Sensitivity Analyses of Woody Species Exposed to Air Pollution Based on Ecophysiological Measurements

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Abstract

Goal, Scope and Background. Air pollution has been of a major problem in the Pearl River Delta of south China, particularly during the last two decades. Emissions of air pollutants from industries have already led to damages in natural communities and environments in a wide range of the Delta area. Leaf parameters such as chlorophyll fluorescence, leaf area (LA), dry weight (DW) and leaf mass per area (LMA) had once been used as specific indexes of environmental stress. This study aims to determine in situ if the daily variation of chlorophyll fluorescence and other ecophysiological parameters in five seedlings of three woody species, *Ilex rotunda*, *Ficus microcarpa* and *Machilus chinensis*, could be used alone or in combination with other measurements for sensitivity indexes to make diagnoses under air pollution stress and, hence, to choose the correct tree species for urban afforestation in the Delta area.

Methods. Five seedlings of each species were transplanted in pot containers after their acclimation under shadowing conditions. Chlorophyll fluorescence measurements were made in situ by a portable fluorometer (OS-30, Opti-sciences, U.S.A). Ten random samples of leaves were picked from each species for LA measurements by area-meter (CI-203, CID, Inc., U.S.A). DW was determined after the leaf samples were dried to a constant weight at 65°C. LMA was calculated as the ratio of DW/LA. Leaf N content was analyzed according to the Kjeldhal method, and the extraction of pigments was carried out according Lin et al.

Results and Discussion. The daily mean F_v/F_m (F_v is the variable fluorescence and F_m is the maximum fluorescence) analysis showed that *Ilex rotunda* and *Ficus microcarpa* were more highly resistant to pollution stress, followed by *Machilus chinensis*, implying that the efficiency of photosystem II in *I. rotunda* was less affected by air pollutants than the other two species. Little difference in daily change of F_v/F_m in *I. rotunda* between the polluted and the clean site was also observed. However, a relatively large variation of F_v/F_m appeared in the other two species, particularly in *M. chinensis*, suggesting that they were more sensitive to air pollutants than *I. rotunda*.
The mean LA was reduced for all species growing at the polluted site. The mean LMA for all species exceeded the sclerophylly threshold given by Cowling and Campbell and increased for those under pollution stress, which could be explained as one of the acclimation strategies for plants to air pollution stress.
Little difference in leaf chlorophyll content was observed in *F. microcarpa* and *M. chinensis*, while remarkable differences were found in *I. rotunda* growing at the polluted and the clean site. Content of leaf carotenoids was largely reduced in *I. rotunda* growing at the polluted site, but increased in *F. microcarpa* and *M. chinensis*, compared with plants growing at the clean site. Plants growing at the clean site had a lower leaf N content than those growing at the polluted site. In addition, species with a higher resistance to pollution stress showed less difference in leaf N content than those sensitive species.

Conclusion. Based on F_v/F_m measurements of the three woody species, *I. rotunda* showed the highest resistance to air pollutants from ceramic industries, followed by *F. microcarpa*. *M. chinensis* was the most sensitive species to air pollution, had lowest capacities to cope with the air pollution stress, which was consistent with visual injury symptoms observed in the crown profiles of plants at the polluted site. F_v/F_m, LAM, LA, leaf pigments and N content could be used alone or in combination to diagnose the extent of the physiological injury. The ratio of F_v/F_m, however, was the best and most effective parameter.

Recommendation and Outlook. Tree species which have higher air-pollutant resistance, as diagnosed by such ecophysiological parameters, should be considered first and planted widely for urban afforestation or forest regeneration in areas where the forest was seriously degraded or forest health was markedly effected by the same kind of air pollutants.

Keywords: Air pollutants; chlorophyll fluorescence; *Ficus microcarpa*; *Ilex rotunda*; *Măchilus chinensis*; leaf N; leaf mass per area (LMA)

1 Introduction

The measurement of chlorophyll fluorescence has been used for decades as a sensitive, reliable, and rapid method to determine the effects of environmental stresses, like drought, temperature, excessive light and air pollution on green plants (Bolhàr-Nordenkampf et al. 1989). The fluorescence parameters, such as variable fluorescence (F_v), minimum fluorescence (F_o) and maximum fluorescence (F_m) were used to evaluate environmental stress on plants (Brennan and Jefferies 1990). The ratio of F_v/F_m, which is equal to $(F_m-F_o)/F_m$, was considered as a quantitative indicator of the photochemical capacity of photosystem II (Björkman and Demming 1987) and has been suggested to be a suitable parameter for the assessment of stress damage in plants (Bolhàr-Nordenkampf et al. 1994, Lanaras et al. 1994, Mèthy et al. 1996, Toivonen and DeEll 1998). Air pollution related reduction of the F_v/F_m level has been published for the lichen species of *Hypogymnia physodes*, *Pseudevernia furfuracea*, *Platismatia glauca* and *Lobaria pulmonaria* (Jensen 1999). Adverse effects of O_3 exposure on cabbage (Calatayud et al. 2002), tomato (Calatayud and Barreno 2001), field-grown oats (Carrasco-Rodriguez and Valle-Tascon 2001) and poplar trees (Giacomo et al. 1999) has also been reported. However, most of the research mentioned have been operated in open top chambers (OTCs) or in modeling conditions. The increase of leaf chlorophyll concentration in polluted urban environments has already been reported by Saarinen (1993), Carreras et al. (1996) and Gratani et al. (2000). Some leaf traits such as leaf area (LA), dry weight (DW) and the leaf mass per area (LMA, in terms of DW/LA ratio), and macronutrient content in broadleaved trees, have been proved to express the degree of leaf hardness, which could be considered as specific indexes of stress (Bussotti et al. 2000).

The Pearl River Delta in south China has been characterized by a rapid development of industry as well as by serious environmental pollution during the last two decades. The effects of environmental pollution on woody species used for afforestation in south China aroused the concern of the public and of politicians. Five seedlings of three species, *Ilex rotunda, Ficus microcarpa* and *Machilus chinensis,* which were widely used as urban afforestation in the area, were utilized for the measures at both the polluted and the relatively clean environments. The objective of this study was to determine in situ if daily variation of chlorophyll fluorescence and other ecophysiological parameters of three woody species could be used alone, or in combination with such other measures as sensitivity indexes, to make diagnoses under air pollution stress and, hence, to choose the correct tree species for urban afforestation in the Pearl River Delta in south China.

2 Materials and Methods

2.1 Site description

Two sites were selected for experiments, one of which is located in Nanzhuang of NanHai County, the other is located in South China Botanical Garden of Guangzhou, representing heavily polluted and relatively clean sites, respectively. Both sites are similar in geolocation and belong to lower-subtropical monsoon humid weather with warm and humid southeast and southwest winds in summer, and rich rainfall in the summer and early autumn. Nanzhuang is one of the most rapidly developed areas dominated by ceramic industries during the last two decades, as well as one of the most seriously polluted areas in the Pearl River Delta. Emissions of air pollutants, including sulfur, fluorine compounds and suspended particles from these industries have already led to damages in local vegetation. Visible injuries have been observed in both evergreen species (*Eucalyptus exserta, Eucalyptus urophylla, Syplocos lancifolia, Mallotus apelta, Celtis sinensis, Aporosa dioica, Pinus massoniana, Carallia brachiata, Ficus hirta, Dalbergia balansae*) and deciduous trees (*Broussonetia papyrifera, Trema orientalis, Vitex negundo*) (Wen et al. 2002). Visible injuries for most species occurred first at the apical part or the edge of leaves, then extended to inner parts of leaves at a later stage, which were consistent with the symptoms of leaf injuries caused by fluoride (Kong et al. 1988). The reference site in the Botanical Garden was far away from a pollution source, and the air was much clean than the polluted site (**Fig. 1 A and B**).

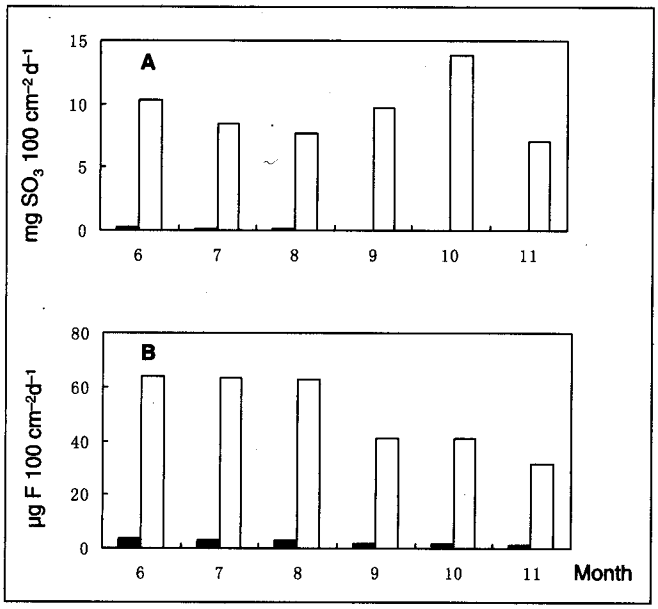

Fig. 1: The rate of sulfating (A) and the deposition of fluorine compound (B) at the two sites from June to November of 2002. The mean values of the rate of sulfating at Nanzhuang (□) were 78 times that found at the Botanical Garden (■), and the deposition of fluorine compound at Nanzhuang (□) was 24 times that of the Botanical Garden (■). The monitoring and measurements were carried out according to methods presented by Ministry of Urban and Rural Construction and Environmental Protection, PRC (1986)

2.2 Plant materials

Seedlings of each woody species, ovate leaf holly (*Ilex rotunda*), small fruit fig (*Ficus microcarpa*) and Chinese machilus (*Machilus chinensis*) were used for the measurements of chlorophyll fluorescence and morphological test. These species are widely used for urban afforestation in subtropical China. For the experiment, five seedlings of each species were transplanted to pot containers (60 cm in diameter and depth) in early May and placed at the polluted and relatively clean sites in late May of 2002 after their acclimation under shadowing conditions. Seedlings for all species were all 3-year-old and 70–100 cm in height. All seedlings were cultivated under adequate irrigation and proper fertilization was guaranteed throughout the growth period.

2.3 Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were made in situ using a portable fluorometer (OS-30, Opti-sciences, U.S.A) from 7:00 to 18:00 at every 2 hours on clear days in late October and early November of 2002. The daily tendency of chlorophyll fluorescence was measured in 5 leaves of the same tree. The leaves selected for measurements were just mature and without visible injuries, and were carefully cleaned to remove the dust from the surface with paper prior to measurements. Fully expanded leaves were dark-adapted for 15 min using clips with dark room, and then measurements were made on the apical parts of their upper surface. The run time was 2 seconds, and the saturating light intensity was 3000 μmoles $m^{-2}s^{-1}$. After the dark adaptation, the parameters F_o, F_v, and F_m were measured, and the ratio F_v/F_m was calculated.

2.4 Leaf traits, N content and pigments

Ten leaves for each species were taken from each treatment and used for leaf area tests (LA, measured by CI-203 Area-meter, CID, Inc., U.S.A). These samples were immediately placed into polyethylene bags, and brought to the laboratory at the same day as the collection. DW was determined after the leaves were dried to a constant weight at 65°C. LMA was calculated by $DW \cdot LA^{-1}$ ($mg\ cm^{-2}$).

Leaf N content was analyzed according to the Kjeldhal method after the leaves were finely ground. An additional sample of four leaves was selected from each species at each site for analysis of foliar pigments. These leaves were placed in plastic bags and stored in an ice-filled box on the same day as they had been collected. The extraction for pigments was carried out according to the methods described by Lin et al. (1988).

3 Results

3.1 Diurnal change of chlorophyll fluorescence

Values of daily F_v/F_m in *I. rotunda* ranged from 0.767 to 0.823 at the polluted site, and from 0.749 to 0.828 at the clean site. The daily change of F_v/F_m in *I. rotunda* growing under air pollution appeared to be somewhat consistent with that observed at the clean site, except at 10:00, 13:00, 14:00 and 15:00 (**Fig. 2A**), when the F_v/F_m at Nanzhuang was slightly higher than that at the Botanical Garden, implying that the *I. rotunda* were injured less by the air pollutants from the ceramic factories, and the photochemical efficiency of the photosystem **II** was effected only slightly. This result might suggest that *I. rotunda* was a resistant species to this kind of air pollution. The lowest F_v/F_m at 15:00 at both sites might result from the inadequate sunlight, because it was partially cloudy at that time.

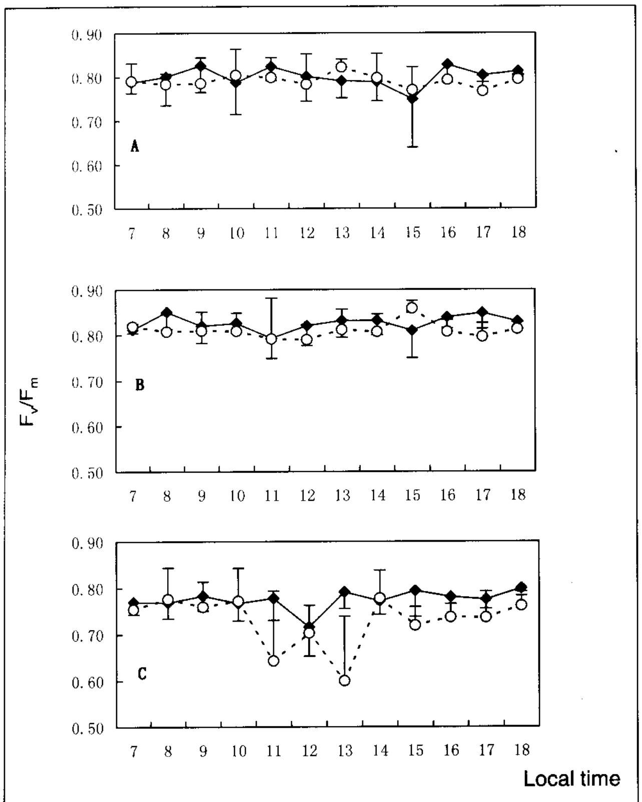

Fig. 2: Diurnal change of F_v/F_m ratio at Nanzhuang (O) and the Botanical Garden (◆) of *I. rotunda* (A), *F. microcarpa* (B), and *M. chinensis* (C). The treatment effect was tested with a univariate analysis of variance (ANOVA), tests of significance were made at the 95% confidence level. The bars shown in the figures represented the standard deviation

In *F. microcarpa*, F_v/F_m ranged from 0.790 to 0.859 and from 0.793 to 0.831 at the polluted and the clean site, respectively. It was slightly lower at the polluted site than that at the clean site during the whole period except at 15:00 (**Fig. 2B**). It's interesting that the ratio values showed reverse trends at both sites. This result implied that the injuries induced by pollution on the efficiency of the photosystem **II** in *F. microcarpa* were slightly heavier than that in *I. rotunda*.

In *M. chinensis*, the F_v/F_m at the clean site were maintained relatively stable during the daytime, and no significant difference ($p=0.05$, $n=60$) was observed. Pollution stress, however, led to a general decrease in F_v/F_m, particularly significant at 11:00 and 13:00 ($p=0.05$, $n=5$), suggesting that the efficiency of the photosystem **II** and the capacity for plants to capture photon flux was decreased, especially at noon time, as compared with the same species at the clean site.

Generally, the F_v/F_m for all species growing at the clean site were maintained relatively stable during the daytime, and no significant differences ($p=0.05$, $n=5$) in the diurnal course of F_v/F_m were detected in *I. rotunda* and *F. microcarpa* between the different sites. On average, *M. chinensis* had an overall higher F_v/F_m at the clean site (0.775 ±0.021) than at the polluted site (0.717±0.056), and no significant differences ($p=0.05$, $n=60$) were found between different sites for the other two species. As a whole, we concluded that *M. chinensis* was sensitive to air pollution, and had lower capacities to cope with the air pollution stress than *I. rotunda* and *F. microcarpa*.

3.2 Leaf morphology

Fig. 3 showed the morphological measurements of the three species growing at the polluted and the clean sites. LA for all species was generally reduced under the pollution stress (Fig. 3A). The mean LMA for plants at the polluted site, however, was higher than that at the clean site (Fig. 3B).

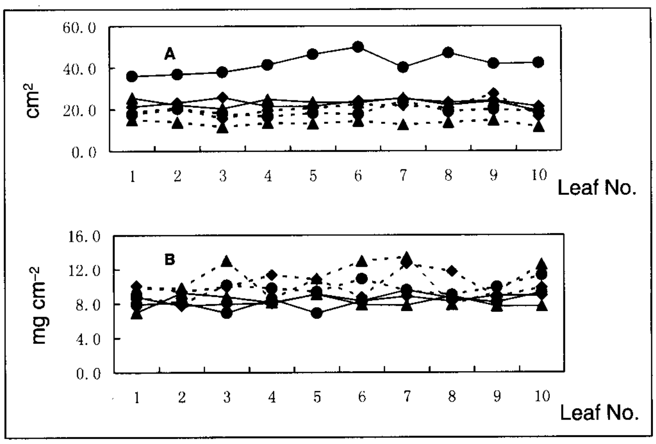

Fig. 3: The LA (A) and LMA (B) of the *Ilex rotunda* (▲), *Ficus microcarpa* (◆) and *Machilus chinensis* (●) at Nanzhuang (------) and the Botanical Garden (—)

3.3 Leaf pigments

Fig. 4A showed that the content of leaf chlorophyll did not differ significantly for both *F. microcarpa* and *M. chinensis* growing at different sites, but it decreased by 19% for *I. rotunda* at the polluted site, compared with that at the clean site. The content of leaf carotenoids in *I. rotunda* at Nanzhuang was decreased by 21%, but increased by 11% and 12% in *F. microcarpa* and *M. chinensis* at Nanzhuang, respectively in comparison with corresponding values at the Botanical Garden (**Fig. 4 B**).

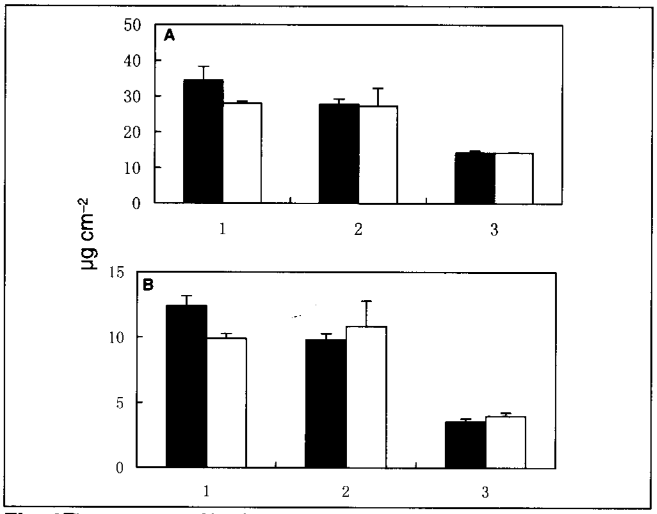

Fig. 4 The content of leaf chlorophyll (A) and carotenoids (B) of the 3 species at Nanzhuang (□) and the Botanical Garden (■). 1 is *I. rotunda*, 2 is *F. microcarpa* and 3 is *M. chinensis*

3.4 N content

Leaf N content in trees growing in the relatively clean environments was lower than that of those growing in the polluted environments. The highest N content was measured in the leaves of *F. microcarpa* and the lowest was observed in the leaves of *M. chinensis* at the Botanical Garden, while the highest leaf N content appeared in *M. chinensis* growing under the air pollutants. The difference of leaf N content in *M. chinensis* was much larger than that of *I. rotunda* and *F. microcarpa* at the two sites. The leaf N content in *M. chinensis* at Nanzhuang was 40% higher than that found at the control site, while in *I. rotunda* and *F. microcarpa* growing at Nanzhuang, the leaf N content were 17% and 2.6% higher than that of at the reference site, respectively (**Fig. 5**). The comparison of leaf N content in the same species at different sites suggested the conclusion that if species were higher resistant to environmental stress, the variations of macronutrient content might be smaller than that of sensitive ones.

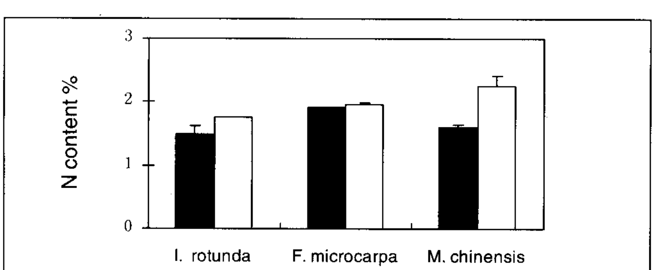

Fig. 5: The leaf N content of the species at Nanzhuang (□) and the Botanic Garden (■)

4 Discussion

F_v/F_m, representing the photochemical efficiency of photosystem II, is one of the most widely used fluorescence parameters and is considered to be an ideal measure for the determination of the influence of stress on the health of the photosystem II mechanism, such as chilling injury to membrane (Lurie et al. 1994). Since it provided the most consistent results with regards to other environmental stresses, including air pollution (Peng et al. 2002, Su et al. 2002), F_v/F_m and its diurnal variation will be the main fluorescence parameter discussed in this paper.

In the present study, *I. rotunda* and *F. microcarpa* had a higher stability to air pollution as indicated by the similar trends of F_v/F_m and small differences of its diurnal courses between different sites. However, *M. chinensis* growing at the pollution site exhibited a general decline in F_v/F_m from 8:00 to 17:00 than that at the clean site, particularly at midday, suggesting its lower capacity to cope with the air pollution. These results agreed with those obtained by Deltoro et al. (1999) in the lichens *Evernia prunastri* and *Ramalina farinacea* and by Guidi et al. (1997) in two bean cultivars (*Phaseolus vulgaris*, cv. Pinto and cv. Groffy), and was well consistent with the discoloration and injury symptoms in leaves of *M. chinensis* growing at the polluted site.

The reduction of diurnal F_v/F_m in *M. chinensis* at Nanzhuang might be related with the pollutant-induced photosystem II inactivation or damage of photosystem II, and responsible for the photosynthetic down-regulating process, since the F_v/F_m ratio indicates the photochemical efficiency of photosystem II and its decrease in this parameter is a reliable sign of photoinhibition (Krause 1988). At 11:00 and 13:00, F_v/F_m for *M. chinensis* at the polluted site appeared particularly lower than that at the clean site. Since photoinhibition may cause a decrease in photosynthesis and lead to limitations in daily carbon gain (Ögren and Sjötrön 1990), it can obviously be concluded that *M. chinensis* is more sensitive to the strong incident light combined with air pollutants, and reveals a more limited capacity to pollution stress than the other two species.

Depression of photosynthesis at midday could be the consequence of excess excitation energy under conditions of high photosynthetic photon flux density (PPFD), leading to photoinhibition of photosystem II photochemistry (Ort 2001). Barber and Anderson (1992) found that photoinhibition severity was not only determined by light intensity, but also by the combination of other environmental stress, such as high temperatures, water availability or CO_2 supply. Thus, we speculated that large reductions in F_v/F_m ratios for the polluted *M. chinensis* at 11:00 and 13:00 were probably dependent on strong solar radiation accompanied with high leaf temperatures. In fact, the pronounced leaf temperature was 38–41°C at midday.

A decreased F_v/F_m was observed in all three species under pollution for most daytime measurements, particularly significant in *M. chinensis* at midday. However, daytime changes in photosystem II photochemistry were reversible for all species, suggesting that plants under the long-term exposure of pollutants exhibited adaptive or protective mechanisms for photoinhibition. Plants subjected progressively to ozone (Barnes et al. 1990) and Cd (Krupa et al. 1992) were capable of adapting to the stress and decrease in F_v/F_m was not observed, which was similar to the results from *I. rotunda* and *F. microcarpa*, but not for *M. chinensis*.

Among the morphology parameters, LMA is particularly interesting. Based on the definition by Cowling and Campbell (1983), leaves with an LMA greater than 7.5 mg cm^{-2} are to be considered as sclerophyllous. The mean LMA for *M. chinensis*, *F. microcarpa* and *I. rotunda* was 9.73±1.05, 8.48±0.50 and 8.21±0.75, respectively. All the studied species can be described as sclerophyllous. Pollution, however, reduced the mean LMA in *M. chinensis* by 20%, increased by 32% and 22% in *I. rotunda* and *F. microcarpa*, respectively. The change in LMA may be related with the protective or adaptive mechanisms of plants under pollution stress, partially reflecting the correlation of species sensitivity to pollution, thus supporting the statement that LMA was an environmental index given by Bussotti et al. (2000). Under stressed conditions, in fact, plants build mechanical defense tissues and expand the apoplastic fraction (Gutschick 1999).

The content of chlorophyll is a valuable index to determine the injury degree of plants. Tanaka et al. (1988) reported the degradation of leaf chlorophyll caused by SO_2, which was supported by Su et al. (2002) who showed that the leaf chlorophyll content from the industrial environment was almost 50% lower than that from the relatively clean environment. The increase in chlorophyll content in polluted urban environments has already been reported by Saarinen (1993), Carreras et al. (1996) and by Gratani et al. (2000), and can probably be explained by the triggering of metabolic compensation mechanisms. In our study, we found that the effects of pollution stress on chlorophyll content were not apparent, since nearly no differences in chlorophyll content were observed between the polluted and the clean sites for *F. microcarpa* and *M. chinensis*, although it decreased by 19% for *I. rotunda* at the polluted site (see Fig. 4A). Thus, no specific relationships were established between the change of chlorophyll content and plant injury. The contents of carotenoids in all species exposed to air pollution, except *I. rotunda*, were higher than those of the same species growing in the clean environment. The organelles, such as chloroplasts were considered the most sensitive target of a variety of atmospheric pollutants, such as SO_2 (Schiffgens-Gruber and Lüz 1992) and fluorides (Elefteriou and Tsekos 1991).

Atmospheric chemical composition affects foliar chemical composition. In general, leaf N concentration of plants is decreased under elevated CO_2 (Koricheva et al. 1998), marginally reduced under elevated O_3 (Lindroth et al. 2000), which disagreed with the results of Scherzel et al. (1998), who showed that single pollutants, such as O_3, didn't significantly alter foliar N concentration. When treated in combination with CO_2, however, leaf N concentration was decreased by 18% to 40% (Scherzel et al. 1998). In our study, we found that pollution stress generally increased leaf N concentration for all the studied species, particularly significant in the low-tolerant species *M. chinensis*. The possible explanation would be (1): to compensate for the physiological damage caused by the pollutants for surviving; (2) to reallocate N from injured or discolored leaves to nearby healthy leaves. Fang and Tao (1995) showed that the net photosynthetic rate increased with the increasing nitrogen content in the leaves of rice plants.

5 Conclusion

Based on F_v/F_m measurements of the three woody species, *I. rotunda* showed the highest resistance to air pollutants from ceramic industries, followed by *F. microcarpa*. *M. chinensis* was the most sensitive species to air pollution and had the lowest capacities to cope with the air pollution stress, which was consistent with visual injury symptoms observed in the crown profiles of plants at the polluted site. F_v/F_m, LAM, LA, leaf pigments and N content could be used alone or in combination to diagnose the extent of the physiological injury, the ratio of F_v/F_m, however, was the best and most effective parameter. When choosing the tree species to be applied in urban afforestation or for restoration of seriously degraded land affected by similar air pollutants, species with a higher resistance or tolerance like *I. rotunda* or *F. microcarpa* should be considered first and extended widely to enhance the environmental quality.

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