Chemical Analysis of Transplanted Aquatic Mosses and Aquatic Environment during a Fish Kill on the Chungnang River, Seoul, Korea

Joohyoung Lee, Perry Johnson-Green[1], and Eun Ju Lee*

School of Biological Sciences, Seoul National University, Seoul 151-742, Korea;
[1]Department of Biology, Acadia University, Wolfville, NS B0P 1X0, Canada

Key Words:
Aquatic moss
Fish kill
Biomonitoring
Industrial effluent
Transplant

In mid-April, 2000, hundreds of thousands of fish floated dead on the Chungnang River, one of the small branches of the Han River in Seoul. We examined the causes of the accident in detail, through analysis of monitoring data from the Han River Monitoring Project, which employed the transplanted aquatic moss, *Fontinalis antipyretica*. This allowed investigation of another possible cause of the fish kill: release of trace metals into the river from industrial sources during the rainfall event. In addition, we aimed to verify the usefulness of aquatic mosses as bioindicators of the event. Water samples collected 48 h after the fish kill exhibited low pH and high Total-N and Total-P, indicating that acidic compounds rich in nitrogen and phosphorus might be a major contaminant. BOD and COD were also very high. On the whole, the conditions of the river water were degraded at that time. Distinct trends were not observed in the chlorophyll phaeophytinization quotient and photosynthesis rate of transplanted mosses. However, mosses sampled soon after the accident exhibited the lowest values for those variables ($p < 0.01$), suggesting that stress factors in the river were diluted out over time. Heavy metals with characteristics of industrial effluents (Cr, Pb, Zn, Fe, Cu, and Cd) increased ($p < 0.01$), indicating that they were unlikely to be major causes of the accident.

On April 21, 2000, hundreds of thousands of fish (mostly carps and catfishes) floated dead on the Chungnang River, one of the small branches of the Han River in Seoul, South Korea. Soon after the accident, a big campaign using air and water vehicles transported millions of fish to upstream reaches of the Han River. This accident drew our attention because the Chungnang River was included in our Han River Monitoring Project, which uses transplanted aquatic mosses as biomonitors. A sewage disposal plant is located 2 km upstream of the accident site. Its working capacity is 70,000 t/h of sewage, but when the accident happened, the inflow to the plant was over 140,000 t/h because of a rainfall (9 mm/day). A total of three hundred thousand tons of untreated sewage was discharged to the Chungnang River. This may have been the primary cause of the fish kill according to the government's official conclusion.

Another possibility is that the fish kill was caused by intentional or unintentional release of trace metals into the river from the industrial complex located on the upstream, during the rainfall event. In order to examine the causes of the accident, we analyzed monitoring data from the site of the fish kill, including physicochemical analysis of the water and the aquatic moss *Fontinalis antipyretica*.

Biomonitors can be used to obtain time-integrated information about biological impacts of trace pollutants. In contrast, obtaining water samples representative of the temporal pollution pattern is a labor-intensive and expensive exercise. In addition, water samples, whether collected instantaneously or continuously, do not provide accurate information about the bioavailable, *i.e.* the potentially hazardous, fraction of trace pollutants (Mouvet, 1984; Mersch and Pihan, 1993).

Unfortunately, naturally occurring indicator species are rare or even absent under biologically adverse conditions next to the discharge point of an effluent. In receiving water body, an assessment of trace pollution is possible only further downstream, after considerable dilution. As such, it is necessary to employ a transfer technique of an adequate biomonitor for direct effluent testing. The transfer technique has been used (1) when no indigenous mosses are available, (2) to obtain indications representative of a limited period of time, and (3) to provide comparable data from different

* To whom correspondence should be addressed.
Tel: 82-2-880-6673, Fax: 82-2-872-6881
E-mail: ejlee@plaza.snu.ac.kr

sites with heterogeneous biological materials (Mouvet, 1984; Kelly et al., 1987; López et al., 1994; Mersch and Kass, 1994; Fernández et al., 2000; Reinmann et al., 2001).

Among freshwater biomonitors, the above prerequisites are fully satisfied by aquatic bryophytes. Mosses have been successfully employed to monitor trace metals in surface waters under contrasting environmental conditions (Goncalves et al., 1992; Mersch and Pihan, 1993; Mersch et al., 1993a). Combined with a high accumulation capacity of trace metals, rapid uptake kinetics allows mosses to provide a reliable response after short exposure durations. Thus, immersion periods between 1 and 10 days have been considered sufficient (Mouvet, 1984; Kelly et al., 1987; López et al., 1994). It has been shown that transplantation *per se* has no substantial effect on the accumulation process of trace metals (Kelly et al., 1987), nor on the vitality of mosses (López et al., 1994).

The objectives of this study were to reveal the trend and extent of changes in biological and physicochemical characteristics of transplanted bryophytes by deteriorated water quality, and to verify their usefulness for biomonitoring methods in Korean environment.

Methods

Study site

Seoul (127°00′E, 37°32′N) is located in west-central Korea. The Han River runs across the city (605 km^2), dividing it into 'River-North' and 'River-South' with almost equal areas. The Chungnang River comprises over ten tributaries starts from North of Seoul, Kyung-Gi Province, and meets the Han River in the center of the city. The study sites are shown in Fig. 1.

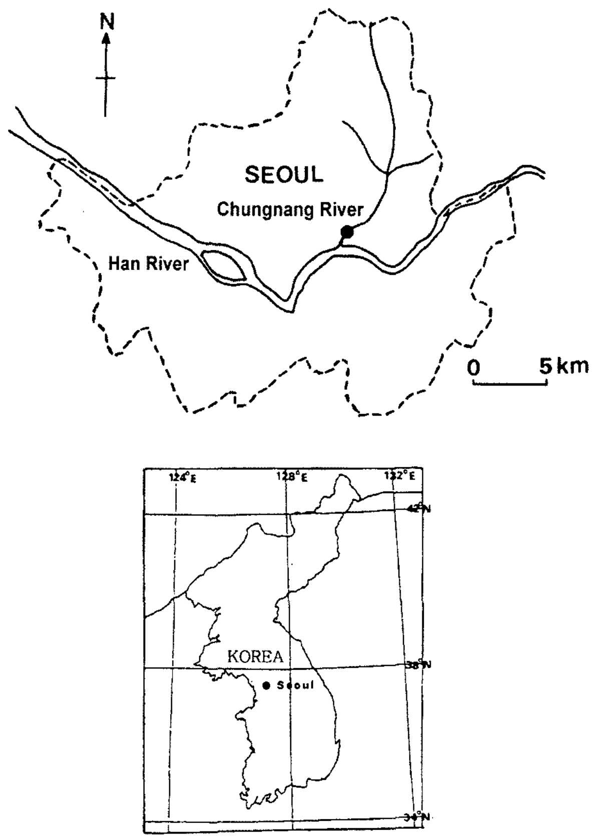

Fig. 1. A map of the study site on the Chungnang River, Seoul.

Moss transplant

Aquatic bryophytes (*Fontinalis antipyretica*) were identified (Choi, 1980) and collected at several small streams of Chungpyung, which is located in an unpolluted source stretch of the Han River.

They were transplanted (n = 5) (500 g for each event), as being attached to the buoy which is bound to the bank, on April 19, and collected on April 22 (the accident occurred during the night of April 20). Mosses were transplanted and recollected every two days until April 30. There was no more rainfall during this period.

Mosses were first rinsed in river water and then, in the laboratory, washed with distilled and deionized water consecutively, and stored for 24 h in a refrigerator prior to analysis.

Sample analysis

Each moss sample was divided into two subsamples; one was used for metal analysis, and the other for photosynthetic assays. The 'metal' subsample was rinsed thoroughly in distilled water before analysis and dried at 60℃ for 24 h. Enough materials were taken to give a final dry weight of 250-300 mg per sample. Plants were then placed in acid washed mini-autoclaves of Teflon and digested with 15 mL of 7.2 M HNO_3 at 140℃ for 4 h. The digest was made up to a final volume of 25 mL with distilled deionized water. Trace metals were measured (n = 5) using atomic absorption spectrophotometry (AAS) and inductively coupled plasma emission spectroscopy (ICP-AES) according to the previously described procedures (Goncalves et al., 1992; Mersch et al., 1993b; Mersch and Pihan, 1993). The chlorophyll phaeophytinization quotient (D665/D665a) ratio was measured in the remaining subsample (López and Carballeira, 1990). Photosynthesis-respiration rates were determined by the light/dark bottle technique (Carballeira et al., 1998). Water samples (500 mL) in which the mosses were submerged were collected the moment the mosses were recollected. The basic analysis of river water followed standard methods, and measured once (APHA, 1985; Carballeira et al., 1998; Gagnon et al., 1998). ANOVA test was employed for the statistical analysis (SAS;1999).

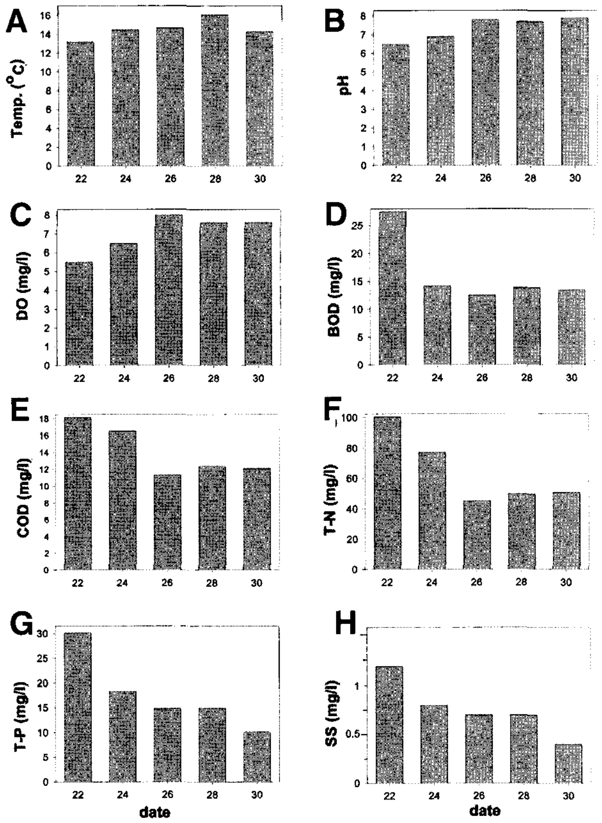

Fig. 2. Temperature (A), pH (B), DO (C), BOD (D), COD (E), Total-N (F), Total-P (G), and Suspended Solids (H) of Chungnang River, measured every two days from 22 April to 30 April, 2000 (n = 1).

Results and Discussion

Basic water conditions

Temperature, pH, DO, BOD, COD, Total-N, Total-P, and SS (suspended solids) of the river water, measured every two days from April 22 to 30, are shown in Fig. 2. The low pH and high T-P, and T-N of day 22 samples indicated that nitrogen and phosphorus-rich materials had contaminated the river. Microbial breakdown of sewage would be expected to release large amounts of nitrogen and phosphorus, and to drive down the pH. Such microbial activity would also be expected to lead to low levels of oxygen in the river. Indeed, DO was very low in this measure. Levels of BOD and COD were also high in the initial samples. Temperature was slightly lower than average values initially, because of rain. Overall, the conditions of the river water were degraded immediately following the rainfall event. After some fluctuations all the variables returned to the normal range at the final sampling time.

Stress parameters

The chlorophyll phaeophytinization quotient (D665/D665a) and other pigment-based indices are reliable

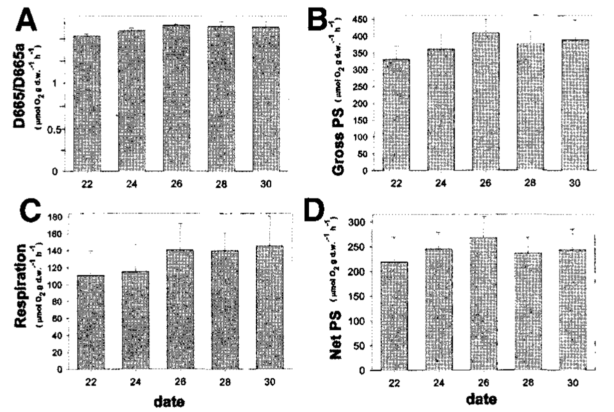

Fig. 3. Chlorophyll phaeophytinization quotient (A), gross photosynthesis (B), respiration (C), and net photosynthesis (D) of the transplanted mosses from 22 April to 30 April, 2000 (n = 5).

measures of stress in aquatic bryophytes. Especially they are low-cost, rapid, and effective (Peuelas, 1984; López and Carballeira, 1990; López et al., 1994). Decreasing quotient reflects increasing stress, and small but persistent effects on pigment ratio can be associated with marked effects on photosynthesis and the long-term survival. Pigment indices, however, do not necessarily exhibit simple relationships with photosynthesis. For instance, net photosynthesis is best predicted from D665/D665a ratio by an exponential function (Carballeira et al., 1998). In this study the chlorophyll phaeophytinization quotient and photosynthesis rate were used as indicators of stress. Photosynthesis in aquatic bryophytes is largely controlled by three factors: temperature, light intensity, and inorganic nutrient availability (Maberly, 1985a, 1985b; Dilks and Proctor, 1975). Due to the higher chlorophyll content of aquatic mosses, photosynthesis in general is saturated at lower levels of light intensity than that in other small plants (Kallio and Karenlampi, 1975; López and Carballeira, 1990). Therefore aquatic mosses are suitable as the short-term bioindicators. Low photosynthetic rates indicate physiological or structural impacts imposed by hostile environmental conditions.

No clear trends were observed in photosynthetic variables (Fig. 3). However, mosses sampled on the first occasion after the accident had the lowest levels for most variables ($p < 0.001$) except D665/D665a value, indicating stress factors in the river that were soon diluted out (Table 1).

In addition, despite only a two-day exposure in the river, the transplanted mosses suffered from adverse environmental conditions. Light green to yellowish colored leaves with brown edges were observed.

Metal accumulation

The pattern of accumulation of metals exhibited two distinct patterns (Fig. 4). The concentrations of Cr, Pb, Zn, Fe, Cu, and Cd increased with time, whereas

Table 1. Weighted Poisson ANOVA test about the changes of D665/D665a, gross photosynthesis (PS), respiration, and net photosynthesis (PS) values

Values	*df*	*F*	*P*
D665/D665a	4	1.22	0.3332
Gross PS	4	1265	0.0001
Respiration	4	432	0.0001
Net PS	4	449	0.0001

Table 2. Weighted Poisson ANOVA test about the changes of heavy metal accumulation values

Heavy metals	*df*	*F*	*P*
Cr	4	157.37	0.0001
Pb	4	338.25	0.0001
Zn	4	1204.60	0.0001
Ca	4	6.07×10^{-5}	0.0001
Mn	4	5.19×10^{-6}	0.0001
Fe	4	1.35×10^{-7}	0.0001
Cu	4	576	0.0001
Cd	4	9.69	0.0002

those of Ca and Mn decreased ($p < 0.001$) (Table 2). It would be expected that heavy metal contents in the river increased, because the rainwater influx diluted the basic contents at first, then the river was restored to the usual level. However, Ca and Mn decreased in spite of the dilution effect. This means that they came into the river in a large quantity during the rainfall.

The metals that increased with time included those with characteristics of industrial effluents. If heavy metal effluents were discharged during the rainfall event, the first samples should have had the highest values. Therefore, it appears that biohazardous metals were not the cause of the fish kill. Ca and Mn could be a normal constituent of human sewage, and anoxic conditions following the rainfall may have led to the increased reduction and solubilization of Mn.

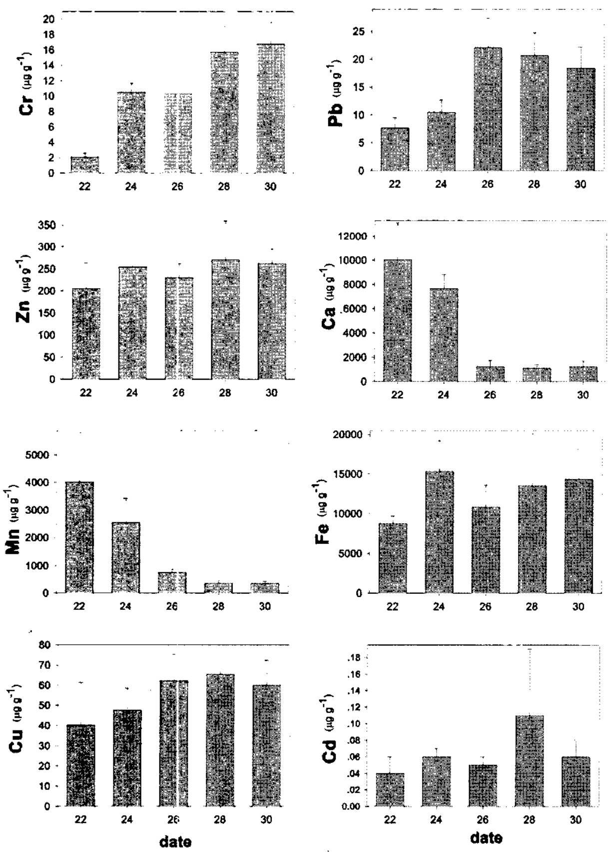

Fig. 4. The accumulations of heavy metals in the transplanted mosses (μg g-1) from 22 April to 30 April (n = 5).

If toxic heavy metals were in anionic forms, they might not accumulate efficiently in the moss. Also, ions in the river may have led to ion exchange of metals absorbed to the moss, or the time of exposure to metals may have been insufficient for the moss to reach equilibrium, even though other studies showed a few hours to days were optimum for equilibrium (Pickering and Puia, 1969; Mouvet, 1987).

Conclusion

For complex effluents, it is difficult to identify the major factors responsible for physiological damage to organisms. From the metal accumulation data it is speculated that the discharge of industrial effluents was not the direct cause of the accident. However, possible causes of organic pollution other than heavy metals (e.g. industrially derived hydrocarbons or other toxic compounds) cannot be excluded. When the accident occurred, fish moved upstream for annual spawning. The density of fishes, therefore, was very high in the contaminated river. This aggravated the accident. If it had rained more heavily, pollutants in the river would have been much diluted, but if the influx of rainwater had been smaller, the sewage disposal plant could have successfully cleared it. Therefore, too much, but not enough, precipitation was one of the causes of the fish kill.

However, the most fundamental problem would be the fact that the Chungnang sewage disposal plant still has a confluence sewer system, and human sewage and runoff are treated and discharged together. The government admits that the system has defects, but does not plan to repair it, because revenue sources cannot be secured in the near future.

Acknowledgements

This study was supported by Brain Korea 21 program and we thank Mr. J. H. Kim for his valuable technical assistance.

References

APHA (1985) Standard Methods for the Examination of Water and Wastewater. 16th Ed. United Book Press, Inc., Baltimore.
Carballeira A, Díaz S, Vázquez MD, and López J (1998) Inertia and resilience in the responses of the aquatic bryophyte

Fontinalis antipyretica Hedw. to thermal stress. *Arch Environ Contam Toxicol* 34: 343-349.

Choi DM (1980) Illustrated Flora and Fauna of Korea. Vol. 24. Korea Ministry of Education, Seoul.

Dilks TJK and Proctor MCF (1975) Comparative experiments on temperature responses of bryophytes: assimiliation, respiration and freezing damage. *J Bryol* 8: 317-336.

Fernández JA, Aboal JR, and Carballeira A (2000) Use of native and transplanted mosses as complementary techniques for biomonitoring mercury around an industrial facility. *Sci Total Environ* 256: 151-161.

Gagnon C, Vaillancourt G, and Pazdernik L (1998) Influence of water hardness on accumulation and elimination of cadmium in two aquatic mosses under laboratory conditions. *Arch Environ Contam Toxicol* 34: 12-20.

Goncalves EPR, Boaventura RAR, and Mouvet C (1992) Sediments and aquatic mosses as pollution indicators for heavy metals in the Ave river basin (Portugal). *Sci Total Environ* 114: 7-24.

Kallio P and Karenlampi L (1975) Photosynthesis in mosses and lichens. In: Cooper JP (ed), Photosynthesis and Productivity in Different Environments, Blackwell, Oxford, pp 393-423.

Kelly MG, Girton C, and Whitton BA (1987) Use of moss-bags for monitoring heavy metals in rivers. *Water Res* 21: 1429-1435.

López J and Carballeira A (1990) A comparative study pigment contents and response to stress in five species of aquatic bryophytes. *Lindbergia* 15: 188-194.

López J, Vázquez MD, and Carballeira A (1994) Stress responses and metal exchange kinetics following transplant of the aquatic moss *Fontinalis antipyretica* Hedw. *Freshwater Biol* 32: 185-198.

Maberly SC (1985a) Photosynthesis by *Fontinalis antipyretica* I. Interaction between photon irradiance, concentration of carbon dioxide and temperature. *New Phytol* 100: 127- 140.

Maberly SC (1985b) Photosynthesis by *Fontinalis antipyretica*. II. Assessment of environmental factors limiting photosynthesis and production. *New Phytol* 100: 141-155.

Mersch J, Gurold F, Rousselle P, and Pihan JC (1993a) Transplanted aquatic mosses for monitoring trace metal mobilization in acidified streams of the Vosges Montains, France. *Bull Environ Contam Toxicol* 51: 255-259.

Mersch J and Kass M (1994) La mousse aquatique *Fontinalis antipyretica* comme traceur de la contamination radioacitve de la Moselle en aval de la centrale nuclaire de Cattenom. *Bull Soc Natur Luxembourg* 95: 109-117.

Mersch J, Morhain E, and Mouvet C (1993b) Laboratory accumulation and depuration of copper and cadmium in the freshwater mussel *Dreissena polymorpha* and the aquatic moss *Rhynchostegum ripariodes. Chemosphere* 27: 1475-1485.

Mersch J and Pihan JC (1993) Simultaneous assessment of environmental impact on condition and trace metal availability in zebra mussels *Dreissena polymorpha* transplanted into the Wiltz River, Luxembourg. Comparison with the aquatic moss *Fontinalis antipyretica. Arch Environ Contam Toxicolol* 25: 353-364.

Mouvet C (1984) Accumulation of chromium and copper by the aquatic moss *Fontinalis antipyretica* L. ex Hedw transplanted in metal-contaminated river. *Environ Technol Letter* 5: 541-548.

Mouvet C (1987) Accumulation et relargage de plomb, zinc, cadmium, chrome et cuiver par des mousses auatiaues en millieu natural et au laboratorie. Laboratoire d'cologie, Universite de Metz, France and Agence de Lau Rhone, Mediterranee-Corse. Lyon, p 122.

Peuelas J (1984) Pigments of aquatic moss of the river Muga, NE Spain, and their response to water pollution. *Lindbergia* 10: 127-132.

Pickering DC and Puia IL (1969) Mechanism for the uptake of zinc by *Fontinalis antipyretica. Physiol Plant* 22: 653-658.

Reimann C, Niskavaara H, Kashulina G, Filzmoser P, Boyd R, Volden T, Tomilina O, and Bogatyrev I (2001) Critical remarks on the use of terrestrial moss (*Hylocomium splendens* and *Pleurozium schreberi)* for monitoring of airborne pollution. *Environ Poll* 113: 41-57.

[Received May 17, 2002; accepted August 30, 2002]