Assessment of roadside air quality in the Tokyo metropolitan area by a novel biomonitoring method

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Abstract

In order to evaluate air quality in Tokyo, a novel biomonitoring method has been developed. This paper aimed to utilize the plant comet assay for determining the integrated effect of urban air quality and environmental stresses on the three plant species: Ginkgo, Pothos, and Periwinkle. The combination of air pollutants caused by traffic was suspected as the main stressor of DNA damage in the urban biota. Therefore, the different patterns between DNA migration ratios of the selected test species in roadside and non-roadside areas in an urban environment were observed. This study also focused on the change in the degree of DNA damage depending on the exposure time due to the environmental stresses, including the additional effect induced by roadside air pollutants.

Keywords: plant comet assay, air pollution, biomonitoring, Ginkgo, Pothos, Periwinkle, Tokyo.

1 Introduction

Tokyo, a city employing over 12 million population and millions of vehicles, is known as one of the largest city in Asian region. The city is densely populated and large amount of people are exposed to motor vehicle exhaust. The assessment of health risk caused by hazardous compounds in vehicle exhaust has been carried out mainly by measuring known hazardous substances in the atmosphere. However, such approach may not be able to find risks by unknown substances or a substance that cannot be measured by instrumentation analyses.
This investigation aimed to apply a novel biomonitoring method in order to assess integrated risk of urban air quality in Tokyo, Japan. The method applied for this investigation was “plant comet assay.” This method enables the measuring of air quality by quantifying the degree of DNA fragmentation of plant cell caused by the hazardous substances in the atmosphere. Test plants for this study were Pothos, Ginkgo leaves, and Periwinkle. The test were carried out by exposing three test plants in roadside and non-roadside areas for a long term (three months), sampling plant leaves, and perform comet assay to measure DNA fragmentation of damaged plant cells.

2 Materials and methods

2.1 Area of study and Experimental set up

The study was performed in Hongo campus of the University of Tokyo located in Bunkyo ward inside Tokyo Metropolitan Area. This area is categorized as the residential zone in an urban environment. The weather of Tokyo is mostly temperate, with 4 seasons: spring (April-June), summer (July-August), autumn (September-November) and winter (December-March). Rain falls throughout the year, but main rainy period usually starts from June to early of July. Typhoons mostly occur in September or October. Meteorological parameters measured by Japan Meteorological Agency were shown in Figure 1.

![Figure 1: Meteorological data of Tokyo, Japan during April to October 2003.](image)

In this study area, temporal variation of particulate polycyclic aromatic hydrocarbons (pPAHs), one of the traffic-related air pollutants, and the dispersion pattern were described [1]. Roadside results showed higher peak and average concentration of pPAHs than non-roadside ones. This probably suggested the widespread dispersion of pPAHs over the entire study area. The result could reflect the transportation of air pollutants from roadside to non-
roadside areas as a result of the convection by local wind characteristics and diffusion by concentration gradient.

To compare the integrated effects in different levels of air pollutants on living species, roadside and non-roadside biomonitoring were conducted. Sampling positions inside the university and on Hongo Street were selected as shown in Figure 2.

The experiment was carried out during April-October 2002. Before setting at sites, stock pothos and periwinkle were analyzed. Their results were used as the initial damages. Roadside samples were collected from a ginkgo tree on Hongo Street and a set of both pothos and periwinkle placed at the ground floor of the 14th bldg. of School of Engineering. In the non-roadside area, a set of both active biomonitorors was placed on the roof of the 1st bldg. of School of Laws with the height of 12 m approximately and a ginkgo tree in front of Yasuda Hall was selected.

Figure 2: Sampling sites in Hongo campus, The University of Tokyo, Tokyo, Japan.

The first set of ginkgo leaves was observed since the beginning of April 2002. With young age, leaf structure was too soft to be sliced by simple mechanical cutting. Then the first ginkgo leaves was sampled at the end of April.
The exposure of Pothos and Periwinkle in roadside and non-roadside areas started on 9 and 13 May 2002 respectively. Test species were placed inside well-ventilated transparent plastic chamber placed in partial-shade area to prevent extreme sunlight, rainfall and wind.

Biomonitors were exposed to the combination of air pollutants and other environmental stresses. The field conditions are illustrated in Table 1.

<table>
<thead>
<tr>
<th>Environmental Factors</th>
<th>Ginkgo</th>
<th>Pothos</th>
<th>Periwinkle</th>
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<tbody>
<tr>
<td><strong>Resource competition:</strong></td>
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<tr>
<td>Growing space</td>
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<tr>
<td>Nutrients</td>
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<td>(planted in Cl-free water)</td>
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<tr>
<td>Sunlight</td>
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<td>(partial shade)</td>
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<td>Water</td>
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<td><strong>Chemical factors:</strong></td>
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<tr>
<td>Toxic elements</td>
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<td>Pesticides</td>
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<td>O</td>
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<tr>
<td>(none)</td>
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<tr>
<td>Air pollution</td>
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<tr>
<td>- Composition</td>
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<tr>
<td>(roadside and non-roadside)</td>
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<tr>
<td>- Exposed position</td>
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<td>(roadside and non-roadside)</td>
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<tr>
<td><strong>Human disturbances</strong></td>
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<td>(remote area)</td>
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</table>

Note: “−”: uncontrolled (natural) condition; “O”: controlled condition; “−/O” Partially controlled condition.

### 2.2 Leaf sampling method

Without any visible injury, 50 leaves from different branches of the selected ginkgo were randomly collected in the range of 1.5-3m height, while 3-4 leaves per type of pothos and periwinkle were taken from unfixed positions. Average leaf sizes of ginkgo, pothos and periwinkle were ranged between 8-10, 4-5, and 1.5-2 cm width, respectively. After harvesting, leaf samples were kept in an icebox and immediately brought to a laboratory for analysis.

### 2.3 Comet assay

The comet assay procedure was followed to the method described in authors’ previous reports [2, 3].
3 Results and discussion

3.1 DNA distribution pattern of three test plants

Figures 3, 4, and 5 show the change in distribution pattern of DNA damage from the beginning stage until the end of exposure. With increasing exposure time, distribution patterns shifted from low to high degrees of damage. In addition, roadside samples yielded higher degree of damage than non-roadside ones. And different results among species could be observed.

3.1.1 Ginkgo (Ginkgo biloba)

During the beginning period of spring, fluctuation of DNA damage was observed in young leaves. Fully developed ginkgo leaves were observed in June. After leaf maturation in June, degree of DNA damage increased exponentially in both roadside and non-roadside samples, but in different rates. This phenomenon probably corresponded to the respiration pattern of deciduous leaves at different
phenological stages [4]. During unfolding stage of leaves, constructional respiration is relatively higher than the maintenance one. It leads to higher exchange rate of all gases, including air pollutants, through the open stomata. With differentiation and maturation of the tissues, respiration activity usually drops to the level of maintenance respiration. In addition, the higher level of repair activity in newly emerged leaves was reported in a model plant [5]. In natural condition, leaves had been continuously exposed to the combination of environmental stresses since they sprang. Accumulative damage might increase with their ages. After leaf maturation, damage inflicted by air pollutants and other stresses might not be completely repaired in mature leaves. Chronic damage could be observed as the exponential increase of degree of DNA damage depending on the exposure time detected in this study.

Figure 4: Results of long-term exposure test on Pothos. (a) Distribution of DNA migration ratios (non-roadside); (b) Boxplot with outlier “o” and extreme “***” values (non-roadside); (c) Distribution of DNA migration ratios (roadside); and (d) Boxplot with outlier “o” and extreme “***” values (roadside).

In order to establish the relationship between degree of DNA damage and the exposure time, fluctuated data of young leaves were excluded. Only mature leaf
data were used to figure out the relationship between degree of DNA damage and the exposure time (days) (Figure 6) as:

\[ \text{Degree of DNA damage} = A \cdot e^{B \cdot \text{time}} \]  \hspace{1cm} (1)

For Non-roadside ginkgo: \[ A = 0.116; B = 0.008 \text{ d}^{-1}; R^2 = 0.958 \]
Roadside ginkgo: \[ A = 0.124; B = 0.010 \text{ d}^{-1}; R^2 = 0.982 \]

Figure 5: Results of long-term exposure test on Periwinkle. (a) Distribution of DNA migration ratios (non-roadside); (b) Boxplot with outlier “o” and extreme “*” values (non-roadside); (c) Distribution of DNA migration ratios (roadside); and (d) Boxplot with outlier “o” and extreme “*” values (roadside).

From the exponential relationship, “A” might be interpreted as the “Restabilized level” reflecting the exposure history of an individual tree, and “B” should be the “response factor” that could indicate the degree of plant response to the environmental stresses. It could be inferred that higher response factor found in roadside samples probably indicated the higher response level of plant under roadside environment.
Figure 6: Time dependent response curve of degree of DNA damage of ginkgo non-roadside and roadside samples (GNH and GRH).

Figure 7: Progressive response of ginkgo induced by roadside air pollution (R/N ratios).
To extract the additional response induced by air pollution to the roadside biomonitor, ratio between degree of DNA damage in roadside sample and that of non-roadside one (R/N ratio) was introduced and calculated based on results from Eq. (1). Damage in non-roadside sample was considered as the background damage mainly from other environmental stresses. Then R/N ratio potentially reflected the additional response of roadside biomonitor to the intensity of roadside air pollutants in a series of time. Increase in additional response was proportional to exposure time (Figure 7). It probably indicated the progressive effect in the exhaustion phase, in which chronic effect of plants under stresses occurred.

Figure 8: Overall time-dependent response curve of degree of DNA damage of pothos non-roadside and roadside samples (PNH and PRH).

3.1.2 Pothos (*Epipremnum aureum*)

Pothos could be planted throughout the exposure period without visible severe injury. Linear increase in degrees of DNA damage of both roadside and non-roadside samples were observed at the beginning stage. However, exponential increases in their DNA damage were detected after a month of exposure. At the final stage of exposure test, stable degree of damage was obtained. The overall response of pothos to the exposure time (days) could be expressed by a sigmoid (S-shaped) curve (Figure 8) as:

\[
Degree \ of \ DNA \ damage = \frac{A}{1 + e^{-(C+B\times time)}}
\]
For Non-roadside pothos: \( A = 0.655; B = 0.096; C = -6.45; R^2 = 0.989 \)
Roadside pothos: \( A = 0.705; B = 0.110; C = -6.04; R^2 = 1.000 \)

![Graph showing progressive response of pothos induced by roadside air pollution](image)

Figure 9: Progressive response of pothos induced by roadside air pollution (R/N ratios).

Time dependent-response pattern found in this study might be associated with the basic concept of dose-response in the toxicological study. Therefore, response pattern of genetic damage of pothos to the environment could be explained as: First, pothos cell structures and functions might be destabilized by environmental stresses. But pothos might have its tolerant ability to repair itself. Thus, fluctuated degrees of damage could be observed during the first stage of exposure. After a period of time that stresses still existed, sudden increase in damage could be detected. However, pothos showed its defensive and adaptive ability resulting in the new stabilized conditions or acceptable damage levels, in which the new constant damage level could be obtained. Shapes and slopes of time dependent-response curves from non-roadside and roadside samples were important in their response differentiation. Slope of curve probably explained the response per unit change in dose or exposure time [6]. Maximum slopes differentiated from equations were used. Roadside pothos with maximum slope of 0.019 d\(^{-1}\) at 55 days after exposure was steeper than non-roadside one with maximum slope of 0.015 d\(^{-1}\) at 68 days after exposure. It probably indicated the higher increase rate of DNA damage or severer situation observed in roadside species.
S-shaped curve probably described exposure time-response pattern of the specific biomonitor in the field before irreversible damage in plant occurred. Thus, constant “A”, “B”, and “C” in Eq. (2) might be interpreted as the “Restabilized level”, “response factor”, and “tolerant ability” of the specific biomonitor.

To clarify the additional damage induced by roadside air pollutants, R/N ratios of calculated values from Eq. (2) were plotted (Figure 9). Trend of R/N ratios might be compared to the common response phase of plant under environmental stresses. Progressive response to the air pollution was observed in the initial stage of exposure. It might be explained by the intensification of the impairment from roadside air pollutants since pothos was introduced into the monitoring area. Additional damage reached its maximum at 44 days after exposure. The decrease of additional damage might indicate the effect of other environmental factors on both pothos plants in roadside and non-roadside areas. As discussed previously, plants could protect themselves with defensive and adaptive capabilities. These mechanisms leaded them on to the resistance phase, in which the resistance increases. Constant R/N ratios in the final stage might indicate the re-stabilized condition of roadside pothos after long-term exposure to the roadside air pollution.

Figure 10: Time dependent response curve of degree of DNA damage of periwinkle non-roadside and roadside samples (VNH and VRH).

3.1.3 Periwinkle (Vinca rosea or Catharanthus roseous)
Difference in degrees of DNA damage could be observed clearly between results of roadside and non-roadside samples since the first stage of the exposure. It
became greater in a relative proportion of the exposure time. After 2 month of exposure, roadside periwinkle showed visible injury and died. Rapid damage in DNA of samples from both monitoring sites might lead plants to the irreversible damage or overtaxed stage beyond the defensive and adaptive capacity. Degree of DNA damage increased exponentially with the exposure time (days) illustrated in Figure 10. Thus, the exponential function and the interpretation of constant “A” and “B” in Eq.(1) could be used as well.

For Non-roadside periwinkle: \[ A = 0.176; \quad B = 0.004; \quad R^2 = 0.761 \]
Roadside periwinkle: \[ A = 0.176; \quad B = 0.016; \quad R^2 = 0.919 \]

Similar exposure history of test plants prior to the exposure test could be presented with nearly similar “A” values obtained from roadside and non-roadside samples. Degree of DNA damage in roadside samples increased suddenly resulted in the great difference in the response factors, “B”, between roadside and non-roadside samples. This probably reflected the sensitivity of periwinkle to the roadside environmental conditions. Acute collapse of cell integrity might be high enough to dominate the repair mechanism. Then visible injury or irreversible damage with high degree of DNA damage could be observed before death occurred. Considered in R/N ratios curve (Figure 11), periwinkle showed progressive additional effect caused by roadside air quality.

![Figure 11: Progressive response of periwinkle induced by roadside air pollution (R/N ratios).](image-url)
4 Conclusions

Long-term biomonitoring presented overall responses of test plants to roadside and non-roadside environments. Different response patterns and observation period were detected in different plant species, but higher degrees of DNA damage were observed from all roadside test species. At the beginning stage of the exposure, linear increase in degrees of DNA damage, followed by their exponential increase were observed in fully developed leaf samples of all biomonitoring species. Plant could perform its adaptive and defensive mechanisms, if it is exposed to stresses for a long period. Overall response of plant under long-term stresses could be presented with the S-shape response curve as shown in the pothos case. “Re-stabilized level”, “Response factor” and “Tolerant ability” of pothos were defined from time-dependent response curve. To explain additional responses induced by roadside air pollution, R/N ratio was calculated. Additional damage induced by roadside air quality could be detected since test plants initially exposed to the roadside environment. Then, the other environment factors and defensive mechanisms of plant itself presented their effects on both roadside and non-roadside test plants. R/N ratio decreased and kept constant stable condition with a limit of tolerance. However, irreversible damage or even death might take place with the limitation of plant tolerance and its leaf cycle. Phase of plant response was considered as a significant factor for setting the appropriate monitoring period for each monitoring species. Period of the exposure should be in the stage of damage development before defensive mechanisms and apoptosis in the cell cycle take place. From this study, suitable monitoring period for ginkgo was in June-August or in summer of Tokyo. Too young ginkgo leaves yielded fluctuated result probably related to their high rates of respiration and repair activities. On the other hand, too old leaves in autumn were not recommended for biomonitoring purpose. Additional noise or damage might occur in apoptosis cells. For the active biomonitoring approach, the exposure time could be controlled. In the short-term monitoring, pothos should be set at sites not more than a month in order to avoid the effect from defensive mechanism, while periwinkle was not recommended to be further used as a biomonitor in this study due to it high sensitivity to the experimental condition.

References


