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Studies on the Activity of Superoxide Dismutase in *Miscanthus floridulus* Grown near a Copper Smelter in Taiwan

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ABSTRACT: A simple gradient transect of vegetation analysis was used to study the effect of the pollution caused by a copper smelter in northern Taiwan on the development of vegetation in the area. A clear coverage gradient on the vegetation was found near Liiyueh copper smelter after ten years of operation, and an apparent stress gradient was evident in a series of plots in the vicinity on the basis of species diversity and frequency of vegetation found. *Miscanthus floridulus* was a good indicator plant to gauge the vegetation's response to the stress gradient, and its isozyme pattern of superoxide dismutase was studied in connection with this stress gradient. Four MnSODs and five CuZnSODs were present in the leaves of *Miscanthus floridulus*. Its SOD activity, and particularly its MnSODs, showed obvious accumulation in the stress area. MnSOD of *Miscanthus floridulus* as a biochemical indicator for copper smelter pollution is discussed.

KEY WORDS: Copper smelter pollution, *Miscanthus floridulus*, Superoxide dismutase (SOD).

INTRODUCTION

Rapid industrialization has caused severe environmental pollution problems in some countries, which has resulted in damage to native vegetation (Dassler and Bortitz, 1988). Vegetation analysis often provides useful information for understanding the population change of native plants growing in polluted areas (Shimwell, 1972). Copper smelter pollution frequently puts severe stress on the natural environment with the air pollution and high copper levels generated by the smelter, which forms a stress gradient relative to distance from the smelter (Rebele *et al.*, 1993). A strong local acidification gradient was reported in a birch forest near to a copper smelter in northern Norway (Lobersli and Steinnes, 1988).

Superoxide dismutase (EC 1.15.1.1; SOD) is a group of metalloenzymes, and three types of SOD contain either Mn, Fe or Cu plus Zn as prosthetic metals. SOD is present in all aerobic plants (Bowler *et al.*, 1994). Selective inhibition of SOD by potassium cyanide and H_2O_2 makes it possible to distinguish the three types of SOD in the crude homogenates of any plant after separation by electrophoresis (Droillard *et al.*, 1989). SOD catalyzes the dismutation of superoxide radicals ($O_2^{\cdot -}$) to form molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), and is considered as the major enzymatic defense against oxygen free

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Abbreviations: SOD, superoxide dismutase; PAGE, polyacrylamide gel electrophoresis.

radicals (Fridovich, 1975). The unstable $O_2 \cdot^-$ is generated in biological systems by several pathways (Fridovich, 1978; Halliwell, 1987). And a large quantity of $O_2 \cdot^-$ was proposed to form in the cells under stress conditions (Scandalios, 1992). Several transgenic plants having high SOD activity were reported to have high tolerance for environmental stresses (Allen, 1995).

SOD and peroxidase have been studied in spruce (Polle *et al.*, 1993), *Arabidopsis* (Sharma and Davis, 1994) and *Schima superba* (Sheu, 1994) to determine the effect of ozone and sulphur dioxide pollution on them. These antioxidant enzymes have been suggested as indicators in grain crops, spruce, and pine subjected to the various pollutants such as heavy metals (Nashikkar and Chakrabarti, 1994), sulphur dioxide (Polle *et al.*, 1994), and ozone (Polle *et al.*, 1993). However, the effect of the environmental stress factors on the local vegetation of any place and on the specific isozymes of antioxidant of the indicator plant has not been reported.

The object of this study was to determine the feasibility of using the Liiyueh copper smelter in Taiwan as a model system to study the antioxidant responses in *Miscanthus floridulus,* in order to assess the specific isozyme as an indicator in response to environmental pollution. As a first step toward this goal, we analyzed vegetation close to the Liiyueh copper smelter and characterized SOD and peroxidase in *M. floridulus.*

MATERIALS AND METHODS

Liiyueh copper smelter and study area

The Liiyueh copper smelter is located on the west side of a hill named Tzyygehshan in northern Taiwan. The smelter was opened in 1981 (Lin, 1983) and remained in operation until 1992.

Vegetation survey

A simple gradient transect was chosen to determine the direct gradient analysis in this experiment (Whittaker, 1967). Ten plots at intervals of 50 m were used for analyzing the vegetation at progressively greater distances from the copper smelter through an observable environmental gradient. The area of each plot was about 25 m^2 was divided into 20 squares each 0.1 m^2 according to the method of the Danish School (Shimwell, 1972). This study was carried out from 1989 to 1991, which was the last two years of smelter in operation (Wei, 1991). We used frequency and coverage as ecological parameters to analyze the vegetation. Only vascular plants were scored, and the score of each species in a given square was either none or all. The frequency of each species on one plot was also calculated. Coverage of a plot was defined as the percentage of the selected twenty squares occupied by any vascular plant.

Materials for enzyme study

M. floridulus was selected as the indicator plant for the vegetation. Young, healthy plants about 25 to 30 cm in height were collected for enzyme analysis. A plot 300 m away from the smelter was selected and designated as L1, and the other nine plots, L2 to L10, were marked off at 50 m intervals from it moving away from the smelter. The first experiment involved taking five individual plants as one sample from each spot on plots L1 to L8. The second experiment took samples from three different spots of each plot from L2 to L6, each sample contained five inviduals. The collected samples were stored in a handy - freezer and brought back to the laboratory. The middle region area of blades, sheaths and roots were dissected and frozen separately with liquid nitrogen, then stored at –20 °C until used.

Extraction for enzyme assay

Tissues were ground with two volumes of grinding medium containing 150 mM Tris-HCl (pH 7.2), 10% polyvinylpyrrolidone and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 10,000 xg for 10 min at 4 °C. The supernatant was used for isozyme analysis by PAGE (Pan and Yau, 1991).

Electrophoresis

Analytical PAGE (10 or 15%) was performed at 4 °C using a Hoefer mini slab gel unit. Electrophoresis was conducted with a 1.5 mm native gel in 50 mM Tris-glycine buffer, pH 8.3. Appropriate amount of sample extract equivalent to the equal fresh weight about 1.25 mg for all samples was applied to a slot, and electrophoresis was conducted for 20 min at 80 V for stacking gel, and then continued about 1 h at 120 V for running gel (Juang, 1985).

SOD activity staining

Immediately after electrophoresis, the SOD activity was identified by incubating the gel in 2.45 mM nitroblue tetrazolium solution for 15 min and then soaking in 100 mM potassium phosphate (pH 7.0) containing 0.028 mM riboflavin and 28 mM N,N,N',N'-tetramethylethylenediamine for another 15 min in the dark. The gel was illuminated for about 20 min to insure photo-reaction in staining the SOD activity. To identify CuZnSOD activity, 2 mM KCN was included in the riboflavin solution for activity staining. To identify MnSOD activity, the gels were first soaked in 100 mM potassium phosphate (pH 7.0) containing 3 mM H_2O_2 and 0.5 mM EDTA for 30 min, then followed by SOD activity staining (Beauchamp and Fridovich, 1971).

Peroxidase activity staining

Immediately after electrophoresis, the peroxidases were stained by incubating the gel in 100 mM potassium phosphate (pH 7.0) containing 0.266 mM 3,3'-diaminobenzidine and 1.65 mM H_2O_2 for about 5 h at room temperature in the dark (Juang, 1985).

RESULTS

Liiyueh copper smelter and study area

The Liiyueh copper smelter is located on the west side of a hill named Tzyygehshan in northern Taiwan (Fig. 1). It is in a valley close to the East China Sea with the north side facing the sea. From September to February, a north or northeast wind prevails, which made air quality in the local area poor and heavily contaminated with pollutants. Due to the waste from processing and refining, the smelter was the largest source of local SO_2 and Cu, released as heavy metals pollution (Jaw, 1970).

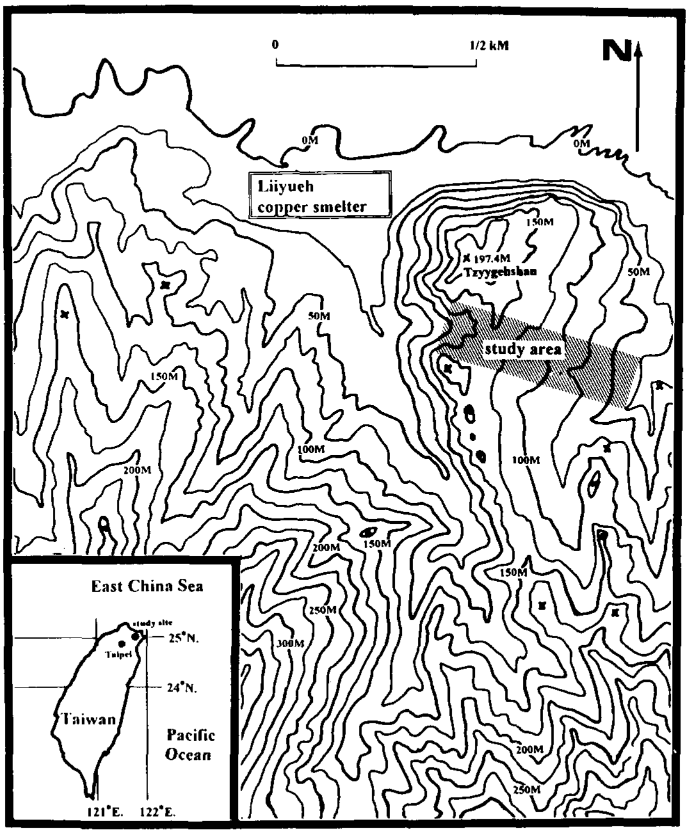

Fig. 1. The geographic diagram around the Liiyueh copper smelter. The smelter located on the west side of Tzyygehshan is surrounded by several hills except on the north side. The study area (25°07'15"N, 121°52'28"E) on the southern hillside of Tzyygehshan is about 500 m long and 80 m wide.

Composition of the vegetation

The hillside of Tzyygehshan is covered with a grassland vegetation, dominant among which is a community of *Miscanthus-Histiopteris,* and there are also some unidentified dead tree trunks. *Miscanthus floridulus* and *Histiopteris incisa* were the two major vascular plants found at the experimental sites. Three tree species and one liana species were found (Table 1). These four species were commonly found in subtropical rain forests in northern Taiwan (Lai, 1978), so it is quite possible that the study site was originally deforested as a result of pollution from the smelter, and that the vegetation found in the area under study was secondary vegetation. A similar observation was made near other copper smelters in Canada (Wood and Nash, 1976).

Vegetation with stress gradient

The experimental plots showed a gradient of vegetation development with one to three species of plants found from L1 to L7, and four to six species of plants from L8 to L10. *M. floridulus* and *H. incisa* were the only two plant species from L1 to L4 (Table 1). Coverage from L1 to L4 was less than 10%, and there was a gradient increase in coverage as distance from the smelter increased. The coverage was 35% at L5 and L6, and over 55% from L7 to

L10 (Table 2). Species diversity for the area, another important ecological indicator, was derived from the composition data collected, and was found to be consistent with the coverage data, as demonstrated by the gradient increase in both the kinds of species found and the frequency of their appearing when moving from L5 to L10. Based on the diversity change and coverage gradient of the vegetation, a pollution gradient was found to generate from the series of experimental plots.

Table 1. Composition of plant species in the plots located at the Liiyueh copper smelter.

	Species										
Plot No.	CA	CC	HI	LJ	MC	MF	PL	PT	SC	SP	TCF
L1			+			+					
L2			+			+					
L3						+					
L4			+			+					
L5			+			+	+				
L6		+		+							
L7			+			+					
L8			+			+		+	+		
L9			+	+	+	+			+		
L10	+	+	+			+			+	+	

Plots L1 to L10 represent the 10 different plots with an internal of 50 m between each. The distance of L1 from the Liiyueh copper smelter is about 300 m. CA: *Centella asiatica* (L.) Urban; CC: *Clerodendrum cyrtophyllum* Turcz.; HI: *Histiopteris incisa* (Thunb.) J. Sm.; LJ: *Lygodium japonicum* (Thunb.) Sw.; MC: *Melastoma candidum* D. Don; MF: *Miscanthus floridulus* (Labill.) Warb. ex Schum. & Laut.; PL: *Pithecellobium lucidum* Benth.; PT: *Persea thunbergii* (Sieb. & Zucc.) Kostermans; SC: *Smilax china* L.; SP: *Symplocos paniculata* (Thunb.) Miq.; TCF: *Torenia concolor* Lindley var. *formosana* Yamazaki. PL, PT and SP are tree species. CC and MC are shrub species. SC is liana species. MF is tall grass species. CA and TCF are short grass species. HI and LJ are fern species.

+: denotes the plot having the indicated species.

Indicator plant for the stress gradient

M. floridulus and *H. incisa* were two major vascular plant species found in the experimental area, and formed a *Miscanthus-Histiopteris* co-dominant grassland. In general, there was a frequency gradient in which *H. incisa* increased from L2 to L10 (Table 2). This species clearly demonstrated its superiority over other plants in growing under heavy copper pollution conditions. It was previously reported that an unidentified fern — presumably *H. incisa*—was often found to be co-present with copper ore (Tan, 1971), this is consistent with this study.

M. floridulus, the other dominant species, was classified into three stages based on the height of the plant: seedling stage, young stage and mature stage. The vegetation coverage was below 10% in the heavily polluted area, which was from L1 to L4. Frequency of the mature stage of *M. floridulus* was under 1% at L1 and L2, but increased to 10% and 15% at L3 and L4 respectively, and then gradually increased from L5 to L10, indicating a clear gradient change in the vegetation. Frequency of the young stage at L1 and L2 was under 1%, but increased to 10% and 25% at L3 and L4, respectively, a pattern quite similar to the mature stage. However, the frequency of the seedling stage at L1 and L2 was over 40%, dropping to 20% at L3 and L4 (Table 2). These results indicated that the seeds of *M. floridulus* could germinate and grow temporarily, as reported previously (Hsu and Chou, 1992), but that not many of the seedlings were able to grow to maturity, presumably because of the cumulative effect of heavy pollution from the copper smelter. Based on these studies, *M. floridulus* was taken as the indicator species for enzyme analysis because it demonstrated a clear response to the gradient of pollution levels.

Table 2. Vegetation analysis of study area beside the Liiyueh copper smelter.

	Frequency of					Sum frequency	Coverage of
		Miscanthus		Histiopteris		of the other	each
Plot No.	seedling	young	mature	young	mature	species	plot
L1	40	+	+	+	10	+	10
L2	45	+	+	+	+	+	+
L3	20	10	10	+	+	+	+
L4	20	25	15	+	5	+	+
L5	10	15	55	5	20	5	35
L6	10	45	40	5	35	+	35
L7	15	20	50	5	40	+	60
L8	10	10	60	0	65	20	55
L9	5	15	70	10	55	25	65
L10	5	5	65	5	75	50	90

Plots L1 to L10 represent the 10 different plots with an internal of 50 m between each. The distance of L1 from the Liiyueh copper smelter is about 300 m.
Unit used in the table is %. When the percentage of certain item is under 1% we mark it as +.
Frequency of certain plant species at specific stage is the percentage of the presence in the selected 20 squares in one plot. Coverage of a plot is defined as the percentage of the selected 20 squares occupied by any vascular plants. *Miscanthus floridulus* (Labill.) Warb. ex Schum. & Laut. was classified into three stages: below 2cm as seedling stage, 2 to 30cm as young stage and above 30cm as mature stage. *Histiopteris incisa* (Thunb.) J. Sm. was classified into two stages: below 30cm as young stage and above 30cm as mature stage.

SOD isozyme pattern

M. floridulus was divided into three parts: blade, sheath and root, and the extracts from different tissues revealed eight bands of SOD activity on 10% native gels. When the gel was treated with H_2O_2 or KCN to indentify SOD isozymes, the result indicated that *M. floridulus* has four MnSOD isozymes, namely MnSODI, II, III and IV, and five CuZnSOD isozymes, I, II, III, IV and V, based on their mobilities from the cathode to anode (Fig. 2). A similar SOD pattern was observed for blades, sheaths and roots. Leaf blades contained a higher SOD activity level than that of either the sheaths or roots on a per fresh weight basis, a situation similar to that of rice (Pan and Yau, 1991).

Enzyme activity change for the series of plots

The first experiment on the isozymes of SOD and peroxidase in the blades of *M. floridulus* showed higher CuZnSODIII and IV activity closer to the smelter at plots L1, L2, and L3, and lower activity farther away from the smelter (Fig. 2). Peroxidase with similar isozyme pattern in the blades of *M. floridulus* in the series of plots showed activity changes (data not shown). The second experiment, which consisted of three independent samplings from L2 to L6, found that L2 had the highest activity level for MnSODI and II, with the activity decreasing at L3 to L4, and not appearing at all for samples collected at L5 and L6 (Fig. 3). The activity level of other SOD isozymes also exhibited changes, but did not display the similar trend seen with MnSODI and II (Fig. 3). The results showed a gradient change in the activity of MnSODI and II in the blades of *M. floridulus* that were collected from the series of plots. Another study using samples of *M. floridulus* collected from a field near the Taiwan Metal Mining Corporation, which is heavily polluted and is located in an old copper mining area in northern Taiwan.We found that the activity of MnSODI and II in the roots of *M. floridulus* increased several-fold in highly polluted areas, and MnSOD activity was far greater than CuZnSOD activity, although CuZnSODII activity showed a slight increase (Fig. 4A and B). In addition, a minor MnSODIII activity present on 15% gel displayed marked changes that corresponded to different pollution levels (Fig. 4B). Thus, MnSODs of *M. floridulus* appear to be more susceptible to environmental change than other SOD isozymes, and as such, may serve as effective markers for pollution pressure.

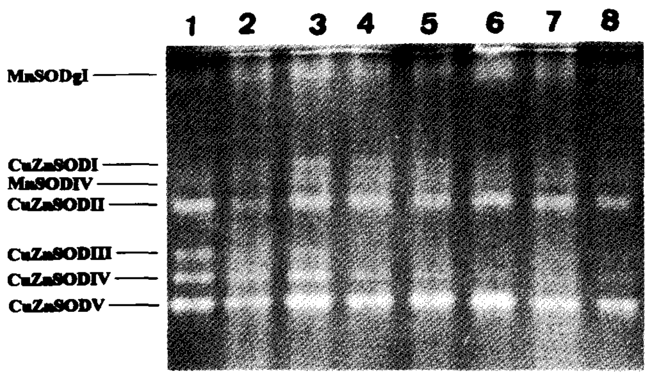

Fig. 2. The isozyme pattern of SOD in the leaves of *Miscanthus floridulus* collected at different plots. SOD isozymes were separated in 15% gel. Lane 1, 2, 3, 4, 5, 6, 7 and 8 represent the samples taken from plots L1 to L8. Each sample contained the same amount of fresh weight. MnSODgI indicated the activity including MnSODI and MnSODII.

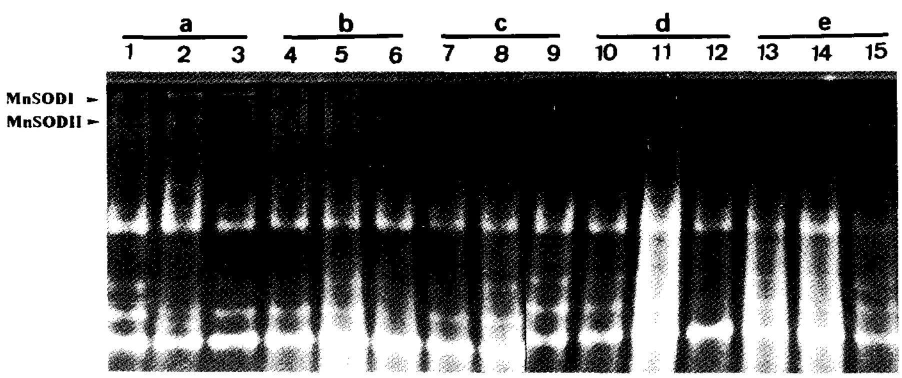

Fig. 3. The isozyme pattern of SOD in the leaves of *Miscanthus floridulus* collected at different plots. Three individual samples were taken from each plot; a, b, c, d and e represent the samples taken from plots L2 to L6 respectively. Each sample was extracted as the text described, then the extracts were run in a 10% gel. Each sample contained the same amount of fresh weight.

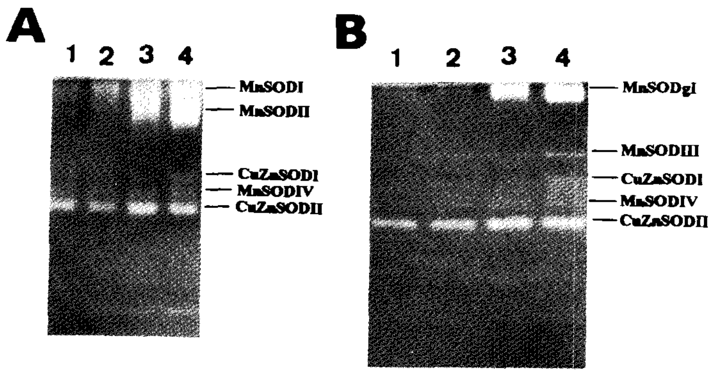

Fig. 4. The isozyme pattern of SOD in the roots of *Miscanthus floridulus* collected from the sites near Taiwan Metal Mining Corporation. A: 10% gel. B: 15% gel. Sampling sites are about 200 m distance from the factory. Lanes 1, 2 and 3 represent three sampling sites were in the middle region of the polluted area, which showed a *Miscanthus-Histiopteris* co-dominant grassland; lane 4 represents the sample taken from a site which was heavily polluted by copper and other metals, with only a few plants surviving. Each sample contained the same amount of fresh weight. MnSODgI indicated the activity including MnSODI and MnSODII.

DISCUSSION

Several lichens have been selected as indicators for testing air pollution in temperate countries, because of their susceptibility to pollution (Lawrey, 1993). *M. floridulus*, a plant with pioneer and euryecious characteristics (Hsu, 1978), is known as a dominant species of secondary succession in most grasslands at low elevation in Taiwan (Chou and Chen, 1990). It has been reported to have a high tolerance for some heavy metals (Hsu and Chou, 1992). In this report, vegetation analysis revealed a stress gradient made evident by the species diversity and coverage of vegetation in the area. *M. floridulus*, one of the co-dominant plant species in this vicinity, was selected as an indicator plant for researching the stress gradient.

In this study, *M. floridulus,* which is taken as an indicator plant of copper smelter pollution, possesses two important characteristics. First, it is able to grow in the whole area of varying stress levels generated by the copper smelter. And second, the change in MnSOD activity of *M. floridulus* possibly reflects the pollution level in the vegetation as a whole. Although the air pollutants in this area was not measured, the major pollutants in copper smelters was collected and analyzed as SO_2 (Sutherland and Martin, 1990; Lukina and Nikonov, 1995). The increased activity of MnSOD may reflect the high activity of mitochondria to generate energy to resist the stressful environment (Bowler *et al.*, 1989). In other reports on the environmental impact of pollution on vegetation, various indicator plants showed accumulation of specific amino acids, such as glutamate, arginine, aspartate and glutamine (McLaughlin *et al.,* 1994), and S-containing moleculars, such as glutathione, cysteine, and glutamylcysteine concentrations in the leaves (Polle *et al.*, 1994).

The use of peroxidase or SOD activity as a biochemical indicator for pollution measurement has previously been assessed (Keller, 1974; Klumpp *et al.*, 1989). Keller (1974) found that the activity of peroxidase could be an indicator of invisible injury to plants caused by air pollution. But, the controversial results showed that SOD activity in spruce and pine did not always increase in response to rising ozone levels or SO_2-mediated stress (Polle and Rennenberg, 1991; Polle *et al.*, 1994). Despite these inconsistent results, antioxidant enzymes may still be considered as reliable pollutant bioindicators if a gradient of vegetation with a suitable indicator plant in the field can be found. This report shows that although all the SOD isozymes in *M. floridulus* changed in response to the local environmental stress, however, if only total SOD activity has been measured, the significant changes in MnSODs activity would quite possibly have been missed. These results, therefore, show that the activity of MnSOD in *M. floridulus* grown near a copper smelter is more susceptible to environmental change, it may serve as an effective marker for stress pressure. Although since SODs are modulated by many different factors, changes in SOD can not be considered as specific markers for copper smelter.

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LITERATURE CITED

Allen, R. 1995. Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. **107**: 1049-1054.

Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. Anal. Biochem. **44**: 276-287.

Bowler, C., T. Alliotte, M. De Loose, M. Van Montagu and D. Inz'e. 1989. The induction of manganese superoxide dismutase in response to stress in *Nicotiana plumbaginifolia*. EMBO J. **8**: 31-38.

Bowler, C., W. Van Camp, M. Van Montagu and D. Inz'e. 1994. Superoxide dismutase in plants. Crit. Rev. Plant Sci. **13**: 199-218.

Chou, C.-H. and Y.-Y. Chen. 1990. Population study of *Miscanthus floridulus*: III. Population variation of *Miscanthus floridulus* in green and orchid islets of Taiwan. Bot. Bull. Acad. Sin. **31**: 223-234.

Dassler, H. G. and S. Bortitz. 1988. Air pollution and its influence on vegetation: Cause, effects, prophylaxis and therapy. Junk Publishers, Dordrecht, DRW, 223 pp.

Droillard, M. J., D. Burrreau, A. Paulin and J. Daussant. 1989. Identification of different classes of superoxide dismutase in carnation petals. Electrophoresis **10**: 46-48.

Fridovich, I. 1975. Superoxide dismutase. Annu. Rev. Biochem. **44**: 147-159.

Fridovich, I. 1978. The biology of oxygen radicals. Science **201**: 875-880.

Halliwell, B. 1987. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplast. Chem. Phys. Lipids **44**: 327-340.

Hsu, C.-C. 1978. Gramineae. In: Li, H.-L., T.-S. Liu, T.-C. Huang, T. Koyama and C. E. Devol. (eds.), Flora of Taiwan. pp. 373-783. Epoch Publishing Co., Taipei.

Hsu, F.-H. and C.-H. Chou. 1992. Inhibitory effects of heavy metals on seed germination and seedling growth of *Miscanthus* species. Bot. Bull. Acad. Sin. **33**: 335-342.

Jaw, T.-H. 1970. What environmental pollutions are caused by copper smelter? Mining & Metallurgy **14**: 76-83.

Juang, R.-H. 1985. The study of sucrose synthetase in rice. Purification, biochemical and immunological study. PhD thesis, National Taiwan University. Taipei.

Keller, T. 1974. The use of peroxidase activity for monitoring and mapping air pollution areas. Eur. J. For. Path. **4**: 11-19.

Klumpp, G., R. Guderian and K. Kueppers. 1989. Peroxidase activity, superoxide dismutase activity and proline contents of spruce needles fumigated with ozone, sulfur dioxide and nitrogen peroxide. Eur. J. For. Path. **19**: 84-97.

Lai, M.-J. 1978. A botanical survey of the flora of Taiwan. Q. J. Chinese For. **11**: 57-66.

Lawrey, J. D. 1993. Lichens as monitors of pollutant elements at permanent sites in Maryland and Virgina. Bryologist **96**: 339-341.

Lobersli, E. M. and E. Steinnes. 1988. Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water Air Soil Pollut. **37**: 25-40.

Lukina, N. and V. Nikonov. 1995. Acidity of podzolic soils subjected to sulphur pollution near a Cu-Ni smelter at the Kola Peninsula. Water Air Soil Pollut. **85**: 1052-1062.

McLaughlin, J. W., D. D. Reed, S. T. Bagley, M. F. Jurgensen and G. D. Mroz. 1994. Foliar amino acid accumulation as a indicator of ecosystem stress for first-year sugar maple seedlings. J. Environ. Qual. **23**: 154-161.

Nashikkar, V. J. and T. Chakrabarti. 1994. Catalase and peroxidase activity in plants: An indicator of heavy metal toxicity. Indian J. Exp. Biol. **32**: 520-521.

Pan, S.-M. and Y.-Y. Yau. 1991. The isozymes of superoxide dismutase in rice. Bot. Bull. Acad. Sin. **32**: 253-258.

Polle, A. and H. Rennenberg. 1991. Superoxide dismutase activity in needles of Scots pine and Norway spruce under field and chamber conditions: Lack of ozone effects. New Phytol. **117**: 335-344.

Polle, A., T. Pfirrmann, S. Chakrabarti and H. Rennenber. 1993. The effects of enhanced ozone and enhanced carbon dioxide concentrations on biomass, pigments and antioxidative enzymes in spruce needles (*Picea abies* L.). Plant Cell Environ. **16**: 311-316.

Polle, A., M. Eiblmeier and H. Rennenberg. 1994. Sulphate and antioxidants in needles of Scots pine (*Pinus sylvestris* L.) from three SO_2-polluted field sites in eastern Germany. New Phytol. **127**: 571-577.

Rebele, F., A. Surma, C. Kuznik, R. Bornkamm and T. Brej. 1993. Heavy metal contamination of spontaneous vegetation and soil around the copper smelter "Legnica". Acta Societatis Botanicorum Poloniae **62**: 53-57.

Scandalios, J. G. 1992. Molecular Biology of Free Radical Scavenging Systems. Cold Spring Harbor Laboratory Press, Plainview, New York, 284 pp.

Sharma, Y. K. and K. R. Davis. 1994. Ozone-induced expression of stress-related genes in *Arabidopsis thaliana*. Plant Physiol. **105**: 1089-1096.

Sheu, B. H. 1994. Effects of sulfur dioxide on growth, photosynthesis and enzyme activities of Chinese Guger-Tree seedlings. Environ. Pollut. **86**: 349-354.

Shimwell, D. W. 1972. The Description and Classification of Vegetation. University of Washington Press, Seattle, 322 pp.

Sutherland, E. K. and B. Martin. 1990. Growth response of *Pseudotsuga menziesii* to air pollution from copper smelting. Can. J. Foro Res. **20**: 1020-1030.

Tan, L.-P. 1971. Characteristic and exploration method in fern-type copper deposits. Mining & Metallurgy **15**: 69-72.

Wei, D.-S. 1991. The study on the feasibility of superoxide dismutase or peroxidase of *Miscanthus floridulus* as a marker enzyme of the environmental pollution. Master thesis. National Taiwan University. Taipei.

Whittaker, R. H. 1967. Gradient analysis of vegetation. Biol. Rev. Cambridge Philosophical Soc. **42**: 207-264.

Wood, C. W. and T. N. Nash. 1976. Copper smelter effluent effects on Sonoran desert vegetation. Ecology **57**: 1311-1316.

台灣禮樂煉銅廠附近五節芒之超氧歧化酶的研究

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摘　　要

以植被梯度分析方法，探討台灣北部禮樂煉銅廠產生之污染對當地植被之影響。由其殘存物種之種歧異度和出現頻度，顯示附近之植被有明顯之梯度變化。五節芒為煉銅廠主要優勢種之一，以其作為偵測當地植被對逆境梯度反應之指標，並研究其超氧歧化酶 (SOD) 之同功酶形式與當地逆境梯度之關係。五節芒葉片中含有 MnSOD 及 CuZnSOD 多種，其 MnSOD 活性隨著當地逆境加強而遞增，最後討論五節芒之 MnSOD 作為煉銅廠污染之生化指標之可行性。

關鍵詞：煉銅廠，五節芒，超氧歧化酶。

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