Electron microscopic observation on leaf-dead narcissused by volatilized heavy oil in Anto, Mikuni, Fukui Prifecture, Japan

ELECTRON MICROSCOPIC OBSERVATION ON LEAF – DEAD NARCISSUSES BY VOLATILIZED HEAVY OIL IN ANTO, MIKUNI, FUKUI PREFECTURE, JAPAN

Kazue TAZAKI[1], Ayumi AOKI[2] and Hiroaki WATANABE[3]

[1] Department of Earth Sciences, Kanazawa University, Kanazawa, Ishikawa 920-1192, JAPAN; e-mail: kazuet@kenroku.kanazawa-u.ac.jp
[2] #402, 1-1-12 Matsugawa, Naha, Okinawa 902-0062, JAPAN; e-mail: aokiayumi@hotmail.com
[3] 354-2 Oshidate, Inagi, Tokyo 260-0811, JAPAN

ABSTRACT

The effects of heavy oil spill on the air and land plants by means of SEM – DEX were studied. Shrinkage or reduction of plant stomata, deformation of surface and fine granular adhesion materials were observed on narcissus exposed in volatile substances from the C – typed heavy oil and contaminated by solvent used for cleaning up heavy oil spill. The leaves' surface was covered with sulfur – and phosphorus – rich slicks originally from gas of heavy oil and detergent, which could have caused not only the leaf – dead but also human health.

INTRODUCTION

The Russian Tanker, *Nakhodka*, the cause of heavy oil spill gave tremendous environmental damages to the shore of Hokuriku District, North – Central of Japan. Heavy oil rushed at the coastal areas influenced the ecosystem heavily, which also left great concerns about sea birds and oceanic organisms. We also grew to concern about health conditions of people doing heavy oil removal task, since volatile organic solvent was used as "detergent" for the purpose of washing off heavy oil. In this study, we observed micro particles in the air collected in Sodegahama, Wajima City and Nagahashi seashore, Suzu City, Ishikawa Prefecture on January 26th, 1997. During the follow – up investigations of April 8th, 1997 along the seashore in Anto, Mikuni Town, Fukui Prefecture where the bow – part of the *Nakhodka* had reached, we also recognized those leaf – dead narcissuses growing below a certain height of sea cliff. Narcissus was one of plants which blossom in early spring. Tazaki *et al.* (1996) reported that narcissus was sensitive to air pollution and exhaust fumes of a diesel engine caused narcissus's leaf – death. These suggested that the gas volatilized from heavy oil would have some effects not only on human health but also on land plants. However, there had been no report on land plants influenced by heavy oil contamination. Thus, it was remarkable to observe leaf – dead narcissuses on the scene of heavy oil spill, as well as dust particles in the air collected by a portable air sampler, due to discuss the acute toxicity of C – typed heavy oil.

In this study, we have observed the leaf surface of narcissuses with scanning electron microscope (SEM) and did qualitative analyses with EDX to study the causal relationship between leaf – dead narcissus and heavy oil spill. At the same time, we also observed narcissuses grown with C – typed heavy oil in the green house of Kanazawa University for comparison. Moreover, we examined the dust in the air collected by a portable air sampler in Sodegahama, Wajima City and Nagahashi seashore, Suzu City in Ishikawa Prefecture.

SAMPLES AND METHOD

Narcissuses used for this study were collected in Anto, Mikuni Town in Fukui Prefecture on April 8th, 1997. These leaf – dead narcissuses were found on the polluted seacliff in approximately three meters above the sea level (Fig. 1 right). Some parts of the tip leaves had turned into brown (Fig. 1, left and Fig. 2). In Kakuma campus, Kanazawa University, we prepared two pots of narcissuses: one with heavy oil in plastic covering, while the other outside of building without heavy oil for a control (Fig. 3). The narcissus which had been tested with heavy oil for one month resulted in turning black in many parts of leaves.

Dusts in the air were collected by a portable air sampler at Sodegahama, Wajima City and Nagahashi seashore, Suzu City in Ishikawa Prefecture on January 26th, 1997 due to study the effect on air environment by drifted heavy oil. Air sucking was taken place for seventeen hours at Sodegahama and for two hours at Nagahashi by sucking speed 530 l/ min with setting dust sampling filters (ADVANTEC TOYO) in the high volume air sampler, HVC – 500 (Shibata Scientific Technology Ltd.).

Three to four square – millimeters of both dead and alive leaves were cut off, and were fixed on carbon double tape. The dust collected by the air sampler was put on the carbon double tape directly from the filter paper. These samples were coated with carbon, and observed with an SEM (JEOL – JSM – 5200LV) and analyzed by EDX (Phillips – EDAX PV 9800STD), running by 15 kV.

RESULTS

Narcissuses exposed with heavy oil

Both narcissus leaves grown outside (Fig. 4A) and put with heavy oil (Fig. 4B) in Kakuma campus, Kanazawa University showed flat and smooth surfaces, although B appeared to be with more harsh adhesions. EDX showed high background with K and Ca peaks in outside narcissus, while Cl, K, and Ca peaks in narcissus with heavy oil. Most adhesions showed Na, Mg, Al, Si, P, S, Cl, K, and Ca peaks by means of EDX. As a result of SEM – EDX analyses, the adhesions around stomata showed heavy oil components, such as S, associated with aerosol dust components of Al and Si.

Narcissuses on the scene of drifted heavy oil

Leaf surface of most narcissuses in Mikuni Town where the bow – part of the *Nakhodka* had reached, were covered with films or adhesions, and some surfaces had relatively rough and sandy adhesions (Fig. 5A, B). The micromorphology of the leaf surface shows various types, such as spotted, spherical, flake – like, or needle – like shapes, and often including diatoms and halite. Fig. 5A shows spotted and flake – like films (arrow). In Fig. 5B, the stoma was buried in those filmy substances. The EDX analysis of the flat part showed high back ground with Na, Mg, Si, P, S, Cl, K, and Ca peaks (Fig. 5a). S and Ca suggest coming from the filmy substances (Fig. 5A, B arrow). The electron microscopy reveiled presence of fine and infinitesimal holes on the spotted films, which were widespread. Flakes (Fig. 5A, B) composed of clear Al, Si, P, S, Cl, K, and Ca elements (Fig. 5b). Granular adhesions showed remarkable Al and Si or Al, Si, K, and Fe peaks, suggesting mineral aerosol dust. Sometimes weak P, S, and Cl peaks were found. Some granular adhesions in 10 to 20 μm size showed high background with strong S peak, which suggested oil accessory ingredient. Some flakes (Fig. 5B arrow) also showed strong S and P peaks of organic materials. Given the above, films and adhesions apparently showed S and P were common elements in oil.

Dusts in the air around heavy oil spill

The SEM – EDX observation showed most air dusts collected by the air sampler were including halite particles which were originated from oceanic salts. Most halite grains are cubic structure and aggregated each other. The halite grains were attached with flat and granular adhesions, which the EDX resulted in Na and Cl from halite and strong S, K, and Ca peaks from gypsum and sulfate minerals. In addition, carbon soot and platy mineral substances which showed Al and Si were also determined.

DISCUSSION

Some local people and volunteers who joined in the cleaning task around the area of heavy oil spill reacted to vaporized kerosene used for washing off heavy oil, complaining some symptoms, such as headache, dizziness and nausea. Volatile substances in oil are benzene, toluene, and xylene, which were tested comparing with potable collector and fixed collector. The concentration of volatile substances using portable collector were two to three times higher than in fixed one (Ando *et al.*, 1997). This suggested that the volunteers working on cleaning task used to be exposed high concentration of vaporized kerosene. Also, Sawano (1997) pointed out that some toxic solvents were in use as "detergent" on removal sites. Sulfur in the common C – typed heavy oil is 0.1 to 3.0 %, which is a major factor of air pollution. It was no surprise that sulfur was detected in the emulsified heavy oil sample by means of EDX (Ueda and Ando, 1997; Tazaki, 1997).

The major substance, halite in the dust collected by air sampler was attached with an amount of adhesions which possibly were gypsum or Mg – K sulfate minerals, and films with rich S. In general, halite has flat and smooth surface as a dust in the air with few adhesions (Watanabe *et al.*, 1992). Zhou *et al.* (1996) reported that gypsum as a common mineral in aerosol reacts to calcite and sulfur while transferring in the air. In this study, almost all halite crystals had adhesions like gypsum and films, probably as a result of reacting with sulfur vaporized from heavy oil and Mg, K, and Ca in seawater.

SEM observations of the leaves of narcissus which showed leaf – dead in Anto, Mikuni Town clarified that the leaf surface and the stomata were covered with adhesions and filmy substances. This could prevent the narcissus from growing healthy since stomata are very important to photosynthesize. EDX analysis showed that those adhesions and films were consisted not only by Al – and Si – clay minerals originally from soils but also by P, S, Cl, and K, where Cl is originally from seawater. Additionally, the emulsified C – typed heavy oil showed strong S peak in EDX analysis, and so did the substances attached around the stomata of narcissus grown in Kanazawa University, which suggested that the sulfur of adhesions and films on narcissus leaves could be a result of diffusing fumes from drifted heavy oil. The P and K peaks were considered to have the same origin, which was vaporized from detergents.

Although it had been half a year since the heavy oil had damaged the seashore in Mikuni Town in 1997, we still could see some evidences of heavy oil contamination on land plants around Mikuni Town (Figs. 1, 2). Therefore, it would be very important to investigate the area continuously.

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Figure 1 Leaf – dead narcissuses (left) in Mikuni wharf, Anto, Fukui Prefecture, Japan (right). The heavy oil reached on April 8th, 1997.
The leaf – dead narcissuses were found on the polluted sea cliff in approximately three meters above the sea level.

Figure 2 Leaf – dead plants on the scene (upper) and the samples for scanning electron microscopic observations (down right).

Figure 3 Normal, healthy, and beautiful narcissuses in Kanazawa City on April 11th, 1997 (upper), and experimental exposure of C – typed heavy oil (down right), whereas the left for a control sample.

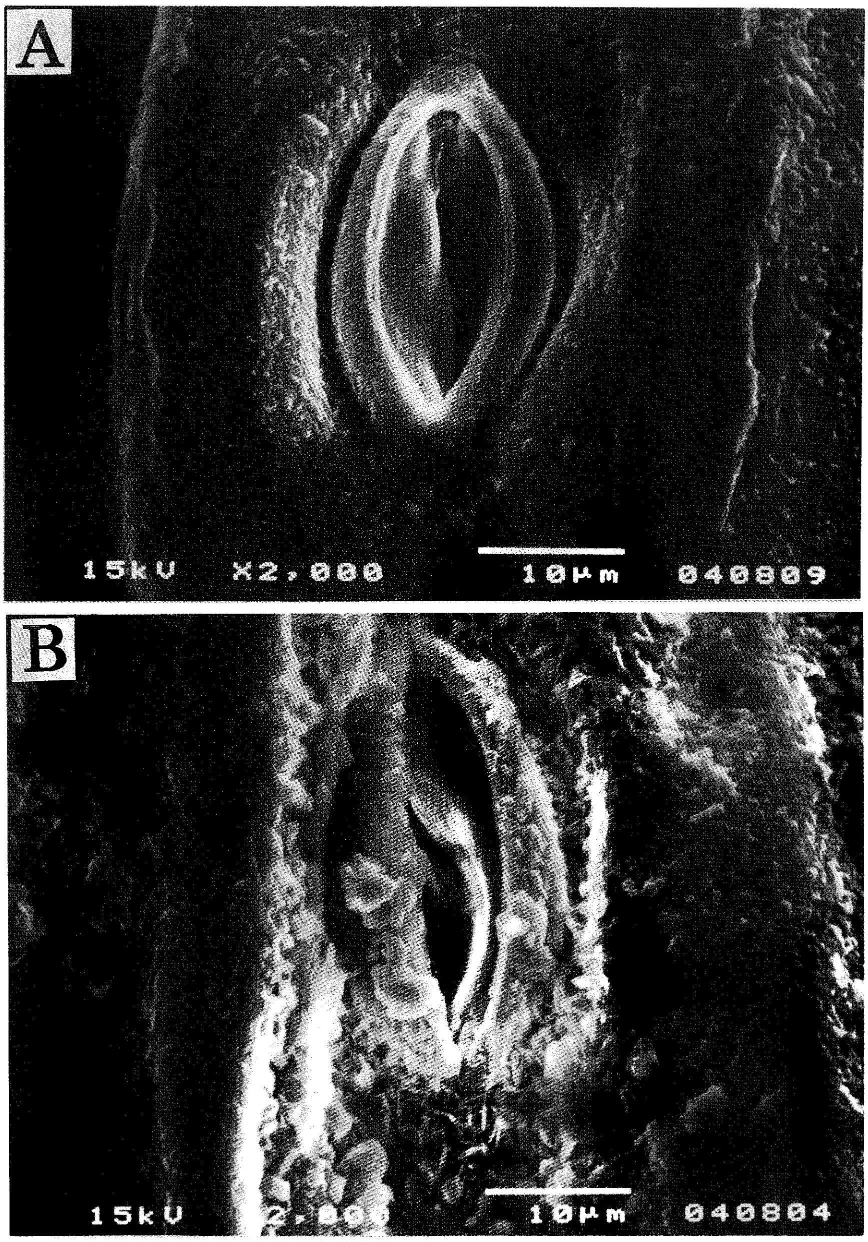

Figure 4 Scanning electron microscopic images of narcissus leaf. A; stoma of normal narcissus in Kanazawa City. B; stoma of narcissus after experimental exposure of heavy oil. Note that stuck with micro granules.

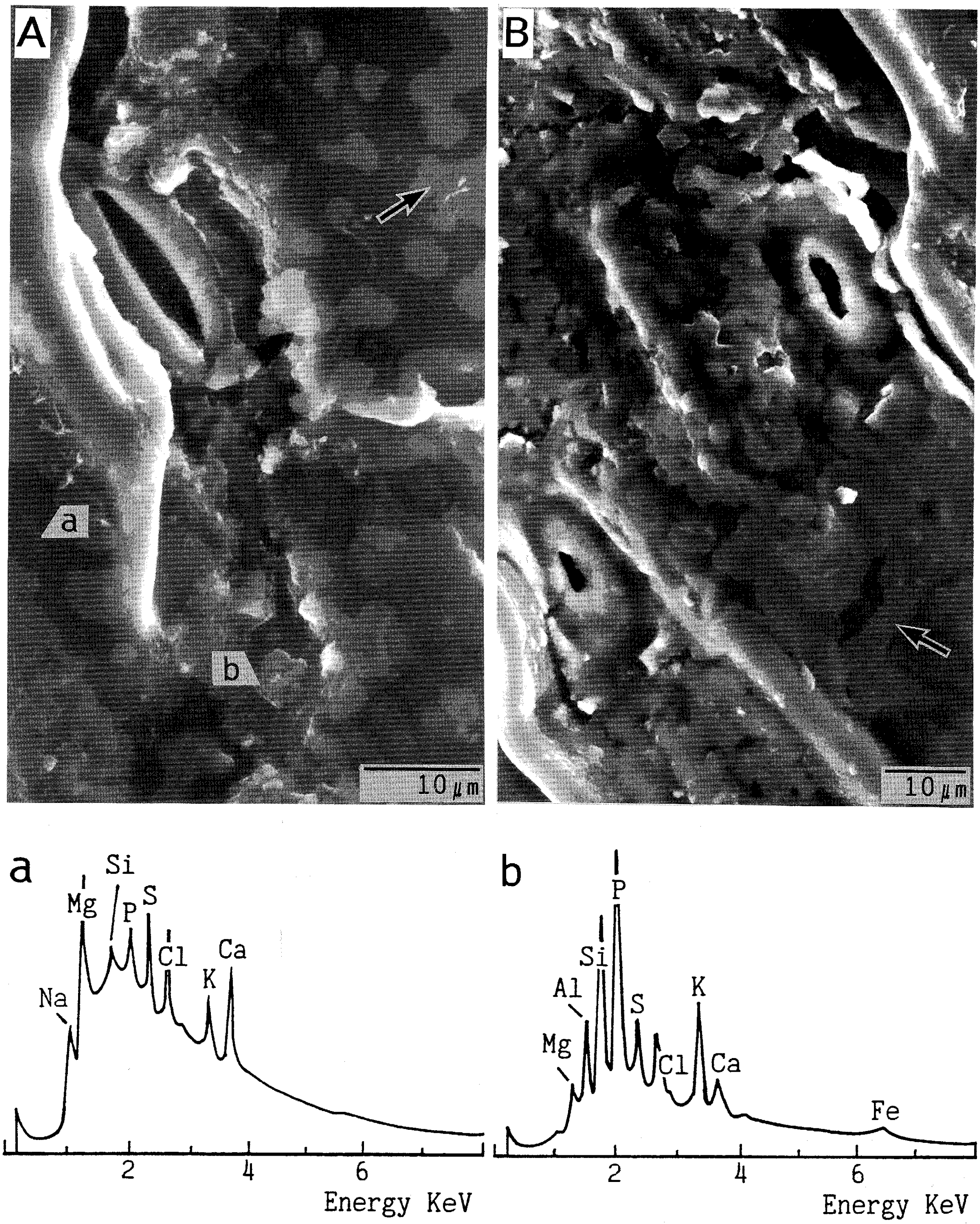

Figure 5 Scanning electron micrographs of leaf – dead narcissus in Anto, Mikuni Town (which had tremendous damage by heavy oil), and the EDX analyses (a, b). Micro substances covering around stomata and all over the leaf surface (arrows).