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SO_2-Induced Change of Spectrum in Low-Level Chemiluminescence from Leaf of *Populus tomentosa*

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Sulfuric dioxide is a major air pollutant. At present, its sources exist widely and the releasing quantity of that gas is large. It severely affects human living environment. It is proved that plants are sentitive to SO_2 pollution. Generally speaking, dose of that pollutant which does not reach a level to harm animals or human beings can often hurt some plants (Commission of Japanese Environ-Ecolgy, Environment and indicating biology 1989) Therefore, indicating SO_2 pollutant with some plants becomes extremely significant. It is shown evidently by efficient method that leaf is a kind of plant organ, which has a function in exchanging gases with environment. Large area of leaf surface is in contact with air directly, and SO_2 can readily enter the plant together with air through their holes. So, the damage of SO_2 is displayed at leaves firstly. Prof. Yu (Yu Shuwen 1981) pointed out that when SO_2 acute damage occurred in broad-leaf plant, there are some irregular shape spots with different sizes emerged on leaves between veins. Moreover, leaf polluted by SO_2 can emit a special fluorescence which may be a cheracteristic being able to be used to diagnose the damage. With low-level luminesecence measurement technique, we have conducted some exoeriments of antificial somking leaves of Populus tomentosa with SO_2 and other harmful gases, and found that the address luminescence of SO_2--smoked leaf is related to its concentration. Although the investigation showed the possibility of detecting air pollution by using plant indicator, it is not possible to distinguish the SO_2 from other air pollutants, because there is lack of the specificities of SO_2 influence. Theoretically, low -level luminescence from living object is associated with organism energy metabolism, oxidation and toxicity effect. It is a kind of weak emission of photon during the processes of normal and special pathological metabolism. Thus, plant leaf damage caused by air polluant can result in their luminescence characteristic

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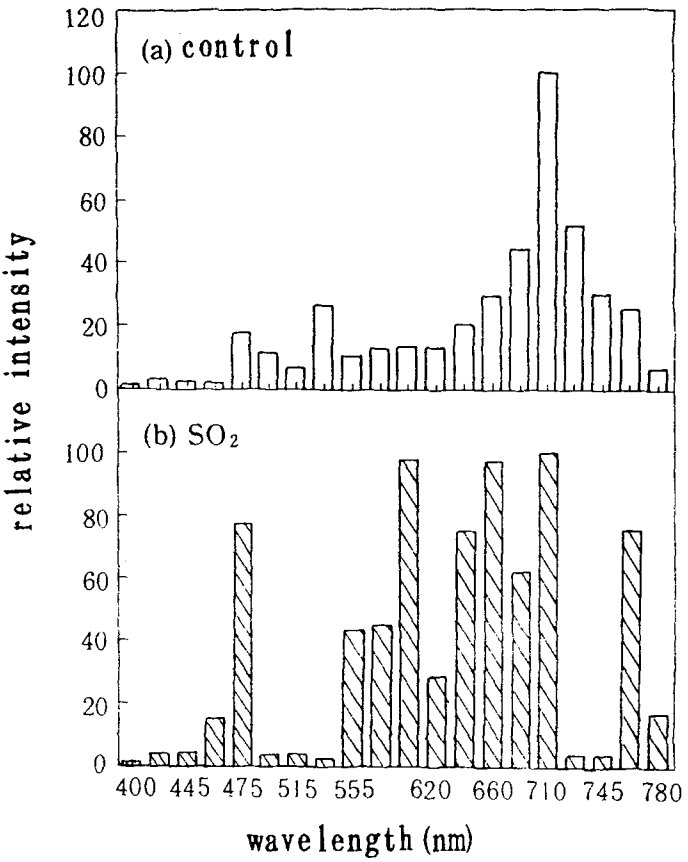

Figure 1. Emission spectrum of Populus tomentosa leaf smoked with SO_2.

changes. Through analyzing the characteristic emission spectra of leaves contaminated by various pollutants, it is possible to determine the characteristic luminescence spectrum of SO_2 damage. This work is the further study following our previous one (Ma Yuqin 1992) and the aim is to indicate SO_2 pollution with Populus tomentosa leaf by its low- level chemiluminescence spectrum.

METHODS AND MATERIALS

SO_2 or other air pollutants smoking experiment was done directly on the branches of Populus tomentosa tree. Measured leaves were chosen from the middle leaves of smoked branches, with similar surface area and ripened in June or July. Sampling is taken from 7 to 9 o' clock AM. The temparature is 25-35℃; humidity is about 80%. Leaves are detected immediately after they are picked from tree. (Ma Yuqin 1992)
SO_2, NO, CO and NO_2 used in the smoking are all standard gases (National). Measured wavelengh ranged from 400 to 800nm by a set of interfering filters with 15--20 nm interval

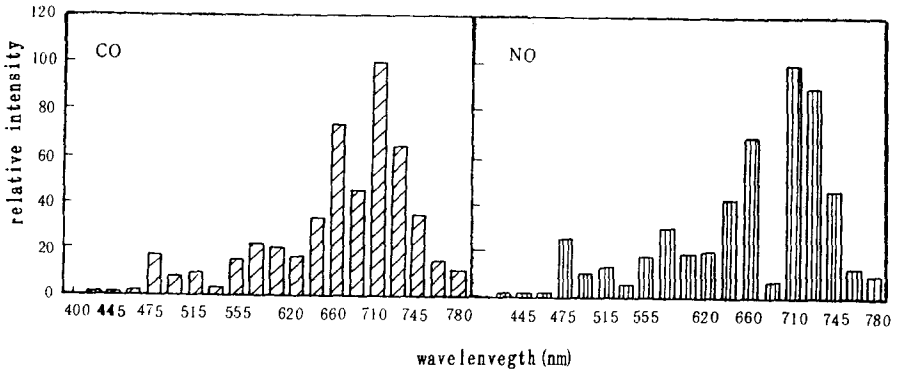

Figure 2. Emission spectrum of Populus tomentosa leaf smoked with CO and NO

RESULTS AND DISCUSSION

Change in emission spectrum of low- level chemituminescence from Populus tomentosa leaf an hour after it was smoked with 2% SO_2. The leaf emission spectrum showed in Figure 1. There were acute damage on these leaves and their luminescence increase dramatically in above conditions. The graph a in Figure 1 indicated that, the emission spectrum of Populus tomentosa leaf seemed like Gaussian distribution curve under normal physiological condition and its photon emission was between 620 and 780nm, which accounted for eighty percent of the total luminescence intensity. The maximum peak was at 710nm. In addition, there were two small peaks at 475nm and 535nm. Oppositely, after leaves were smoked with 2% SO_2, the emission spectrum of leaf varied obviously due to the occurrence of acute damage. The emission spectrum became multiple-peak curve (graph b). Although the maximum peak were still at 710nm, but there were three new peaks at 595nm, 660nm and 760nm. Besides the spectrum showed distinct blue shift. For example, the luminescence intensity at 475nm increased four times, which indicated the characteristic change caused by SO_2.
Influence of CO and NO on the emission spectrum of Populus tomentosa leaf. CO is other pollutant in air which is emitted to air when coal burning occurs. When the leaves were smoked with 2% CO (equal to SO_2 concentration), its emission spectrum shape is still like Gaussian distribution (Fig. 2 graph a). Unlike SO_2 influence on leaf, CO did not induce change of leaf emission spectrum but luminescence intensity of 660nm increaased from 30% to 70%. There was no clear change in blue area.
Nitrogen oxide are another important air pollutant including NO, NO_2 and N_2O_3. They are usually expressed as NOx. In general, NOx belonging to photochemical substance can permeate through cuticle of leaf. Its toxicity is only a tenth part of SO_2. Beacuse NO is easily oxidated, it was chosen in the experiment to test the influence of NOx on leaf. The results showed in graph b. The emission spectrum of leaf smoked with NO was similar to Gaussian distribution and its maxiumum peak was at 710nm and the change of intensity at 475nm was not observed.
When SO_2, NO and CO were mixed with equal concentration to smoke leaves for an hour, it was difficult to detect any change of the smoked leaf emission spectrum because the most smoked leaves were dropped and dead and

the luminescnce was very low. There is relationship between the singlet oxygen and the change in emission spectrum of Populus tomentosa leaf smoked with SO_2. It was confirmed that whether animal, plant or microbe, the major internal light source of their low- level chemiluminescence is singlet oxygen. NaN_3 is a special quencher of singlet oxygen. The quecking phenomena can prove the existence of singlet oxygen which directly result in decrease of luminescence. Fig3 showed the emission kinetic curve of Populus tomentosa leaf soaked with NaN_3 after smoked with SO_2. The luminescence intensity of leaf varied with time and wavelength. The change of luminescence characteristics are as follwing:

(a) The luminescence curve a of control group is nearly like quasi-exponent law decrease, only at 575nm and 710nm, there was a delay about 30 min and the luminescence decay law was unchanged.

(b) There were some different changes after leaf smoked with SO_2. the curve c and d in the fig.3 shown that the intensity tended to reach a stable value during 150 min after leaf soaked with NaN_3; the intensity of the first 60 min reduced about 20% in curve b (575nm), then the intensity rose again, but its valve was also less 10% than that of initial; besids, we could find that leaf intensity decayed with time according to quasi-exponential law in curve a (475nm) and curve e (710nm) after the leaf soaked with NaN_3.

As shown in figure 4, the value of 475/710nm in the Populus tomentosa leaf of control group was 0.75; the same ratio with NO smoked was 1.1; the value of CO was 0.85; the ratio of 475/710nm of SO_2 in same conditions was 3.5. Therefore, it is possible that the luminescence intensity from leaf of Populus tomentosa may use as an indicator to express SO_2 pollution, especialy when the ratio of 475/710nm from leaf rises greatly.

Because low- level chemiluminescence from biological system is too small to study on photon emission spectrum, 2% SO_2 concentration is necessary for analysis of photon emission spectrum. At this time luminescence intensity increased was more than 10 times under this conditions. Emission spectrum of low- level chemiluminescence from our results was a wide Gaussian curve, which was coincided with Ables' experiment result (Ables, F. B. 1986). Spectrum of leaf distributed mainly in red light area. Photon emission is largely resulted from the formation of singlet oxygen :

A, $^1O_2$ double-molecule transition

$$^1O_2 + {}^1O_2 \longrightarrow 2\,{}^3O_2 + h\nu \qquad (575\ \ 640\ \ 703nm)$$

B, Reaction of $^1O_2$ with double- band ($>C=C<$) produce excited carbonyl which can emit photon when it not excited,

$$\longrightarrow C=O + {}^*C=O \qquad (380\text{-}460nm)$$

(dioxetane)

C, Activated oxygen groups (O_2^-, $\cdot OH$, H_2O_2, $ROO\cdot$ etc.) react to singlet oxygen.

From above reactions, it could be concluded that acute damage occured on leaves. The luminescence ratio between 475 and 710nm was related to photon emission caused by singlet oxygen. When acute damage by SO_2 occured at Populus tomentosa leaves, the maximum peak of its luminescence was still at 710nm. Other three peaks emerged at 595, 660 and 760nm. The photon emission at there three wavelengthes also belong to red or infra- red area. So, the action of SO_2 induced the redistribution of intensity at some wavelengthes

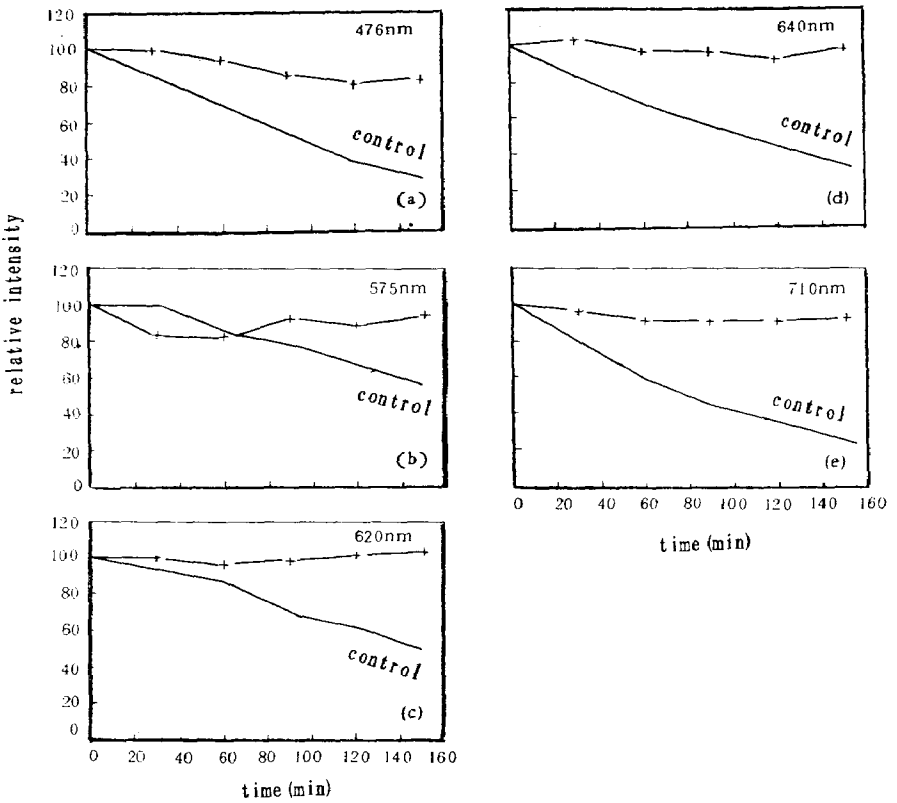

Figure 3. Luminescence kinetic curve of Populus tomentosa leaf soaked with 1mg/ml NaN_3 solution after smoked with 2% SO_2

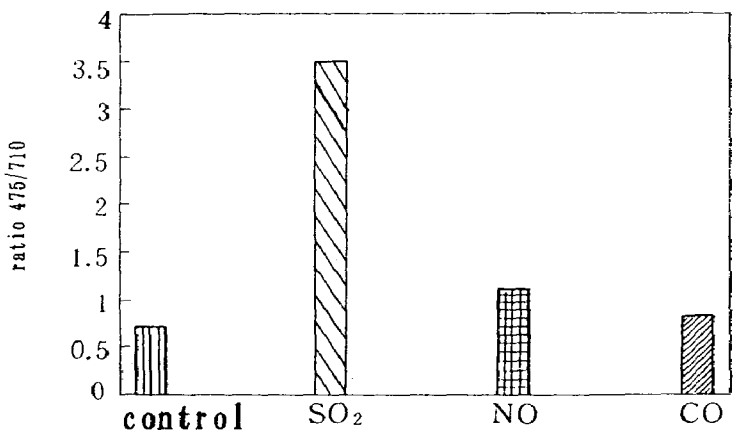

Figure 4. Change in the nm of luminescence intensity from leaves smoked with different gases

in infra- red area of leaf emission spectrum. In blue area, when acute demage occurred at leaves polluted by SO_2, leaf respiration and transpiration become difficult and abnormal.

The reaction rate of activated oxygen group, such as O_2^-, H_2O_2 and OH may be accelerated. Luminescence intensity near 475nm increased which resulted in increase of luminescence ratio (475/710nm). As some published papers indicated, the luminescence intensity from Populus tomentosa leaf is linear related with SO_2 concentration under certain experiment condition. When leaves were dropped, there was flash emission. The above phenomena are good indexes for detecting SO_2 pollution. Therefore, it may be used to detect air pollution, especially its luminescence ratio of 475/710nm from leaf of Populus tomentosa.

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