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The Separated Distribution of the Two Varieties of *Achnanthes minutissima* KUETZ. According to the Degree of River Water Pollution

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Abstract

The ecological characteristics of *Achnanthes minutissima* KUETZ. var. *saprophila* H. KOB. & MAYAM., reported as one of the most pollution-tolerant diatoms by the present authors in 1982, were investigated in detail in the river waters of Tokyo and vicinity. In contrast to the distribution of the nominate variety, var. *saprophila* was found only in heavily to excessively polluted waters and even occurred as dominant or subdominant taxon in some samples. Vigorous growth was observed in the strongly polluted stretch of the Minamiasa-kawa (Minamiasa River), as shown by cell numbers in a given area.

1. Introduction

Achnanthes minutissima KUETZ var. *minutissima* is a cosmopolitan taxa, as characterized by HUSTEDT (1938), and its occurrence has been repeatedly recorded by many taxonomists and ecologists mostly from less polluted waters. BUDDE (1930) mentioned that it was mesosaprobe. HUSTEDT (1957) classified this taxon into the oligosaprobic group. SLÁDEČEK (1973) considered it an indicator of oligosaprobic to β-mesosaprobic conditions, and FUKUSHIMA and KO-BAYASHI (1975) reported this entity to be an indicator of β-mesosaprobic environments. LANGE-BERTALOT (1978, 1979a, 1979b) and SCHOEMAN (1979), who paid special attention to pollution-tolerance rather than to the pollution-affinity of diatoms, regarded it as one of the most sensitive taxa, and found it useful together with the other members of LANGE-BERTALOT's group 3 diatoms for distinguishing moderately polluted β-mesosaprobic conditions from heavily polluted α-mesosaprobic ones in the estimation of water quality.

LOWE (1972) also stated that the taxon might indicate a lack of tolerance for organic pollution. However, there are few reports in which its occurrence was noted in α-mesosaprobic water (*e.g.*, FJERDINGSTAD, 1965) and in the one locality of FOGED (1948) which was assumed to be polysaprobic by FJERDINGSTAD (1950).

Because of the fine striation of the valves and the extremely wide variaiton in valve shape, identification of this taxon has long been fraught with difficulties. However, recently, LANGE-BERTALOT and RUPPEL (1980), who examined not only type material of *A. minutissima* var. *minutissima* using electron microscopy but also many type specimens of taxa related to it, made its circumscription clear, based on many light and electron microphotographs.

Achnanthes minutissima KUETZ var. *saprophila* H. KOB. & MAYAM. was described as a new taxon from a severely polluted Minamiasa-kawa (Minamiasa River), of which BOD_5 was estimated to be $24\,mgO_2 \cdot l^{-1}$ (KOBAYASI and MAYAMA, 1982). Under light microscopy, the valves of the variety can only be distinguished by the broader valve ends. However, the variety was clearly distinguished from the nominate variety by electron microscopical criteria. From the applied hydrobiological point of view, it seems very important to clarify the ecological difference between var. *saprophila* and var. *minutissima*. In the present study, the differences in distribution of these two varieties in the rivers in Tokyo and the vicinity are discussed with regard to the degree of pollution of these waters.

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2. Materials and Methods

In order to examine the distribution of *Achnanthes minutissima* var. *saprophila*, 141 samples of benthic diatoms were collected from 52 stations of the 25 rivers which were estimated as α-mesosaprobic, α-meso/polysaprobic and polysaprobic by the Bureau of Environmental Protection of the Tokyo Metropolitan Government (TOKYOTO KANKYO HOZENKYOKU, 1981, 1983a, 1983b) and by the authors from 1980 to 1982.

During the same period, 29 samples of benthic diatoms were also taken from 15 stations estimated to be oligosaprobic, oligo/β-mesosaprobic and β-mesosaprobic on the Tama-gawa (Tama River) and the Minamiasa-kawa, in order to compare the pattern of distribution of the nominate variety.

As the degree of pollution of a river fluctuates from point to point due to the inflow of sewage and self-purification, it is effective to examine in detail how the relative abundance of diatom valves changes along a river stretch in connection with the estimation of the ecological characteristics of the individual diatoms. In Minamiasa-kawa, therefore, sampling stations were established at short intervals such as 0.1–1.7 km and then 57 samples of diatoms were collected in series on 23 May 1981, 8 July 1981, 16 October 1981 and 4 March 1982.

Most of the samples of benthic diatoms for qualitative analyses were taken from flat surfaces of stones more than 10 cm in diameter at a depth of 10–20 cm, and a few were taken from river-walls at locations with a deeper riverbed, and then were preserved with formalin. The diatoms in these samples were cleaned either by the usual method with sulfuric acid and hydrochloric acid or by applying ultraviolet radiation (SWIFT, 1967), and mounted in Pleurax. When the identification of living or nonliving cells was necessary, GOTOH's procedure (1978) modified by the authors was employed, *i.e.*, acetocarmine was added to preserved diatoms, and after 24 hr they were washed with distilled water and heated on a cover glass, then mounted in Pleurax (MAYAMA and (KOBAYASI, 1982).

All species encountered in a number of transects across the prepared slide were identified and counted until a minimum of 600 valves had been scored (KOBAYASI and MAYAMA, 1982).

Samples of benthic diatoms for quantitative analyses were collected from $5 \times 5\ cm^2$ quadrates established at random on flat surfaces of submerged stones, 15–20 cm in diameter (KOBAYASI, 1961), and cleaned in the same manner as described above. They were placed in clean vials and additional water was added to bring the volume to 5 m*l*. Further appropriate dilution was made to ease the counting procedure, and 0.1 m*l* of each diluted suspension was dried on a cover glass and mounted in Pleurax. All individuals on each slide were identified and counted.

Measurement of BOD_5 was carried out using water samples taken at the same time as the diatom samples whenever it was possible. If not, conductivity was employed as a parameter of the water quality.

3. Results and Discussion

Achnanthes minutissima var. *saprophila* was found in the living state in 115 samples taken from 19 rivers including 46 sampling stations out of a total of 52 stations examined in Tokyo (Fig. 1). The highest value of the relative abundance measured in each station is listed in Table 1. As seen in Fig. 1 and Table 1, this taxon occurred widely in severely polluted waters, and it was even the dominant or subdominant taxon in sample K-1436 (62.1%, $BOD_5=17$), K-1076 (25.3%, $BOD_5=11$) and K-1145 (14.7%, $BOD_5=27$). However, it did not universally occur in heavily to excessively polluted waters in high proportion, and was not observed in six rivers (Ayase-gawa, Kanda-gawa, Sumida-gawa, Syakujii-gawa, Tachiai-gawa and Uchi-kawa), among the 25 rivers examined. The ecological feature of var. *saprophila* seems to be the same as that of other taxa of the most pollution-tolerant diatom group (LANGE-BERTALOT, 1980; KOBAYASI and MAYAMA, 1982).

The results of comparative analyses on the occurrence of the two varieties of *A. minutissima* are shown in Table 2, based on the materials collected on 16 May 1982 from 16 stations of the Tama-gawa. Values of the relative abundance of the two varieties and conductivity at the time of collection of each water are arranged in accordance with the distance in kilometers

Table 1. The list of sampling stations where var. *saprophila* was found more than 1% in relative abundance. At each sampling station where more than one sample was taken, the highest value of the relative abundances measured is listed.

Sampling station no.	Sample no.	Date	River	BOD_5* (O_2mg • l^{-1})	Relative abundance (%)
1	K-995	30 November 1980	Yanase-gawa	—	20.7
2	K-996	30 November 1980	Yanase-gawa	36	6.8
3	K-1607	11 December 1982	Yanase-gawa	[15] (19)	4.5
4	K-1608	11 December 1982	Karabori-gawa	[44] (40)	9.4
5	K-1609	11 December 1982	Kurome-gawa	[12] (21)	7.5
6	K-1546	03 July 1982	Shirako-gawa	[49] (48)	5.4
7	K-1551	30 June 1982	Meguro-gawa	—	2.0
8	K-1549	30 June 1982	Nomi-gawa	[21] (24)	2.7
15**	K-713	1 March 1980	Tama-gawa	32	9.1
25	K-1436	9 March 1982	Zanbori-gawa	[36] (17)	62.1
26	K-1138	6 July 1981	Hatsusawa-gawa	7	17.9
27	K-1094	23 May 1981	Funada-gawa	—	3.1
28**	K-1145	8 July 1981	Minamiasa-kawa	27	14.7
47	K-712	21 February 1980	Asa-kawa	—	2.1
48	K-915	22 July 1980	Asa-kawa	[6] (11)	6.0
49	K-1435	10 March 1982	Hodokubo-gawa	[13] (10)	19.3
50	K-758	27 April 1980	Ohguri-gawa	8	2.2
51	K-1421	14 December 1981	Ohguri-gawa	[8] (10)	8.5
52	K-691	11 January 1980	No-gawa	[12] (10)	20.0
53	K-1071	10 February 1981	No-gawa	[11] (12)	23.0
54	K-1014	10 December 1980	Sen-kawa	25	3.2
55	K-1076	14 February 1981	Sen-kawa	[18] (11)	25.3
56	K-1563	29 September 1982	Tsurumi-gawa	— (7)	10.7
57	K-794	11 March 1980	Tsurumi-gawa	22	2.2
58	K-874	30 July 1980	Onda-gawa	20	1.1
59	K-1604	9 December 1982	Sakai-gawa	[26] (27)	15.7

* At the stations where BOD_5 was not measured at the time of collection, values measured at another time in the same month and annual average values taken by the Bureau of Environmental Protection of the Tokyo Metropolitan Government (TOKYO-TO KANKYO HOZENKYOKU, 1981, 1983a, 1983b) are shown in brackets and in parentheses respectively.

** Stations of which results are discussed in detail in the later parts of this paper, and one representative datum from each river is shown.

Table 2. Changes in the relative abundance of *A. minutissima* var. *minutissima* and var. *saprophila* based on the materials collected on 16 May 1982 from the Tama-gawa, along a 88 km length of the water course from the mouth.

Sampling station no.	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
River-km from the mouth	88	82	71	64	59	54	51	46	40	35	31	27	23	18	12	5
A. minutissima var. *minutissima*	4.4	1.6	20.6	8.9	5.5	23.9			4.1							
A. minutissima var. *saprophila*							8.3	5.9	12.1	7.5	4.2	1.6	7.4	3.0	1.6	3.0
Cond. ($\mu S \cdot cm^{-1}$ at 25°C)	98	96	99	85	120	115	272	259	392	354	372	418	372	390	392	8200

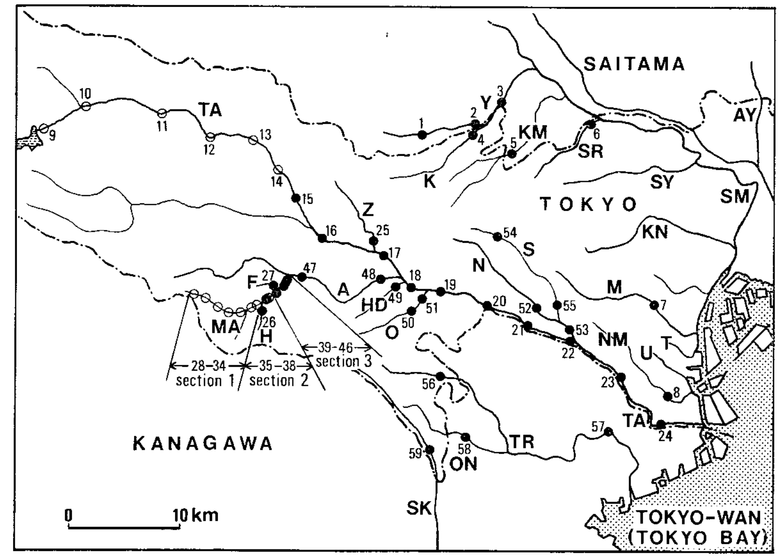

Fig. 1. Map of study area showing sampling stations in the rivers running through Tokyo. ○ with station numbers: *Achnanthes minutissima* var. *minutissima* (occurring more than 1%); ● with station numbers: *A. minutissima* var. *saprophila* (occurring more than 1%); stations where both varieties occurred less than 1% are not plotted; A: Asa-kawa; AY: Ayase-gawa; F: Funada-gawa; H: Hatsusawa-gawa; HD: Hodokubo-gawa; K: Karabori-gawa; KM: Kurome-gawa; KN; Kanda-gawa; M: Meguro-gawa; MA: Minamiasa-kawa; N: No-gawa; NM; Nomi-gawa; O: Ohguri-gawa; ON: Onda-gawa; S: Sen-kawa; SK: Sakai-gawa; SM: Sumida-gawa; SR: Shirako-gawa; SY: Syakujii-gawa; T: Tachiai-gawa; TA: Tama-gawa; TR: Tsurumi-gawa; U: Uchi-kawa; Y: Yanase-gawa; Z: Zanbori-gawa.

from the mouth of the river. Sampling Station 9 is situated at the foot of the Ogohchi Dam which is 88 km from the mouth. The water from here to Station 14, located just above the Hamura Intake Dam, was clear. The Bureau of Environmenatl Protection of the Tokyo Metropolitan Government (TOKYO-TO KANKYO HOZENKYOKU, 1983b) reported that the annual average BOD_5 in this section was less than 1 $mgO_2 \cdot l^{-1}$ and maximum conductivity measured in this section by the authors was 120 $\mu S \cdot cm^{-1}$ even at Station 13 in the town area of Ohme city. At Station 14, almost all of the river water is removed for water service and the river section below this point recovers its water mass by inflow from many tributaries derived mainly from domestic sewage. Therefore, conductivity measured was higher in the lower stretch. At Station 15, in fact, conductivity was 272 $\mu S \cdot cm^{-1}$, which was over two times as high as that of Station 14; at Station 20 it reached 418 $\mu S \cdot cm^{-1}$, but an excessively high value (8200 $\mu S \cdot cm^{-1}$) recorded at Station 24 near the river mouth was clearly due to the effect of sea water. Though var. *minutissima* was detected at Station 17, its distribution was consentrated in the upper section, from Station 9 to Station 14, and showed a maximum relative abundance of 23.9% at Station 14. These results seem to agree with the statements of many investigators characterising this taxon as oligosaprobic or β-mesosaprobic. On the other hand, var. *saprophila* was not found in the upper section in contrast to its concentric occurrences in the lower section below Station 14. Relative abundances were more than 3% at eight points out of ten and reached 12.1% at Station 17.

There are all kinds of conditions from oligosaprobic to polysaprobic along a short stretch of 11 km in the Minamiasa-kawa. Therefore, further detailed surveys were carried out in its main course. As shown in Figs. 2 to 5, seventeen sampling stations in total were selected at short intervals along the stretch, and the materials collected from each site were analysed mainly in connection with the occurrence of the two varieties of *Achnanthes minutissima*.

The river course was divided into three sections mainly according to its water quality. The uppermost, Station 28, was located 11 km above the confluence of the Asa-kawa (Fig. 1). The river stretch from this site to Station 34 was called Section 1. There are few houses in the basin of Section 1, and the water of this section was in good condition; its annual average BOD_5 was 1.5 $mgO_2 \cdot l^{-1}$. In Section 2 including Station 35 to Station 38, the water became slightly worse caused by domestic sewage from the houses. Its annual average BOD_5 was 5.3 $mgO_2 \cdot l^{-1}$. In Section 3, the water quality was markedly worse, being 21.6 $mgO_2 \cdot l^{-1}$ in the BOD_5, caused by a considerable amount of inflow of secondary treated water from waste water treatment plants for about 7,000 inhibitants and untreated domestic waste water from about 6,100 estimated inhibitants (Hosoya and Ogura, 1982).

In the first investigation carried out on 23 May, the nominate variety occurred mainly in Section, 1, and a maximum value of 11.4% was recorded from Station 29 (Fig. 2-A). On the other hand, var. *saprophila* was only found in samples taken from Sections 2 and 3 (Fig. 2-B). Though the relative value of 4.2% at Station 39 proved lower, a marked increase in the values of this variety was suspected to be caused by the considerable amount of inflow of the above mentioned organic load. The riverbed of Section 3 consisted of large gravel and is bulldozed once or twice a year by the local government in order to widen the flow and accelerate its self-purification capacity. Therefore, a decrease in value at Station 40 seemed to be a result of the self-purification at that section. Indeed group 1 diatoms (most tolerant taxa) declined and group 2 diatoms (less tolerant taxa) increased at this site. In contrast to the decline of the Station 40, the decline at Station 44 was caused by an increase of *Nitzschia palea* (Kuetz.) W. Sm., the other member of the group 1 diatoms. The relative value of the group 1 diatoms at this point was highest at 91.5%.

In order to differentiate the ecological properties of var. *saprophila*, the same values of *Navicula minima* Grun. of the group 1 diatoms are shown in the same graph (Fig. 2-B). Though the percentages of *N. minima* are about eight times as large as that of var. *saprophila*, similar changes in these taxa seemed to indicate their similar tolerance or adaptation to organic pollution.

In the second survey on 8 July, materials were collected below Station 33. The nominate variety occurred only at low levels at sites above Station 37 and the var. *saprophila* was restricted to sites below Station 39 (Fig. 3). In this case, *N. minima* changed its relative values the same as var. *saprophila*. The marked

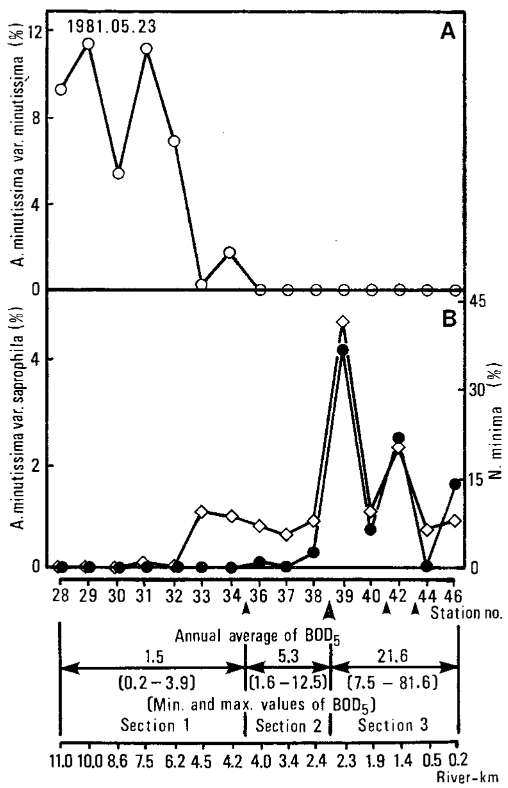

Fig. 2. Changes in the relative abundance of diatoms at each sampling station in the Minamiasa-kawa with various degree of water pollution (23 May 1981).
Section 7—from Station 28 to 34; Section 2—from Station 35 to 38; Section 3—from Station 39 to 46; ○-*Achnanthes minutissima* var. *minutissima*; ●-*A. minutissima* var. *saprophila*; ◇-*Navicula minima*. Arrowheads indicate main organic load inflow.

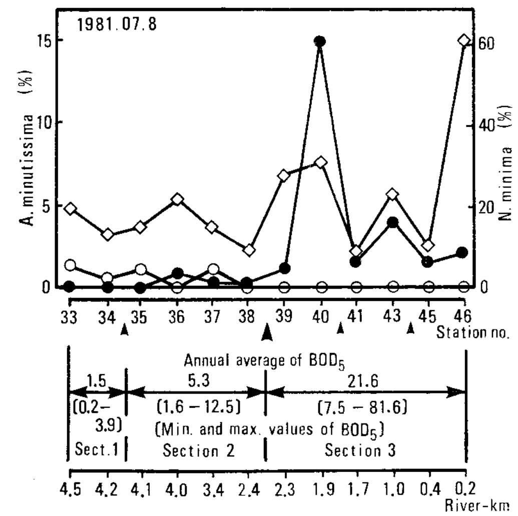

Fig. 3. Changes in the relative abundance of diatoms at each sampling station in the Minamiasa-kawa (8 July 1981). ○-*Achnanthes minutissima* var. *minutissima*; ●-*A. minutissima* var. *saprophila*; ◇-*Navicula minima*. Arrowheads indicate mein organic load inflow.

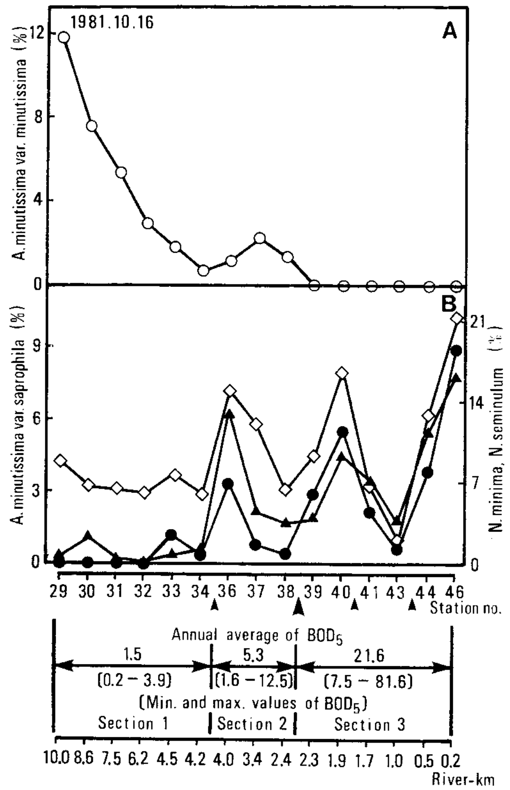

Fig. 4. Changes in the relative abundance of diatoms at each sampling station in the Minamiasa-kawa (16 October 1981). ○-*Achnanthes minutissima* var. *minutissima*; ●-*A. minutissima* var. *saprophila*; ◇-*Navicula minima*; ▲-*Navicula seminulum*; Arrowheads indicate main organic load inflow.

declines of these two taxa at Station 41 and Station 45 were also caused by the marked increase of *Nitzschia palea* in these communities. The relative abundance of the latter was 78.2% at Station 41 and 79.8% at Station 45 respectively.

The results of the third survey are shown in Fig. 4. In contrast to the high proportion of occurrences of the group 1 diatoms such as var. *saprophila, Navicula minima* Grun. and *N. seminulum* Grun. (Fig. 4-B), the nominate variety could not survive in the polluted waters lower than the Station 39 (Fig. 4-A).

The results of 4 March are shown in Fig. 5. The nominate variety was also abundant in Section 1 and showed a maximum of 28.5% at Station 29, but could not survive in Section 2 and 3 (Fig. 5-A). However, var. *saprophila* was found at all sampling sites below Station 37 and it occurred with a maximum value of 9.6% at Station 40. The condition of the river water was worst in March at almost all sampling stations especially in Section 2 and 3. The BOD_5 was 60.0 $mgO_2 \cdot l^{-1}$ at Station 39 in March, however, var. *saprophila* increased in relative abundance there. The other species appearing in this site associated with var. *saprophila* and showing similar changes in occurrence with var. *saprophila* were *Navicula atomus* (Kuetz.) Grun. and *Navicula minima* (Fig. 5-B).

As shown in Fig. 5-A, the maximum value of 28.5% of var. *minutissima* at Station 29 suggests that it was well adapted to this site where water was in the best condition. However, the small community size of this variety at the site (Fig. 5-C) does not suggest well adapted growth.

On the other hand, both curves of var. *saprophila* showed similar changes. The high values in both curves at Site 40 and 44 seemed to indicate the good adaptation of this variety

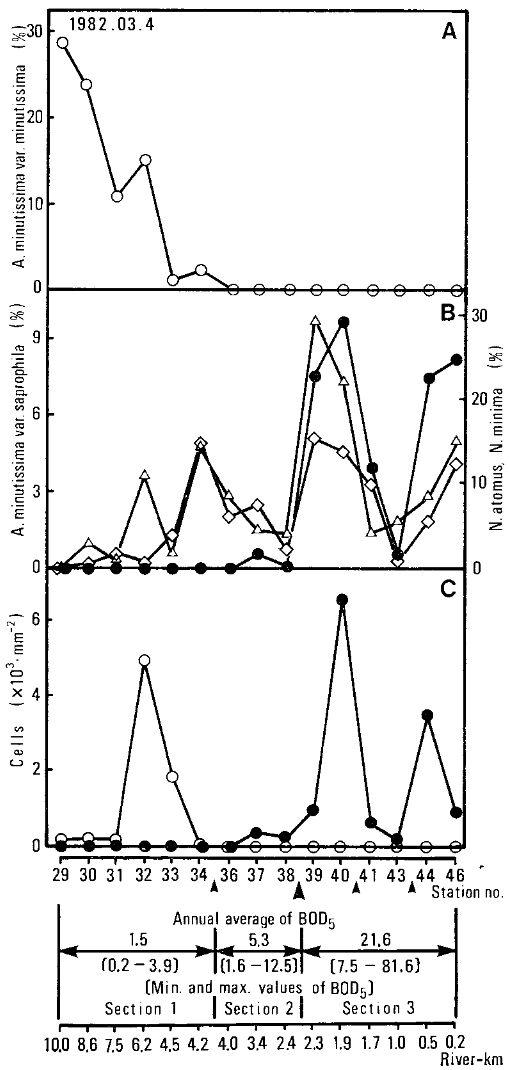

Fig. 5. Changes in the relative abundance (A, B) and cell number (C) of the diatoms at each sampling station in the Minami-asa-kawa (4 March 1982). ○-*Achnanthes minutissima* var. *minutissima*; ●-*A. minutissima* var. *saprophila*; △-*Navicula atomus*; ◇-*Navicula minima*. Arrowheads indicate main organic load inflow.

to severely polluted environments (Fig. 5-B, C).

Although there are many reports where the occurrence of *A. minutissima* var. *minutissima* or some forms identified as this taxon is mentioned, only a few of them are accompanied with the elements which are necessary for confirmation of the correct identification and for evaluation of the ecology of this taxon, *i.e.* microphotograph, relative abundance in a given community and a description connected with water quality (*e.g.* Kobayasi 1962, 1964; Watanabe 1981). In these reports, this taxon occurred abundantly in clear water. In the present study also, it hardly appeared in the α-mesosaprobic environment but was found chiefly in oligosaprobic and β-mesosaprobic river waters. This taxon seems to be very sensitive to pollution, as mentioned by other diatomists (*e.g.* Lowe 1972; Lange-Bertalot 1978, 1979a, 1979b; Schoeman 1979). However, it was confirmed that var. *saprophila* is widely distributed mainly in polysaprobic and α-mesosaprobic river waters. This taxon seems to be well adapted to strongly polluted waters, as it was hardly found in the waters better than β/α-mesosaprobic.

In recent studies, Descy (1979) who classified *A. minutissima* into his index of sensitivity to pollution to be 4 and indicating value to be 1, has represented the mean relative abundance of this taxon graphically at various degrees of water pollution. This graph showed two clear peaks, of which one was in clear water and another in heavily polluted water, in contrast to the other graphs with one peak. Watanabe (1982) stated that the optimum BOD_5 of *A. minutissima* was 10.6 $mgO_2 \cdot l^{-1}$ in his "pollution spectrum," but its occurrence was plotted separately in two ranges of less than 6 $mgO_2 \cdot l^{-1}$ and more than 20 $mgO_2 \cdot l^{-1}$. Though there are no photomicrographs showing species in the above mentioned two papers, it is suggested that the two varieties of *A. minutissima* have been mixed up and treated as one taxon.

摘　要

強腐水域より新変種として報告された，強汚濁耐性ケイソウの1つである *Achnanthes minutissima* var. *saprophila* H. Kob. & Mayam. (Kobayasi and Mayama, 1982) と，汎布種として知られる *A. minutissima* Kuetz. var. *minutissima* の生態的特性を明らかにするため，東京と，その近郊を流れる河川のさまざまな汚濁域から得た試料を用いて，定性・定量分析を行なった．承名変種が β/α-中腐水より清冽な水域にのみ出現したのに対し，var. *saprophila* は α-中腐水または強腐水とされた25河川中，19河川の水域に広く分布していた．また後者は，これらの水域でしばしばケイソウ群落の優占種となり，単位面積あたりの細胞数も増加していたことから，強い有機汚濁に対し，適応性を保有していることが考えられる．また変種 *saprophila* の承名変種とは大きく異なる分布は，同時にこの種類の存立にとって十分な根拠を提供するものと考えられる．

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