A VIDEO PROGRAM SHOWING THE PROCEDURE FOR COLLECTION AND OBSERVATION OF DIATOMS USED FOR EVALUATION OF RIVER WATER QUALITY

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There has been no adequate teaching material to inform high school students about the procedure of diatom collection, cleaning, preparation and observation for evaluation of river water quality. Some dry-labs using diatoms have been devised to show one of the methods to assess water quality, but the procedure from sample collection all the way to microscopic observation was replaced by micrographs of diatoms in these teaching materials. In this study, an easy and safe procedure was selected from reviewed methods, and then a video program showing it was produced. This program will be useful to introduce the actual procedure and the basic knowledge of diatoms to students who have never studied diatoms. By a combination of this video program with the dry-lab for evaluation of river water quality, a better understanding of this subject is expected.

Key words: biology education, diatoms, environmental education, river diatoms, video program, water quality evaluation.

INTRODUCTION

Environmental protection is a worldwide problem today. Biology teaching can effectively give students information for better understanding of this subject, however, useful teaching materials for laboratory experiments are still scarce. Ueyama and Kobayasi (1986) and Kobayasi and Ueyama (1993) devised teaching materials for evaluation of river water quality using diatoms and showed that these teaching materials were useful for environmental education in high schools. Recently, an example of water quality assessment using diatoms was supplied in a textbook of high school biology (Fujii et al. 1993). These teaching materials are the dry-lab models composed of diatom photographs. If students actually collect diatoms from rivers and observe them without any difficulty, it is expected that their interest in diatoms will increase and consequently their understanding of the water pollution problem also will be deepened. However, there has been no adequate text for students showing the actual procedure of diatom collection and microscopic observation. As diatoms are classified based on their valve morphology, it is necessary to remove protoplast from the silicified frustule, namely "cleaning", before identification. However, routine methods used by researchers are dangerous and inappropriate for students as they employ strong acid and high temperature. In this study, we selected an appropriate method for diatom collection, cleaning and slide preparation for high school students. The result is shown in the video program entitled "The Diatoms -Collection and Observation", which was devised for teaching in environmental education.

SELECTION OF AN EASY AND SAFE METHOD FOR STUDENT EXERCISE

(1) Sampling season and diatom collection

Diatoms occur through out the year in rivers as attached algae and this is one of the advantages to using the diatom assemblage as a bioindicator for water quality assessment (Patrick and Hohn 1956). Therefore, students can observe diatoms at any time. However, the biomass of the diatoms varies according to the season. The biomass measured by chlorophyll *c* decreases during summer in the Tokyo area (Bureau of Environmental Protection of the Tokyo Metropolitan Government 1987, 1994). Conversely, green algae and blue-green algae generally increase in this season. As monthly precipitation indicates (Tokyo Astronomical Observatory 1976), there are few rainy days from winter to spring in Tokyo. Therefore, winter and spring are the appropriate season, to collect diatoms from rivers in the Tokyo area.

The attached diatoms occur everywhere regardless of where it is the upper or lower stream. However, species diversity is higher in slow current than fast current (Mayama and Kobayasi 1982). The standing crop of diatoms is also higher in the slow, so it is recommended to choose the slow current (ca. 50 $cm \cdot sec^{-1}$ in current velocity) for diatom collection.

Diatoms are collected from the surfaces of stones. Round (1993) suggests the use of stones of 5–10 cm in diameter. However, small stones usually do not hold a dense community in Japanese rivers. Therefore, a flat stone of 15–20 cm in diameter is usually used for the collection of diatoms (e.g. Mayama 1982, Mayama and Kobayasi 1984, Kobayasi et al. 1985). This stone size was selected for collection in our video program. When the surface of the stone is dirty with mud or silt, the stone is lightly swung in the river water to wash it off. There are several ways to take diatoms from the stone. Van Heurck (1896) described the use of a copper spoon with a screw clamp to fasten to the end of a walking-stick for scraping diatoms off the substratum. Still some European researchers use a spoon for diatom collection. A wire brush is often used for collection, however, partly diatom frustules can be easily broken by this method. We prefer to use a toothbrush for scraping diatoms off the stone.

(2) Sample cleaning

When the living cell of the diatom is observed with a microscope, it is hard to detect the ornamentation of the valve, namely striae, raphe, etc. In addition, raw material usually contains other algae, fungi, bacteria and much detritus of organic and inorganic materials. They always interfere with the observation of diatoms. Therefore, cleaning of the sample is necessary for valve observation. There are many cleaning methods as reviewed by Nagumo (1987). Among them, heating the material with concentrated sulfuric acid or nitric acid in a test tube (e. g. Mayama 1993) and boiling the material with sulfuric acid and potassium permanganate using a water bath (Patrick and Reimer 1966) are relatively popular methods. However, these methods are dangerous for students and require a special treatment facility for waste chemicals. Burning up organic materials on a cover slip is often employed in science teaching (e.g. Endo 1989). However, it is not safe to use fire for a long time and the material is not completely cleaned with this method.

Nagumo and Kobayasi (1990) described a new cleaning method using sodium hypochlorite solution (NaClO) and demonstrated a procedure to clean a single cell under a light microscope. Mayama (1993) modified this method to get larger quantities of material. The cleaning method using sodium hypochlorite does not use a burner, so that it is safer than the method using strong acid. Conveniently, domestic

bleach liquid containing sodium hypochlorite can be used alternatively. However, the domestic bleach, in which a surface active agent is added, is not so useful, as many bubbles occur during cleaning. In this method, put 1 ml of material and 5 ml of bleach in a test tube first, and then stir well for 20 min with a pipette. Teachers are requested to pay attention that students not stir too strongly, because a splashed drop will decolorize their clothes. After stirring, the sample is rinsed with distilled water and centrifuged for 1 min. This operation is repeated four to six times and ultimately cleaned valves are obtained. This method may be inadequate for the samples containing many green and blue-green algae, because cellulose and other wall materials are not easily decomposed. Therefore, the best result can be expected when the method is applied to samples collected from the surfaces of the river stones.

(3) Slide Preparation

The suspension of the cleaned valves is diluted by distilled water to get an adequate concentration. A slightly turbid suspension is suitable for observations. Some drops of the suspension are dried up, not on a slide, but on a cover slip using a hot plate, because the outer surfaces of the valves are attached to the under surface of the cover slip when observed; e.g. the valve ornamentations are set at the nearest position to an object lens.

The valve is made of silica $(SiO_2)_n \cdot nH_2O$ and its refractive index is near those of water and Canada Balsam. Therefore, the fine valve ornamentation can hardly be observed in the specimens mounted in these media. Pleurax (Hanna 1949), Hyrax (Hanna 1930) or Naphrax, which all have a high refractive index, are adequate media for mounting diatoms. Pleurax is a medium synthesized from sulfur and phenol and a commercial product is available.

After a small amount of the medium is dropped on the slide, the cover slip with dried specimens is inverted and put on the medium. The slide is heated weakly (under 90°C) on a hot plate until the volatile solvent (ethanol in Pleurax) evaporates. When a medium with a high refractive index is not available, the turned cover slip can be put on a slide directly and stabilized with adhesive tape at two ends of the cover. In this case, air is a substitute medium and gives a relatively good image of the valve with high contrast.

OTHER INFORMATION INCLUDED IN THE VIDEO PROGRAM

Introducing basic knowledge of diatoms is another aim of this video program. The following information was considered for this aim.

(1) Taxonomy

Diatoms are unicellular algae with yellow chloroplasts and are classified into Chromophyta. The wall is composed of two silicified valves and many bands, which are sophisticatedly ornamented. The taxonomy of diatoms is carried out based on the valve morphology. About 10,000 species are recognized as bona fide taxa, but the number of diatom species is presumed to be about 20,000 (Werner 1977). Some researchers estimate the number to be as many as 100,000 as refered by Volcani (1981).

(2) Ecology

Diatoms are one of the important producers in the aquatic ecosystem. They are usually the main food for herbivorous fish and benthic animals in rivers.

Diatoms occur as periphyton or plankton in various habitats, namely rivers, lakes, ponds, seas, estuaries, moors, wet mosses and soil surfaces. The species composition of diatoms is different among these habitats. Even in the same habitat, it varies according to the ecological tolerance of the individual species toward environmental conditions. According to Hamm (1969), river waters are classified into seven distinct levels of pollution, i. e. oligosaprobic (BOD < $0.5mgO_2 \cdot l^{-1}$, negligibly polluted), oligo/β-mesosaprobic (BOD < 2.0, slightly polluted), β-mesosaprobic (BOD <4.0, moderately polluted), β/α-mesosaprobic (BOD < 7, critically polluted), α-mesosaprobic (BOD < 13, heavily polluted), α-meso/polysaprobic (BOD < 22, very heavily polluted) and polysaprobic (BOD => 22, excessively polluted). Each diatom species has its own tolerance towards water pollution (Kobayasi and Mayama 1981, 1982, 1989, Kobayasi et al. 1985., Mayama and Kobayasi 1984).

(3) Locomotion

There are motile species and non-motile species in diatoms. The motile diatoms possess a raphe system, transapical slit in the valve, and secrete mucilaginous fibrils through it (Edgar and Pickett-Heaps 1984). As the fibrils move along the raphe, diatoms can glide on substrata. This motile function is seen only in diatoms among algae and is one of the characteristics of diatoms. Students seem to be impressed when shown this movement.

MAKING OF VIDEO PROGRAM

Based on the selected methods and information mentioned above, the video program entitled "Diatoms -Collection and Observation" was made as follows:

Locations for taking video were chosen based on previous studies concerning water quality and diatoms (Kobayasi and Mayama 1982, Mayama and Kobayasi 1984, Kobayasi et al. 1985, Bureau of Environmental Protection of the Tokyo Metropolitan Government 1994). Tama-gawa (Tama river) in Fuchu City (β/α-mesosaprobic level) Karabori-gawa (Karabori river) in Kiyose City (polysaprobic level) and a mountain stream in Fujiyoshida City (oligosaprobic level) were chosen for the locations. Diatom collection was recorded by video in January and April, 1994.

For sample cleaning, a commercial domestic bleach was used. Pleurax (Mount Media, Wako Pure Chemical Industries, Inc., Osaka) was used for the mounting of the diatoms. Nikon Optiphot microscope equipped with Olympus OV100 video camera was used in the microscopy and the diatom valves were observed with Plan Apo object lens (X100, oil). For the comparison of the species composition among polysaprobic, α-mesosaprobic and oligo/β-mesosaprobic rivers, the dry-lab plates published by Ueyama and Kobayasi (1986) were recorded in the video program. All scenes except the microscopy were taken with Victor GR-SZ1 and Fuji FX100 video camera, and edited with Victor BR-S382 Video Cassette Recorder in S-VHS mode.

CONCLUSION

The methods for diatom collection, cleaning, preparation and observation were quickly reviewed and appropriate methods for students were selected among them. Basic information was also prepared to introduce diatoms to students who have never studied diatoms. A video program showing the actual procedure from sample collection to the microscopic observations was made. It seems that more effective

teaching of water pollution problems will be performed by the combination of this video program with the dry-lab models already published (Ueyama and Kobayasi 1986, Kobayasi and Ueyama 1993).

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