Bull. Environ. Contam. Toxicol. (1992) 49:906–913

Environmental Contamination and Toxicology

Influence of Rain and Sulphur Dioxide on Low Level Chemiluminescence from Leaf of *Populus tomentosa*

Ma Yuqin,[2] Zhao Kejian,[1] Zhang Zhonglun,[1] Su Zhen,[1] Ma Bin,[1] Zheng Yanzhen,[1] Liu Chengxiang,[1] and Wang Yingyan[1]

[1]Institute of Biophysics, Academia Sinica, 15 Datun Rd., Beijing100101, People's Republic of China and [2]Beijing Municipal Research Institute of Environmental Protection, Beijing, People's Republic of China

We can directly measure the intensity of Populus tomentosa leaf in a high sensitivity photon counter after fresh leaves are picked from trees. This kind of light , which can be detected without introducing any external influences, such as electric, magnetic field, or other chemical factors, is called low level chemiluminescence related with the biochemical metabolism of organism, especially the oxidative metabolism. Its intensity is in direct proportion to the square of radical concentration of RO_2 e.g. $I=KQ(RO_2)^2$. The formation of singlet oxygen is responsible for the production of the light(Slawinska et al.1985). This is an advanced optical electric technology for photon measurement. Although very dim ($10-10^4$ photons/sec/cm^2), low level chemiluminescence is internally associated with organism contamination or detoxification effect, and also is sensitive to chemical toxin, temperature and even the microscopic environmental change in biological creatures. As a result, recently,many of photochemists and photobiologists suggest that low level chemiluminescence from living creatures can be used to indicate environmental pollution. Professor Inaba, a Japanese scientist, has already directed a reseach group to start the work (Swinbanks 1986) . In our experiments we selected the long growth period, large leaf and extensively planted broadleaf tree— Populus tomentosa as experimental material, and studied the basic characteristics of low level chemiluminescenc from leaves, such as the effect of "acid-rain" and sulphur dioxide ect.. The purpose of this paper is supposed to provide a new method for detection of air pollution and biological evaluation.

MATERIALS AND METHODS

Because there is a difference in density of leaf growth in a tree, a piece of leaf in the tree can not reflect the all ones . With a ratio of the numbers of collected leaves (Dense:Sparse=1 : 3), We collected the same leaves in size to do experiment.The measuring equipment was made by the Institute of Biophysics, Academia Sinica, and Colony Corporation. Its sensitivity is nearly one count per second which is correspondent to 10^{-}[illegible] ampere optical electric current. Standard error is smaller than 0.8%. Among 10 repeated measurements, the standard error is less than 0.5%. Pluck leaves and put them in sample room with the surface of the leaves

Send reprint requests to Dr. Ma Yuqin at the above address.

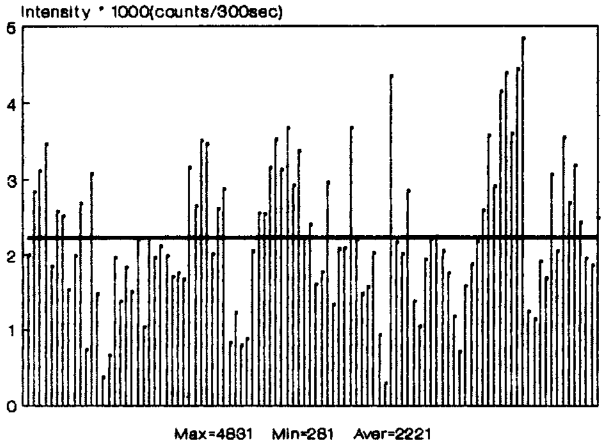

Figure 1. Intensity of Populus tomentosa leaf

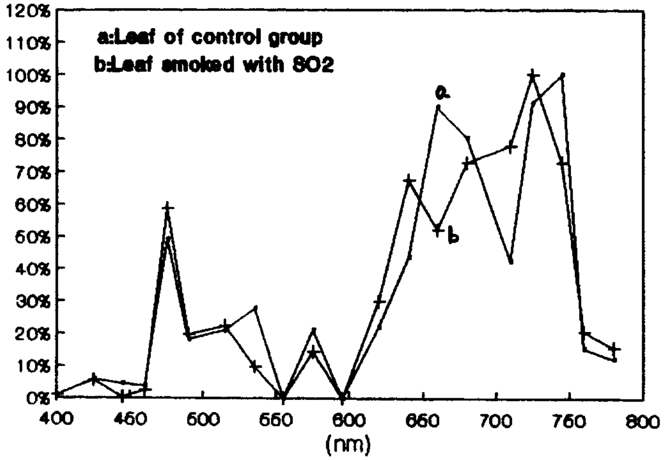

Figure 2. Emission spectrum from leaf of Populus tomentosa

directly facing detector. Measurement time is 500 seconds in which the first 200 sec are measurement of induced or decay luminescence and the last 300sec are measurement of low level chemiluminescence. The intensity is expressed as counts / 300sec. Emission spectrum is analysed with 20 interferential filters which are in range of 400 to 780nm.Besides, absorbance and efficiency of photomultiplier are calibrated. Emission spectrum is plotted by computer.

RESULTS AND DISCUSSION

As shown in Figure 1, of the low level chemiluminescence from 100 pieces of leaves of Populus tomentosa, the maximum intensity was 4831 counts/300 sec, the minimum was 281 counts/300sec, the average was 2221 counts/300sec, which was 2.6 times intensity of Juglans cathayensis leaf, 3.2 times intensity of Ginkgo bitoba leaf. But the intensity of Syringaoblata leaf was 1.3 times that of Populus tomentosa which was nearly intensity of Ionicera mocki leaf.

Table 1. Intensity and percentage transmission of various species of leaves

Species	Intensity (Count/300sec)	T%	Weight (g)
Populus tomentosa	1678	0.08	0.45-0.56
Comue alla L.	354	0.25	0.45-0.56
Cralaegus sp	590	0.12	0.45-0.56
Calelpa specioca wand	810	0.10	0.45-0.56
Ginkgo bitoba	521	0.14	0.45-0.56
Fraxinus chinensis	860	0.12	0.45-0.56
Juglans cathayensis	641	0.17	0.45-0.56
Syringa oblata	2159	0.18	0.45-0.56
Hai Xian Hua	2256	0.18	0.45-0.56
Dispyros chinensis	1354	0.22	0.45-0.56
Lonicera macki	1539	0.12	0.45-0.56

In Figure 2a, in the typical emission spectrum of Populus tomentosa leaf in the wavelenth range of 400 to 780 nm, there were four bands of emission spectra in 475, 575, 660 and 745 nm, the maximum peak was at 745 nm. Moreover, it was evident that leaf luminescence was mainly contributed from the red light area ploted in the curve, which intensity accounted for 82.5% of the total intensity. According to the reported papers (Cadenas 1984), the luminescence from red light area resulted from the formation of singlet oxygen:

$RH + {}^{\cdot}OH \rightarrow R^{\cdot} + H_2O_2$
$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$
$ROO^{\cdot} + RH \rightarrow R^{\cdot} + ROOH$
$ROO^{\cdot} + ROO^{\cdot} \rightarrow RO^{*} + ROH + {}^1O_2$
${}^1O_2 + {}^1O_2 \rightarrow 2\ {}^3O_2 + h\nu$ (λ=478, 580, 634, 710 nm)

In addition, the luminescence from near 480 nm may result from the decay of carbonyl excited state to the ground state (${}^3R=O$):

$R^{*}O(C^{*}=O) \rightarrow RO(C=O) + h\nu$ (480 nm)

From the below kinetic curve of Populus tomentosa leaf, we could find that leaf intensity decayed with time by means of quasi-exponential law, then, tended to reach a stable value. Decay emission resulted from induced emission after leaf was put into sample room, and disappeared within about 200 seconds, the rest of luminescence was due to biochemical metabolism. This was the reason that there was luminescence in the last 300 seconds. As in the Figure 4 are shown the intensity changes of Populus tomentosa leaves in several continuous rainfalls in experimental areas during summer, 1991. Using the leaves picked in a bright day, June 4th, as the control group, it can be seen that the intensity of the leaves collected in rainy days of 8th, 10th, 11th of June greatly reduced, but after these rainy days the intensity rose again.

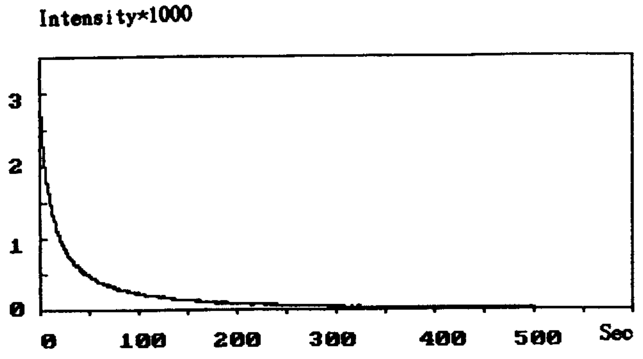

Figure 3. Luminescence kinetic curve of Populus tomentosa leaf

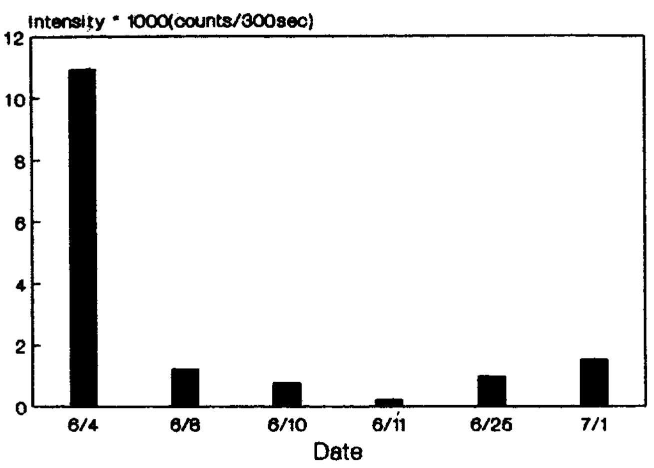

Figure 4. Influence of "acid-rain" on leaf luminescence

The phenomenon that intensity reduced after rainfall was also demonstrated by the intensity changes of leaves picked 10 minutes after or before the rainfall. For example, in June 25 the weather was inclement, before storm came the intensity of leaves was 2051 ± 367 counts/300sec . But the intensity of leaves collected from the same brance of a tree decreased to 1009 ± 284 counts/300sec , which reduced nearly 50. 8%.Why did the intensities of leaves reduce rather than increase after the pollution dust on the surface of leaf was washed away by rainfall with pH 6.5 to 6.8 at that time. Can we assume rain washing as the damage of " acid-rain" to leaves? In order to examine the suspposition, we collected leaves from industrial and hygienic areas to do washing experiments. The results were listed in Table 2 . It was obvious that intensity of leaf washed with water rose, furthermore, the increase in industrial area was greater than that of hygienic area, the former intensity was 2.7 times the latter. It was interesting that why rain can reduce intensity in the same condition such as water washing which can clean dust on the surface of leaf.Evidently, it is necessary to further study the action of H^+ ion on leaf.

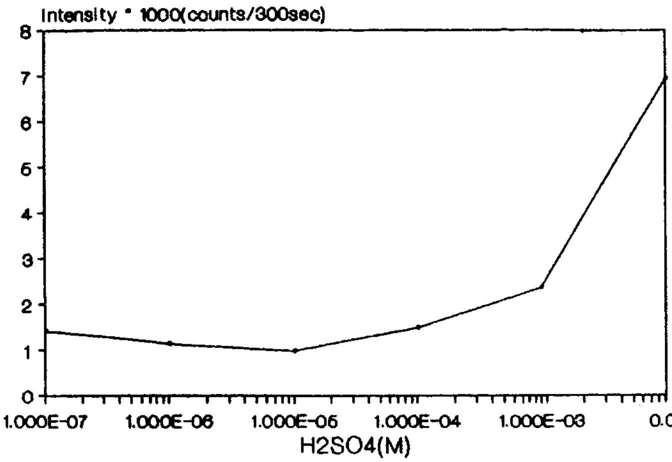

Figure 5. Influence of diluted sulphuric acid on luminescence from leaf of Populus tomentosa

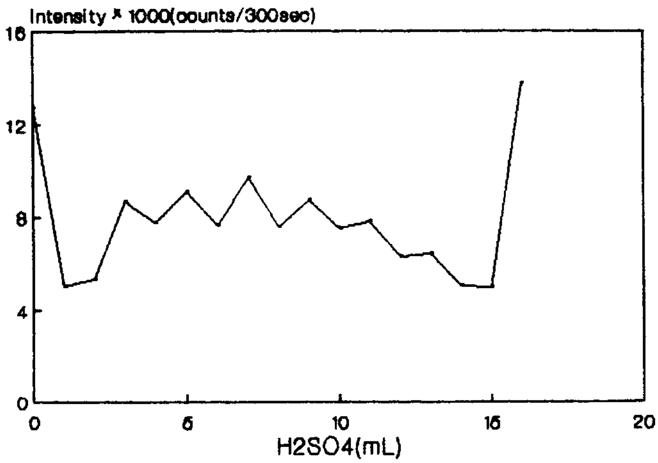

Figure 6. Emission change of Populus tomentosa leaf affected by H_2SO_4

Table 2. Luminescence change of Populus tomentosa leaves after water washing

Area	Intensity (counts／300sec)		Increase of luminescence (%)
	Before washing	After washing	
A(Industrial area)	4110±130	5009±623	24.06
B(Hygienic area)	1609±272	1750±474	8.80

Selecting the same leaves in size, percentage transmission, weight and sampling period, five groups including five pieces of leaves each were immerged in sulphuric acid solution, respectively, and their intensities were measured after 40 minutes soaking. As shown in Figure 5 , compared with the results of water soaking, the intensities of leaves immerged in 10^{-4} to 10^{-5} M sulphuric acid decreased with the increase of acid content, this result was similar to that of "acid-rain". There was no damage on the surface of leaves. But when immerged in greater than 10^{-4} M sulphuric acid, surface of leaf appeared yellow spots and its luminescence increased. While the concentration of sulphuric acid was greater than 10^{-3} M, the leaf was mostly damaged, and its luminescence rose dramatically. Its intensity reached 6954 counts/300 sec , which was correspondent to 4.9 times that of control group. This phenomenon was very similar to " flash emission " reported in references(Popp 1987). To observe the whole process in which luminescence of leaf was changed because of the erosion of acid, the leaf of <u>Populus tomentosa</u> immerged in a cup with 10 mL water was measured. Then, adding 0.1 mL 10^{-2} M H_2SO_4 gradually into the cup , the experiment was repeated as described above. From the results shown in Figure 6 , we can find that after the initial adding of H_2SO_4 solution, the intensity reduced rapidly, but it varied with fluctuation after slight rise of intensity. When the content of acid was so high that leaves suddenly became yellow and blighted. Meanwhile, strong emission was accompanied. The two experimental results discussed above suggested that in the condition of weak acid, for example, the initial toxic reaction of "acid-rain" resulting in the decrease of low level luminescence from leaf was essential for taking measures to reduce or avoid pollution in advance. People often consider sulphuric dioxide as one of the most dangerous toxins. In order to observe the influence of SO_2 pollution on luminescence from leaf of <u>Populus tomentosa</u> , we selected a branch of a tree to do experiments . The branch was covered with a polyethylene film bag which was piped with SO_2 gas produced in chemical reaction by compressed air into the bag. Collecting five leaves in the same time interval from the film bag and measuring intensity. The qualitative resultes were shown in Figure 7. With the experiments, we concluded two results. Firstly, "acid-rain" and H_2SO_4 showed similar effect on leaf luminescence. Luminescence from smoked leaves was reduced. When smoked heavily by SO_2, these leaves tended to be curly, drooped, or perished. At the same time, the luminescence curve showed "flash emission" which meant that the leaves were perished. Secondly, compared with control group(Figure 2a), emission spectrum of smoked leaf at 660 nm was

Table 3. Effect of seasons on leaf luminescence

Measure	In 1991				
	May	June	July	Sept.	Oct.
SO_2 ($\mu g/M^3$)	46	24	13	24	37
Intensity	734±696	1009±284	1506±788	9303±1844	8130±405

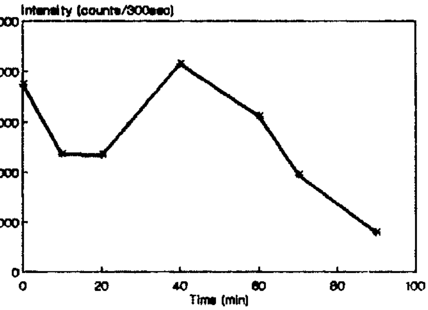

Figure 7. Influence of artificial SO_2 smoke on luminescence from leaf of Populus tomentosa

normal. Its intensity reduced 35%. But there was no distinct difference in blue light area, which was shown in Figure 2b, the result was significant to determine SO_2 pollution on leaves.

As shown in Table 3, the content of sulphur dioxide in air gradually took on decreasing tendency from May to July. However, the luminescence of leaf tended to rise, namely, the higher the content of SO_2, the lower the luminescence of leaf. This result was similar to SO_2 smoke experiment. When sampling took place in fall, the content of SO_2 increased slightly and luminescence of leaf rose greatly. This was possiblly related with that leaf appeared yellow when fall came. In general, the luminescence of Populus tomentosa leaf was changed with season, which decreased in spring and summer, but increased in fall. Compared with hygienic area as control, there were different changes in luminescence from leaves of Populus tomentosa among industrial, commercial and resident areas because of the various air pollution of toxins. For example, because there was shortage of water and annual pollution of SO_2 from burning coal, and even in summer there were some yellow spots and holes caused by pestes on leaf surface , so the intensity was 26% less than that of hygienic area. Besides, the intensity in resident area in October was 2.7 times as much as in June. There was no significant change in hygienic areas. The intensity in industrial area was greater than that of hygienic area.

Table 4. Luminescence from leaves in different functional areas

Measure		Industrial	Commercial	Resident	Hygienic
June	SO_2($\mu g/M^3$)	6-13	6-38	6-44	6-14
	Intensity	1696±108	234±473	1120±230	1494±284
Oct.	SO_2($\mu g/M^3$)	6-83	14-108	20-78	6-22
	Intensity	1238±143		3064±491	2963±535

In short, the low level chemiluminescence from leaf of Populus tomentosa showed certain changes to some extent with "acid-rain " damage and sulphur dioxide pollution, and these changes were related with the content of pollutant or pollution level. Therefore, it was possible that luminescence from leaf of Populus tomentosa may serve as an indicator to express air pollution. Without doubt, this simple, rapid method was more reliable and authentic than regular physical or chemical analysis.

REFERENCES

Cadenas E (1984) Free radicals in biology, Academic Press, New York, Vol 6., p 238

Popp FR (1987) Electromagnetic bio-information, Uradeu Schwarzeubers, Munick, pp 107-122

Slawinska D et al(1985) Chemi-and bioluminescence, Marcel Dekker New York, pp 495-531

Swinbanks D (1986) Body light points to health. Nature 324, 20 November, 203

Received March 12, 1992; accepted June 30, 1992.