Nitrogen fixation rates in volcanic soil on the newly formed island of Surtsey ranged between 2–54 mg $N_2/g/hr$ (23). The nitrogen released by the blue-green algae into the soil environment was

TABLE 4. Relationship between HSO_3^- concentration and nitrogen fixation in *Anabaena*

ppm HSO_3^- (aqueous)	0	0.1	1	10
ppb SO_2 (atmospheric)	0	0.09	9	942
μM C_2H_4/mg Chl *a*	3.81	3.05	2.70	0.88

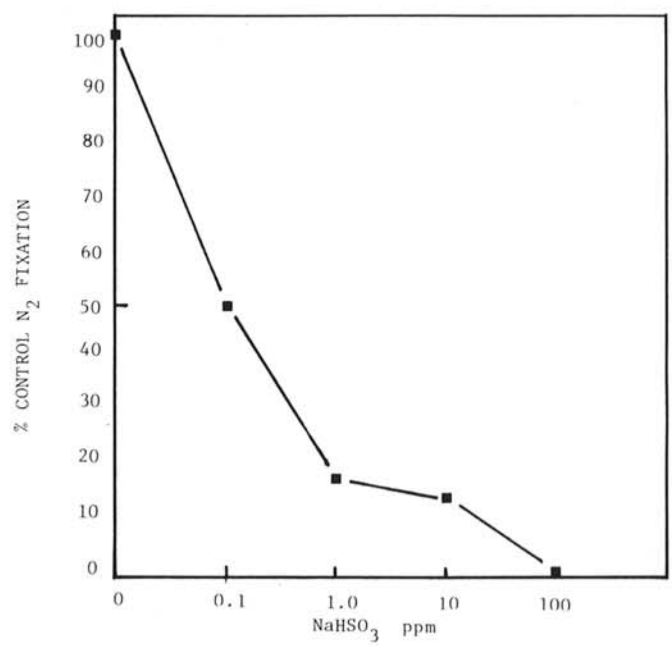

Fig. 4. Relationships between N_2 fixation (C_2H_4) and HSO_3^- concentrations after 24 hr of exposure at pH 7.0.

mainly in the form of polypeptides with lesser amounts as amino acids (14,64). Stewart (66), using $^{15}N_2$, demonstrated the transfer of combined nitrogen from blue-green algae to higher plants. In long term experiments Jones and Stewart (29) demonstrated that the extracellular combined nitrogen excreted by *Calothrix* can be utilized by algae, fungi, and bacteria.

Soil samples taken within a 50-mile radius of the Colstrip Electricity Generating Facility had large populations of soil blue-green algae. The following species have been cultured and were found to fix nitrogen: *Anabaena* sp., *Nostoc microscopium*, and *Tolypothrix ravenelii*. SO_2 forms H_2SO_4 (54) or dissolves directly in water to form H_2SO_3, whereas oxides of nitrogen become HNO_3 (37). The injection of SO_2 or NO_x into the atmosphere produces these strong acids which lower the pH value of rainwater below the carbonic acid-regulated pH of approximately 5.7 (2). Likens et al (37) reported annual pH values in rain which ranged between 3.91 and 4.03 in the Finger Lakes Region of New York State. In southwest Germany, anthropogenic SO_2 sources have markedly increased the acidity of rainwater (3). Recent decreases in rainwater pH over Sweden and Norway have been attributed to SO_2 from the British Isles and Central Europe (11).

In Sweden, acid rains are primarily due to acid sulfates from Great Britain and the Ruhr. Models generated for the Colstrip emission plumes predict pH values of 5.5 at the plume's margin and either pH 4.8 (units 1 and 2) or pH 4.2 (units 1–4) at the plume's center (17). The biological effects of acid rains are not well understood but these problems are currently under intensive study both in this country and in Europe.

Figure 3 shows the relationship of photosynthesis to nitrogen fixation in the soil inhabiting heterocystous blue-green algae *Anabaena* under various pH treatments. The purpose of these experiments was to evaluate the effect of acid rains caused by the oxidation of SO_2 to sulfuric acid on this nitrogen fixing soil organism.

Photosynthesis remained constant at pH 5.6 and then declined approximately 40% at pH 4.8. N_2 fixation was affected at all pH values employed but it was strongly inhibited between pH 5.6–4.8. At pH 4.8 photosynthesis was 40% inhibited while N_2 fixation was 95% reduced.

Table 4 shows the effect of sodium bisulfite treatments on *Anabaena*. The treatment time was 2 hr and the pH 5.3. The concentrations are reported as ppm HSO_3^- in aqueous solution and also as the atmospheric equivalent which is SO_2. The conversion calculations employed the formula SO_2 (w/w aqueous = 10.3 $\sqrt{\text{ppm } SO_2 \text{ v/v air}}$ [46]). Nitrogen fixation, as C_2H_4, decreased with increasing HSO_3 concentrations. The accepted annual average allowed for SO_2 by the State of Montana is 20 ppb with 200 ppb allowable for a 24 hr average 1% of the days or 250 ppb for 1 hr each 4 days.

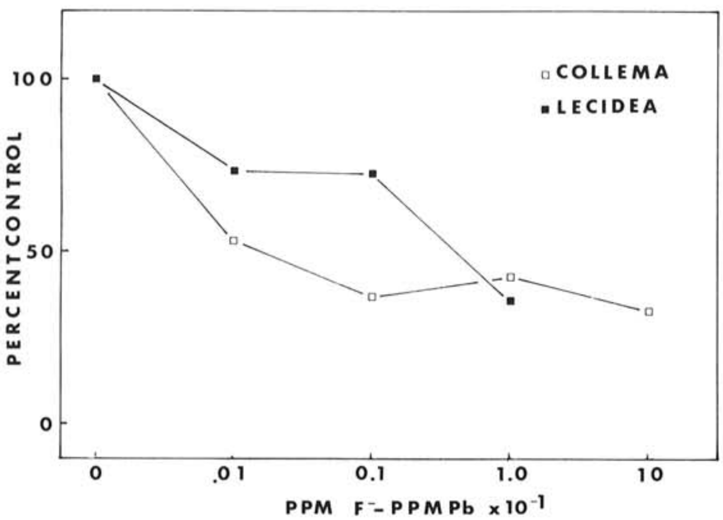

Fig. 5. Nitrogen fixation rates of the native terricolous lichens *Collema tenax* and *Lecidea* sp. after treatment for 24 hr with Pb-F mixture at pH 6.5.

Lichens.—Numerous reports recently have been published concerning the effect of air pollution on lichens. These reports are pertinent to the research reported here in that the Colstrip area has an extensive population of the nitrogen fixing terricolous lichens *Collema tenax* and *Lecidea* sp. It appears that the organisms most sensitive to air pollution are poikilohydric. The ability to absorb moisture as water vapor from the air exposes poikilohydric plants to the effects of gaseous pollutants. Homoiohydric plants, on the other hand, are insulated against water loss and these barriers to water loss help reduce the entrance of gaseous pollutants. Lichens, as poikilohydric organisms, are important in studies involving SO_2 pollution due to their sensitivity to this pollutant. Johnson and Søchting (28) and Hawksworth and Rose (22) arranged several lichen species according to their sensitivity to this gas. Tolerant species were reported from areas fumigated with SO_2 at concentrations of 150 mg/m^3 and sensitive species at approximately 40 mg/m^3. Skye (63) demonstrated damage to the lichen flora in the vicinity of an oil-shale works in Sweden. Similar SO_2 related damages have been reported near an iron-sintering industry in Ontario (49), a steel foundry (48), and a zinc foundry (44). Several reports have documented the paucity of certain lichen species in urban areas (22) and correlated the distribution of lichen species with the concentration of SO_2 (28). Newberry (45) found that fruticose lichen communities were affected by SO_2 from a sulfate-type paper mill within a radius of 21 km. Our studies of a Kraft-process pulp mill showed that the biomass of the sensitive lichen *Alectoria* was inversely related to the total sulfur content of the thallus and that a reduction in biomass could be measured over a distance of 16 km. The biomass reduction was severe enough to warrant declaring the area a "lichen desert" (55). The SO_2 effected a reduction in photosynthesis in lichens exposed to low SO_2 or HSO_3^- concentrations (25,55). The reduction of $C^{14}O_2$ uptake may be attributed to noncompetitive inhibition of the carboxylating enzyme ribulose 1,5-diphosphate carboxylase by SO_2 (74) or the ability of SO_3^- and HSO_3^- ions to behave as nonspecific electron acceptors thereby disrupting normal electron flow between photosystems-1 and -2 (56).

The effects of SO_2 on lichens and bryophytes have received attention because of the sensitivity of these poikilohydric plants to SO_2 (6,16,22,49,72). A "lichen desert" in south Wales resulted from steel mill SO_2 which caused morphological alterations and measureable changes in the species composition of the fumigated communities (48). Studies have begun in northern Sweden and Finland to determine the effects of SO_2 and H_2SO_4 on tundra lichens serving as primary food source of reindeer (31).

During the recent IBP study, Alexander (1) showed that soil lichens contribute between 135-349 mg N/m^2/year. The terricolous lichens *Collema tenax* and *Lecidea* sp. are ubiquitous throughout the northern Great Plains. These lichens form a significant ground cover, and in addition to their N_2 fixing ability, they strongly bind the soil surface.

TABLE 5. Concentrations (ppm) of water- and NH_4OAc-extractable copper, lead, zinc, and cadmium from soils near the Anaconda Smelter[a]

		Distance from smelter			
	Soil				
Metal	depth	0.5 mile		1.0 mile	
	(cm)	H_2O	NH_4OAc	H_2O	NH_4OAc
Cu	1-5	5.9	961	5.0	50.2
	6-10	3.7	548	1.0	45.2
Zn	1-5	13.4	1,080	33.9	62
	6-10	1.7	710	4.1	29
Cd	1-5	0.08	53	0.8	3.8
	6-10	0.08	44	0.8	3.3
Pb	1-5	5.0	168	5.0	2.2
	6-10	···	72	5.0	1.9

[a]Hartman (21).

The experiment shown in Fig. 4 involved treatment of the thallus of *Collema tenax* with solutions of $NaHSO_3$ adjusted to pH 7 in which the predominant ion is HSO_3^- (bisulfite). At the HSO_3^- concentration of 0.1 ppm, nitrogen fixation was reduced 50% and at 1.0 ppm it was 82% of the control.

Both *Collema* and *Lecidea* were treated with a mixture of Pb and F (5.3:1 ratio) which represents the particulate Pb-F from Colstrip Power Plants (Fig. 5). Both genera of soil lichen were inhibited at the lowest concentration of .01 ppm; *Collema* was more sensitive than *Lecidea*.

Angiosperms.—Heavy metals are present in all soils in varying concentrations. These metals are located at the ion exchange sites throughout the soil profile and they also are present as chelated compounds in the soil organic layer. The potential toxicity of soil metals cannot be determined simply by laboratory toxicity studies and compared to soil analysis. The soil metals were determined using water, acetic acid, and hydrochloric extraction techniques. The availability of the metal in the rhizosphere usually is considered to be between the concentration of water extractable and ammonium acetate extractable concentrations. However, the variables including the species tolerance, ecotype tolerance, rhizosphere and soil pH, and available soil water all interact with such complexity that only field studies using analyzed soils present data which appraise the permutations of soil-metal-plant interactions. Table 5 shows the concentrations of copper, zinc, and cadmium water-extracted from soils located near the Anaconda Smelter, Anaconda, Montana. The heavy metals of concern to us are Pb from both coal combustion and smelting (Tables 3, 4) and the Cu, Zn, Cd, Pb emitted by the smelting process (Table 5).

Lead enters plants via both the root and aerial organs and the metal is translocated from the roots to shoots in tomatoes, carrots, potatoes, corn, and lettuce (43). The high lead content of alfalfa leaves suggests that translocation also occurred in that forage crop (35). Lead accumulates in plant tissue and is retained by cell membranes, mitochondria, and the thylakoid membranes of chloroplasts (52). The lead in chloroplasts inhibited photosystem-2 (40) and in mitochondria this metal interfered with normal electron transport (34).

Cadmium enters both the root and aerial foliage (35) and it is readily translocated within the plant organs (53). The phytotoxicity of cadmium may be attributable to its action as a competitive inhibitor of enzymes requiring zinc as an activator (32). Cadmium has induced swelling in mitochondria and disruption of cell membranes (42).

Copper is a potent inhibitor of both amino and sulfhydryl-dependent enzymes (47).

Growth of hydroponically grown soybeans was retarded when treated with zinc at concentrations between 1 and 10 ppm.

Yellow sweet clover (*Melilotus officinalis*) is a European legume which has extensively colonized the Fort Union Basin. Figure 6 illustrates the effect of two heavy metals commonly found in soil (Table 5) near Anaconda, Montana. Threshold for toxicity was 0.01–0.1 ppm for both Cu and Zn, and aeration of the hydroponic solution increased phytotoxicity by 60%.

Alfalfa (cultivar Ranger) treated with cadmium at 100 ppm typically showed considerable leaf damage while treatments with the lower concentrations elicited no visible effects (Fig. 7). by contrast, nitrogen fixation was markedly reduced at concentrations greater than 0.1 ppm Cd. Copper treatment, on the other hand, produced no visual effects of phytotoxicity at 100 ppm, but nitrogen fixation was reduced to approximately 50% of the control at 1.0 ppm. The plants' morphological and nitrogen response to zinc were similar to those of copper.

Irrigation of nodulated roots with a solution containing fluoride or lead (Fig. 8) effected a reduction in N_2 fixation at concentrations greater than 1.0 ppm with Pb and 10 ppm for fluoride.

Soybeans are not an important agricultural plant in the northwestern USA but this legume is extensively cultivated in the mid- and southwestern states where the conversion of natural gas powered electricity-generating plants to coal is imminent. The fumigation of soybean plants with an SO_2 - air mixture for a period of 14 days resulted in a stimulation of nitrogen fixation at the

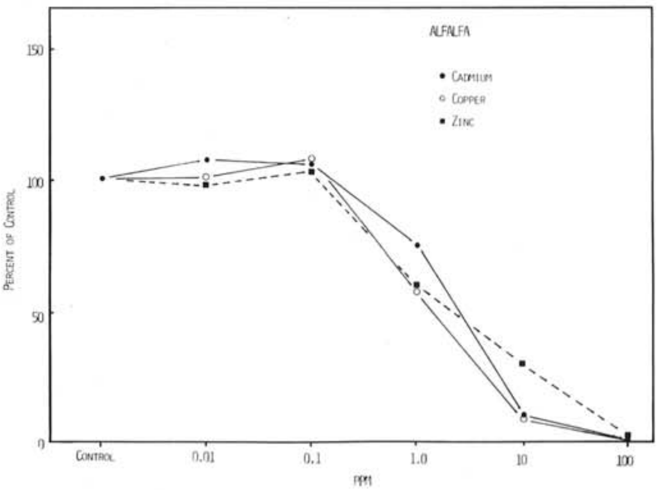

Fig. 7. Rates of nitrogen fixation (C_2H_4) by alfalfa cultivar Ranger after 72 hr treatment in the heavy metals shown (pH 6.5).

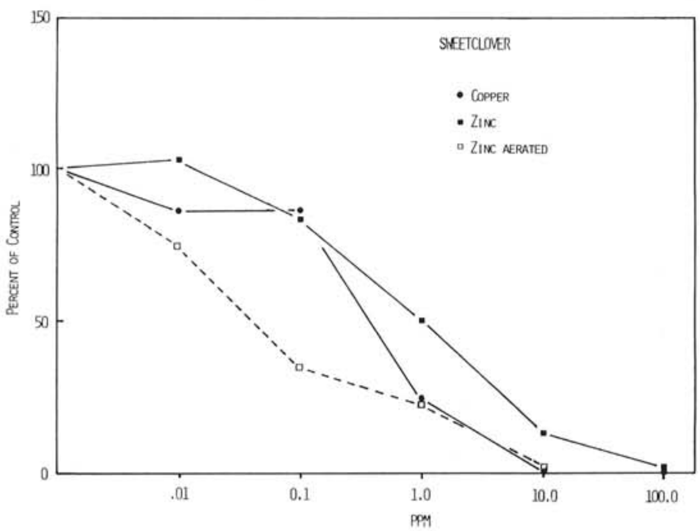

Fig. 6. Nitrogen fixation (C_2H_4) in *Melilotus officinalis* after treatment with Cu or Zn for 72 hr at pH 6.5.

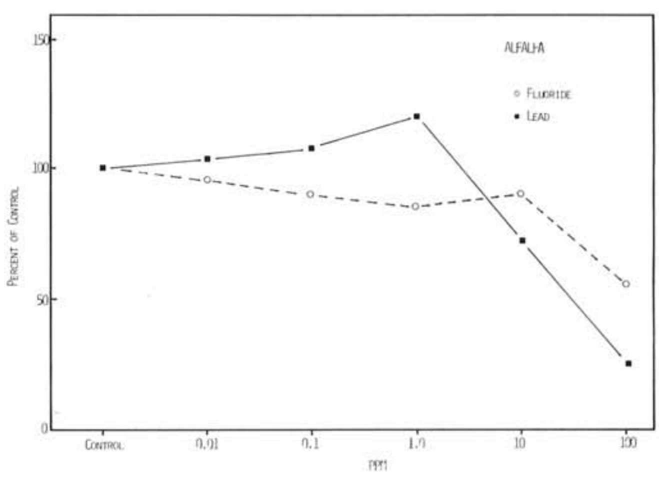

Fig. 8. Acetylene reduction after treatment for 72 hr with NaF (pH 6.5) or lead (pH 5.5) solutions.

lowest concentrations and inhibition at SO_2 concentrations of 50 ppb or higher (Fig. 9). Figure 10 presents data from experiments in which the roots of nodulated plants were treated for 24 hr with NaF at pH 7.0. Nitrogen fixation decreased with increasing fluoride concentrations. Soybeans grown in hydroponic solutions containing either zinc or cadmium first exhibited stimulated rates of N_2 fixation at the lower concentrations and inhibition at higher levels (Fig. 11).

CONCLUSIONS

The soil surface of the Great Plains' biome is extensively colonized by plants having either obligatory symbiotic diazotrophic organisms or are dependent on free-living diazotrophic organisms as a nitrogen source. The importance of diazotrophic organisms in this biome is due to the relatively low available nitrate (2–5 $\mu g/g$) present in the surface soils. Native diazotrophic angiosperms, blue-green soil algae and soil lichens with associated N_2-fixing blue-green algae are an important source of available nitrogen in these ranges and soils. Any deleterious effect by SO_2 or the associated active sulfur-containing molecular species resulting from coal combustion or smelter activity would be expected to effect a reduction in productivity by rangeland grass species and agricultural crops. The potential for the interaction of anthropogenic SO_2, Pb, and F with diazotrophic organisms recently has been made possible in the Colstrip Electrical Generating Facility 1 and 2. The construction of two additional generating facilities has been planned.

This paper presented some of the effects of anthropogenic sulfur compounds along with the phytotoxic elements in the stack emissions from coal-fired electrical facilities and smelters on diazotrophic processes. This is an important subject area for investigation at this time in view of the expanding development of coal-fired generating facilities within the Fort Union Basin and the generally low nitrogen levels of the soils in this region.

The Anaconda Company has operated various smelter facilities in the Deer Lodge Valley since 1884. Historical documents describe the area as choice grazing land with extensive timber reserves. Today, the nearby soils cannot support forest trees and only a sparse grass cover is evident. SO_2 damage to conifers is evident for 48.3 km (30 miles) north of the present smelter site.

Inhibition of nitrogen fixation by SO_2 or its oxidation products involves either a direct effect on nitrogenase or on photosynthesis. In either case, the result is an increased dependence on combined soil nitrogen for growth. Data presented in Fig. 3 for *Anabaena* suggest that nitrogenase is more sensitive to acid rain than is the photosynthetic mechanism. Because these soil cyanophytes grow only on the soil surface they, as well as the poikilohydric lichens, are exposed directly to SO_2 and H_2SO_4 without the benefit of an intervening soil structure as a buffer. Treatment of *Anabaena* with sodium bisulfite solutions at pH 5.3 effected a 30% reduction in N_2 fixation at 1 ppm HSO_3^- which is equivalent to 9 ppb SO_2 (Table 4). We have not yet conducted field studies to quantify the nitrogen contribution to the grassland ecosystem by soil cyanophytes. However, the literature indicates that the range determined for other sites is between 4.3 and 9,560 mg $N/m^2/yr$. The loss of any combined nitrogen in the already nitrogen-poor grassland soils would be expected to reduce yields of rangeland grasses. Similarly,

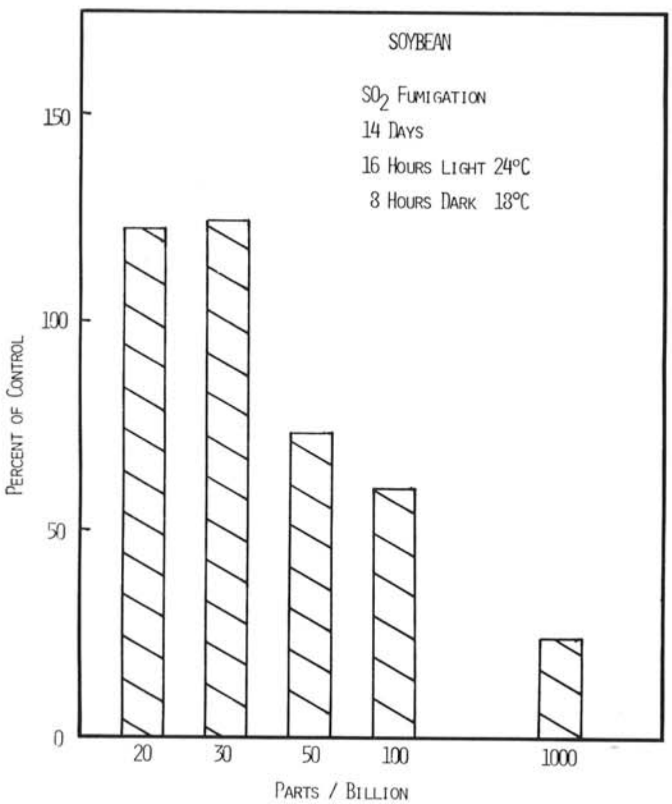

Fig. 9. Direct fumigation for 14 days of *Glycine max* 'Kent' with air-SO_2 mixture.

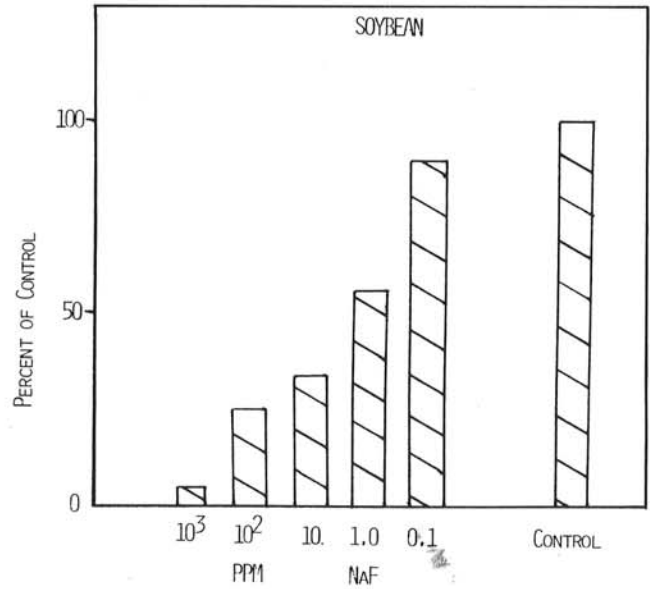

Fig. 10. Nitrogen fixation activity in soybean nodules exposed to NaF solution for 24 hr. Values are averages from the data of seven trials.

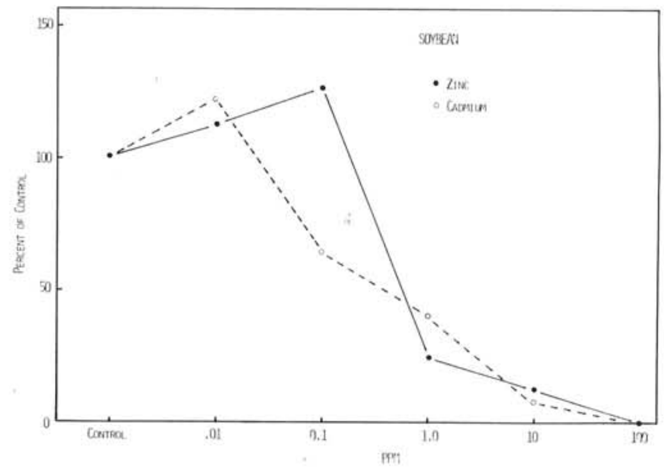

Fig. 11. Zn or Cd effects on nitrogen fixation in plants treated for 72 hr at pH 6.5.

the loss of the nitrogen contributions by the soil-binding terricolous lichens would be serious. The utility industries suggest that any loss of combined nitrogen derived from diazotrophs would be supplied via NO_x from stack emissions as the oxidation products of NO_x would be available to the plants. Recall that the nitrogenase activity is reduced by the addition of combined nitrogen. Our research into this area has just begun; however, the scenario, based on ecological principles, would predict that the diazotrophs would lose their physiological advantage and disappear from a system in which combined nitrogen was supplied by industrial emissions resulting in a dependence on anthropogenic nitrogen which is emitted along with other phytotoxicants. Should the anthropogenic source cease, the stage for a biological catastrophe is set with resulting desertification of the fumigated plains ecosystem.

Fumigation of soybean with SO_2 effected a reduction in nitrogen fixation at levels of 50 ppb or higher. These data, because they involved the fumigation of the aerial portion of the plant, indicate that soybeans are susceptible to SO_2 levels known to occur near coal-fired generating facilities.

It is difficult to extrapolate the laboratory data concerning the inhibitory effects of heavy metals on nitrogen fixation to field conditions. The well known chelating and buffering effects of soil plus the sensitivity of the various plant cultivars and ecotypes vary over such a wide range that each situation must be treated individually. It is clear that the heavily contaminated soils near Anaconda would be expected to effect a reduction in nitrogen fixation by diazotrophic legumes. However, direct field tests are necessary to confirm this hypothesis.

The sensitivity of nitrogen fixation to phytotoxic gaseous and particulate emissions from coal-fired electricity generating plants and smelters when viewed in the context of a rapidly expanding coal economy is a source of great concern and deserves increased attention from the research community.

LITERATURE CITED

1. ALEXANDER, V. 1974. A synthesis of the IBP tundra biome circumpolar study of nitrogen fixation. Pages 109-121 in: A. J. Holding, O. W. Heal, S. F. Maclean, Jr., and P. W. Flanagan,eds. Soil organisms and decomposition in tundra: proceedings of the microbiology, decomposition and invertebrate working groups meeting, U. of Alaska, Fairbanks, Alaska, USA, August 1973.
2. BARRETT, E., and G. BRODIN. 1955. The activity of Scandinavian precipitation. Tellus 7:251-257.
3. BOLIN, G., L. GRANAT, L. INGELSTAM, E. JOHANNESSON, S. ODEN, H. RODHE, and C. TAMM. 1971. Air pollution across national boundaries. The impact on the environment of sulfur in air and precipitation. Stockholm, Royal Ministry of Foreign Affairs, Royal Ministry of Agriculture. 96 pp.
4. BOTHE, H. 1972. Photochemical fixation of nitrogen by a cell-free extract from the blue-green algae *Anabaena cylindrica*. Proc. 2nd Int. Cong., Photos. Res., Stresa, Italy 3:2169-2178.
5. BOTHE, H., and E. LOOS. 1972. Effect of far-red light and inhibitors on nitrogen fixation and photosynthesis in the blue-green alga, *Anabaena cylindrica*. Arch. Mikrobiol. 86:241-254.
6. COKER, P. D. 1967. Effects of SO_2 pollution on bark epiphytes: Trans. Br. Bryol. Soc. 5:341-347.
7. COX, R. M. 1966. Physiological studies on nitrogen fixation in the blue-green alga *Anabaena cylindrica*. Arch. Mikrobiol. 53:263-276.
8. COX, R. M., and P. FAY. 1969. Special aspects of nitrogen fixation by blue-green algae. Proc. R. Soc. Lond. B. 172:357-366.
9. DONZE, M., H. J. Van GORKOM, and A. S. P. RAAT. 1971. Light-dark transients in nitrogen fixation by *Anabaena cylindrica*. J. Gen. Microbiol. 69:XIV.
10. EDDY, R. R., and S. R. POSTGATE. 1974. Nitrogenase. Nature (Lond.) 249:805-810.
11. ELGMORK, K. 1969. Aftenposten, March 17. (Quoted from D. R. Klein. 1971.) Reaction of reindeer to obstructions and disturbances. Science 173:393-398.
12. EVANS, H. J., and L. E. BARBER. 1977. Biological nitrogen fixation for food and fiber production. Science 197:332-334.
13. FAY, P. 1970. Photostimulation of nitrogen fixation in *Anabaena cylindrica*. Biochim. Biophys. Acta 216:353-356.
14. FOGG, G. E., and H. PATTNAIK. 1966. The release of extracellular nitrogenous products by *Westiellopsis prolifica*. Janet. Phycos 5:58-67.
15. FOGG, G. E., W. D. P. STEWART, P. FAY, and E. E. WALSBY. 1978. The blue-green algae. Academic Press, London.
16. GILBERT, O. L. 1970. A biological scale for the estimation of sulfur dioxide pollution. New Phytol. 69:629-634.
17. GORDON, C. C., C. E. CARLSON, and P. TOURANGEAU. 1976. A cooperative evaluation of potential air pollution injury and damage to coniferous habitats on national forest lands near Colstrip, Montana, USDA Forest Service, Northern Region, State and Private Forestry, Missoula, MT, Report No. 76-12.
18. GORDON, C. C., P. C. TOURANGEAU, and P. M. RICE. 1977. Investigation of the impact of coal-fired power plant emissions upon the disease/health/growth characteristics of ponderosa pine-skunkbush eco-systems and grassland ecosystems in southeastern Montana. Third Interim Report, University of Montana, Environmental Studies Laboratory, Missoula, MT.
19. HADFIELD, K. L., and W. A. BULEN. 1969. Adenosine triphosphate requirement of nitrogenase from *Azotobacter vinelandii*. Biochemistry 8:5103-5108.
20. HARDY, R. W. F., and V. D. HAVELKA. 1975. Nitrogen fixation research: A key to world food? Science 188:633-643.
21. HARTMAN, L. M. 1975. Fungal flora of the soil as conditioned by varying concentrations of heavy metals. Ph.D. thesis, University of Montana, Missoula.
22. HAWKSWORTH, D. L., and F. ROSE. 1970. Qualitative scale for estimating SO_2 air pollution in England and Wales using epiphytic bryophytes. Nature (Lond.) 227:145-148.
23. HENRIKSSON, E., L. E. HENRIKSSON, and B. PEJLER. 1972. Nitrogen fixation by blue-green algae on the island of Surtsey/Iceland. Surtsey Res. Prog. Rep. 6:66-68.
24. HENRIKSSON, L. E., P. H. ENCKELL, and E. HENRIKSSON. 1972. Determination of the nitrogen-fixing capacity of algae in soil. Oikos 23:420-423.
25. HILL, D. J. 1971. Experimental study of the effect of sulphite on lichens with reference to atmospheric pollution. New Phytol. 70:831-836.
26. JENSEN, T. E. 1968. Electron microscopy of polyphosphate bodies in a blue-green alga, *Nostoc pruniforme* Arch. Microbiol. 62:144-152.
27. JENSEN, T. E. 1969. Fine structure of developing polyphosphate bodies in a blue-green alga, *Plectonema borynanum*. Arch. Microbiol. 67:328-338.
28. JOHNSON, I., and V. SØCHTING. 1973. Influence of air pollution on the epiphytic lichen vegetation and bark properties of deciduous trees in the Copenhagen area. Oikos 24:344-351.
29. JONES, K., and W. D. P. STEWART. 1969. Nitrogen turnover in marine and brackish habitats. IV. Uptake of the extracellular products of the nitrogen-fixing alga *Calothrix scopulorum*. J. Marine Biol. Ass. U.K. 49:701-716.
30. KENNEDY, C., R. R. EADY, E. KONDROSI, and D. K. REKOSH. 1976. The molybdenum-iron protein of *Klebsiella pneumoniae* nitrogenase. Biochem. J. 155:383-389.
31. KLEIN, D. R. 1971. Reaction of reindeer to obstructions and disturbances. Science 173:393-398.
32. KLOTZ, I. M. 1954. Mechanism of enzyme action. Pages 257-285 in: W. D. McElroy and B. Glass, eds. A symposium on the mechanism of enzyme action. Johns Hopkins Press, Baltimore. 819 pp.
33. KOCH, B., H. J. EVANS, and S. RUSSELL. 1967. Reduction of acetylene and nitrogen gas by breis and cell-free extracts of soybean root nodules. Plant Physiol. 42:466-468.
34. KOEPPE, D. E., and R. J. MILLER. 1970. Lead effects on corn mitochondrial respiration. Science 167:1376-1379.
35. LAGERWERFF, I. V., W. H. ARMIGER, and A. W. SPECHT. 1973. Uptake of lead by alfalfa and corn from soil and air. Soil Sci. 115:455-459.
36. LEX, M., and W. D. P. STEWART. 1973. Algal nitrogenase, reductant pools and photosystem I activity. Biochim. Biophys. Acta 291:64-72.
37. LIKENS, G. E., F. H. BORMANN, and N. M. JOHNSON. 1972. Acid rain. Environment (St. Louis) 14(2):33-40.
38. LOWRY, O., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.
39. LYNE, R. L., and W. D. P. STEWART. 1972. Emerson enhancement of carbon fixation but not of acetylene reduction (nitrogenase activity) in *Anabaena cylindrica*. Planta 109:27-38.
40. MILES, C. D. 1972. Inhibition of photosystem II in isolated chloroplasts by lead. Plant Physiol. 49:820-825.
41. MILLBANK, J. W., and K. A. KERSHAW. 1969. Nitrogen metabolism in lichens. I. Nitrogen fixation in cephalodia of *Peltigera aphthosa*. New Phytol. 68:721-729.
42. MILLER, R. J., J. E. BITTELL, and D. E. KOEPPE. 1973.

The effect of cadmium on electron and energy transfer reactions in corn mitochondria. Plant Physiol. 28:166-171.

43. MOTTO, H. L., R. H. DAINES, D. M. CHILKO, and C. K. MOTTO. 1970. Lead in soils and plants: Its relationship to traffic volume and proximity to highways. Environ. Sci. Technol. 4:231-237.
44. NASH, T. H. III. 1972. Simplification of the Blue Mountain Community near a zinc factory. Bryologist 75:314-324.
45. NEWBERRY, G. 1974. The influence of a sulfate-process paper mill on corticolous lichens. Bryologist 77:561-576.
46. NIEBOER, E., R. D. TOMASSINI, K. J. PUCKETT, and D. H. S. RICHARDSON. 1977. A model for the relationship between gaseous and aqueous concentrations of sulphur dioxide in lichen exposure studies. New Phytol. 59:157-162.
47. OWENS, R. G. 1953. Studies on the nature of fungicidal action. I. Inhibition of sulfyhydryl-, amino-, iron-, and copper- dependent enzymes in vitro by fungicides and related compounds. Contrib. Boyce Thompson Inst. 17:221-242.
48. PYATT, F. B. 1970. Lichens as indicators of air pollution in a steel producing town in South Wales. Environ. Pollut. 1:45-56.
49. RAO, D. N., and F. L. LeBLANC. 1966. Effects of sulfur dioxide on the lichen alga, with special reference to chlorophyll. Bryologist 69:69-75.
50. RAO, D. N., and F. LeBLANC. 1967. Influence of an iron-sintering plant on corticolous epiphytes in Wawa, Ontario. Bryologist 70:141-157.
51. RIPPKA, R., F. NEILSON, R. KUNISAWA, and G. COHEN-BAZIRE. 1971. Nitrogen fixation by unicellular blue-green algae. Archiv. Mikrobiol. 76:341-348.
52. SABNIS, D. D., M. GORDON, and A. W. GALSTON. 1969. A site with affinity for heavy metals on the thylakoid membranes of chloroplasts. Plant Physiol. 44:1355-1363.
53. SCHROEDER, H. A., and J. J. BALASSA. 1961. Abnormal trace metals in man: Cadmium. J. Chronic Dis. 14:236:258.
54. SETHI, D. S. 1971. Photo-oxidation of sulfur dioxide. J. Air pollut. Control Assoc. 21:418-420.
55. SHERIDAN, R. P., and R. ROSENTRETER. 1973. The effect of hydrogen ion concentration in simulated rain on the moss *Tortula ruralis* (Hedw.) Sm. Bryologist 76:168-173.
56. SHERIDAN, R. P., C. SANDERSON, and R. KERR. 1976. Effects of pulpmill emissions on lichens in the Missoula Valley, Montana. Bryologist 79:248-252.
57. SIMON, R. D. 1971. Cyanophycean granules from the blue-green alga *Anabaena cylindrica*: a reserve material consisting of aspartic acid and arginine. Proc. Nat. Acad. Sci. USA 68:265-267.
58. SINGH, R. N. 1961. Role of blue-green algae in nitrogen economy of Indian agriculture. Publication, Indian Council of Agricultural Research, New Delhi. 175 pp.
59. SINGH, R. N., and P. K. SINGH. 1972. Transduction and lysogeny in blue-green algae. Pages 258-261 in: T. V. Desikachary, ed. Taxonomy and Biology of Blue-Green Algae. Madras University, Madras, India.
60. SINGH, R. N., S. P. SINGH, and P. K. SINGH. 1972. Genetic regulation of nitrogen fixation in blue-green algae. Pages 264-268 in: T. V. Desikachary, ed. Taxonomy and Biology of Blue-Green Algae. Madras University, Madras, India.
61. SINGH, R. N., D. N. TIWARI, and V. P. SINGH. 1972. Pages 27-37 in: T. V. Desikachary, ed. Taxonomy and Biology of Blue-Green Algae. Madras University, Madras, India.
62. SKINNER, K. J. 1976. Nitrogen fixation. Chem. Eng. News 54:22-35.
63. SKYE, E. 1968. Lichens and air pollution. Acta Phytogeogr. Suec. 52:1-123.
64. STEWART, W. D. P. 1963. Liberation of extracellular nitrogen by two nitrogen fixing blue-green algae. Nature (Lond.) 200:1020-1021.
65. STEWART, W. D. P. 1966. Nitrogen Fixation in Plants. Athione Press, London, 168 pp.
66. STEWART, W. D. P. 1967. Transfer of biologically fixed nitrogen in a sand dune slack region. Nature (Lond.) 214:603-604.
67. STEWART, W. D. P. 1968. Acetylene reduction by nitrogen-fixing blue-green algae. Arch. Microbiol. 62:336-348.
68. STEWART, W. D. P. 1972. XVI.—Algal metabolism and water pollution in the Tay region. Proc. R. Soc. Edinb. (B) 71:209-224.
69. STEWART, W. D. P. 1973. Nitrogen fixation by photosynthetic microorganisms. Annu. Rev. Microbiol. 27:283-316.
70. STEWART, W. D. P., G. P. FITZGERALD, and R. H. BURRIS. 1967. In situ studies on N_2 fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci., USA 58:2071-2078.
71. STEWART, W. D. P., A. HAYSTEAD, and H. W. PEARSON. 1969. Nitrogenase activity in heterocysts of blue-green algae. Nature (Lond.) 224:226-228.
72. SYRATT, W. J., and P. J. WANSTALL. 1969. The effect of SO_2 on epiphytic bryophytes. Pages 79-85 in: Air Pollution Proceedings of First European Congress on the Influence of Air Pollution on Plants and Animals. Wageningen, The Netherlands.
73. THORNELEY, R. N. F., R. R. EADY, and M. G. YATES. 1975. Nitrogenase of *Klebsiella pneumoniae* and *Azotobacter chroococcum*—Complex formation between the component proteins. Biochim. Biophys. Acta 403:269-284.
74. ZIEGLER, I. 1972. The effect of SO_3^- on the activity of ribulose-1,5,-diphosphate carboxylase in isolated spinach leaves. Planta (Berl.) 103:155-163.