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Most pollution-tolerant diatoms of severely polluted rivers in the vicinity of Tokyo*

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Samples of benthic diatoms were collected from five severely polluted, shallow and small tributary rivers in the vicinity of Tokyo and analysed according to LANGE-BERTALOT's method using differentiating diatom groups. Although dominant species or species composition of an association varied among samples collected in different seasons from the same station, the dominance of group 1 diatoms in all samples collected showed the high reliability of this method at least for the excessively polluted waters in Japan. Ten of eighteen taxa listed in LANGE-BERTALOT's group 1 are recorded and two taxa, *Achnanthes minutissima* var. *saprophila* var. nov. and *Pinnularia braunii* var. *amphicephala*, are proposed to be added to the group for the use of this method in Japan.

Key Index Words: Achnanthes minutissima *var.* saprophila; *diatoms*; *indicator species*; *new variety*; *water quality*.

Since a saprobic system was proposed by KOLKWITZ and MARSSON (1908), many methods using aquatic communities have been devised with respect to the determination of the degree of organic pollution. However, these methods have been repeatedly criticized by other authors as summarized by SLÁDEČEK (1973). LANGE-BERTALOT (1978, 1979a, b) has recently proposed a new method of determining the quality of running water using differentiating diatom species. This method appeared to be highly reliable in an examination of South African waters by SCHOEMAN (1979). Our recent studies (KOBAYASI and MAYAMA 1981) also indicate that his method correlated well with the chemical parameters measured.

Owing to the shallow and steep features of Japanese rivers, it seems to be necessary to reassess individual species of the groups proposed by LANGE-BERTALOT (1979b). With regard to the most pollution-tolerant diatom group, he stated that the occurrence of this group alone cannot indicate a distinct level of saprobity. However, in order to evaluate Japanese species to be included in the LANGE-BERTALOT's group 1 to 3, it is necessary to carry out some research on the most pollution-tolerant diatoms found in the severely polluted waters in Japan.

Materials and Methods

Samples of benthic diatoms and water were collected from five severely polluted rivers in the vicinity of Tokyo (Fig. 1). Due to an increase in housing, a large amount of primary or secondary treated sewage loads are flowing into these rivers. The mean BOD_5 of these rivers measured by the Bureau of Environmental Protection of the Tokyo Metropolitan Government (TOKYOTO KANKYO HOZENKYOKU 1981) showed the polysaprobic level, being 10 to 30 $mgO_2 \cdot l^{-1}$

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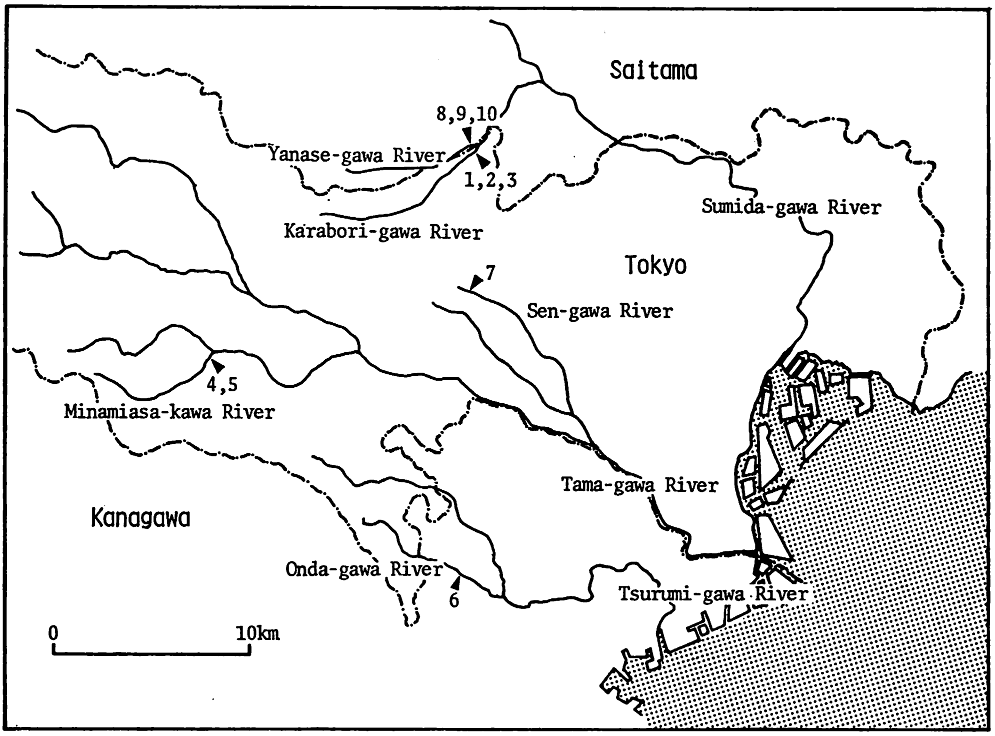


Fig. 1. A map showing sampling stations with sample numbers of the rivers investigated.

or over $30\,mgO_2 \cdot l^{-1}$ through the year. Sample 1, 2 and 3 were collected from the Karabori-gawa River, immediately above its confluence with the Yanase-gawa River. Sample 4 and 5 were collected from the Minamiasa-kawa River in front of the Waterworks Branch of Hachioji City. Sample 6 was collected from the Onda-gawa River above the Yaso-hashi Bridge. Sample 7 was collected from the Sen-gawa River in front of the Koganei Post Office. Sample 8, 9 and 10 were collected from the Yanase-gawa River, before its confluence with the Karabori-gawa River. The results of physical and chemical analysis are shown in Table 1.

Samples of attached diatoms were scraped off from stones more than 10 cm in diameter and fixed with formalin. The diatoms in these samples were cleaned with sulfuric acid and hydrochloric acid and mounted in Pleurax. When identification of living or nonliving cells was necessary, the fixed diatoms were dyed with acetocarmine (GOTOH 1978), and mounted in Pleurax.

All species encountered in a number of transects across the prepared slide were identified and counted until a minimum of 500 valves had been scored (SCHOEMAN 1979). This number was enough to count the diatoms in this study, because the difference of the relative density of each taxon in a sample of the Minamiasa-kawa River was less than 1.5% between the counts of about 8,000 valves and about 600 valves (unpublished data).

In contrast to the continental rivers such as Rhein and Main etc., the Japanese ones are shorter and steeper, and therefore, O_2 can easily dissolve into the water. Sampling at the stations were generally performed within a depth less than 20 cm in this study. Therefore, O_2-saturation rates could not be used for an indicator of the water quality combined with BOD_5. We employed only BOD_5 values for the water quality evaluation.

Physical and chemical determination (Table 1) were carried out on water samples taken

Table 1. Physical and chemical data obtained for water samples from the rivers investigated in the vicinity of Tokyo. K: Karabori-gawa River, M: Minamiasa-kawa River, O: Onda-gawa River, S: Sen-gawa River, Y: Yanase-gawa River.

Sample No.	River	Date	Water temp. (°C)	pH	O_2-saturation (% at 20°C)	Conductivity ($\mu S\cdot cm^{-1}$ at 25°C)	BOD_5 ($mgO_2\cdot l^{-1}$)
1	K	1980/03/16	12.2	7.2	85.2	591	38
2	K	1980/05/14	21.0	7.0	81.1	533	79
3	K	1980/11/16	14.0	7.6	76.6	609	39
4	M	1980/03/1	7.5	7.4	91.3	338	24
5	M	1980/11/14	17.0	7.6	114.4	386	23
6	O	1980/05/7	19.0	7.3	86.0	444	29
7	S	1980/04/9	15.8	8.0	75.8	439	46
8	Y	1980/03/7	9.0	7.6	53.4	624	26
9	Y	1980/05/14	21.0	7.2	73.3	449	22
10	Y	1980/11/30	12.5	7.0	64.0	321	36

at the same time as the diatom samples. All samples for chemical analysis, except pH measured *in situ*, were brought to the laboratory and analyzed using standarized methods (NIPPON KIKAKU KYOKAI 1980).

Result and Discussion

As shown in Table 1, all BOD_5 measured were more than 22 $mgO_2\cdot l^{-1}$ and these values showed the polysaprobic condition of LANGE-BERTALOT's system of classifying water quality (1979b) (Table 2). The high values of conductivity as ranged from 321 to 624 $\mu S\cdot cm^{-1}$ showed also the clear effect of the heavy organic loads. The amounts of dissolved NH_4-N, NO_3-N and PO_4-P measured by the Bureau of Environmental Protection of the Tokyo Metropolitan Government (TOKYOTO KANKYO HOZENKYOKU 1981) were also quite high.

On the other hand, the minimum O_2-saturation rate of 53.4% was measured in the case of Sample 8 and the maximum rate of 114.4 % was in Sample 5. Therefore, these values corresponded to the level of β/α-mesosaprobic or better as indicated in the LANGE-BERTALOT's system (Table 2).

Table 3 is an alphabetical list of all the species encountered in this study with a percentage of their relative abundances at each sample. A total of 39 species and varieties from 8 genera were observed.

As seen in table 3 and Figs 2–13 a dozen taxa including ten LANGE-BERTALOT's group 1 species were recognized to be characteristic to the excessively polluted waters (underlined). This group of species occurred in high percentages in all samples, as shown at the bottom of the table.

Although the Japanese rivers were highly saturated by oxygen even at excessively polluted areas (Table 1), it appeared that the most pollution-tolerant taxa in Europe could also exist with high reproduction rates. Among the taxa composing the group, *Nitzschia palea* (Fig. 12), *Navicula seminulum* (Fig. 7) and *Navicula atomus* (Fig. 3) were most dominant at any one or more samples. *Nitzschia palea* occurred as a dominant species in five samples, *Navicula seminulum* occurred in four samples and *Navicula atomus* occurred in only one station. Any correlation between the occurrence of these three species and the environmental parameters measured as pH, O_2-saturation, conductivity

Table 2. Estimation of water quality from the groups of differentiating diatom species (Group 1-3) as summarized from LANGE-BERTALOT (1978, 1979b).

Percentages of three groups within the community	Water quality class indicated
Group 3 ≧50 % (Group 2 + 1 <50 %)	I-II (β-mesosaprobic), BOD_5 <4mg$O_2 \cdot l^{-1}$, (moderately polluted), O_2-saturation deficit <30 %
Group 3 10 - 50 % (Group 2 + 1 50 - 90 %)	II/III (β/α-mesosaprobic), BOD_5 <7mg$O_2 \cdot l^{-1}$, (critically polluted), O_2-saturation deficit <50 %
Group 3 <10 % Group 2 ≧50 % (Group 1 <50 %)	III (α-mesosaprobic), BOD_5 <13mg$O_2 \cdot l^{-1}$, (heavily polluted), O_2-saturation deficit <75 %
Group 3 <10 % Group 2 10 - 50 % (Group 1 40 - 90 %)	III/IV (α-meso/polysaprobic), BOD_5 <22mg$O_2 \cdot l^{-1}$, (very heavily polluted), O_2-saturation deficit <90 %
Group 3 <10 % Group 2 <10 % (Group 1 ≧80 %)	IV (polysaprobic), BOD_5 ≧22mg$O_2 . l^{-1}$, (excessively polluted), O_2-saturation deficit ≧90 %

and BOD_5 was not found except that with water temperature. *Nitzschia palea* was dominant in the samples collected from warm waters ranging from 15.8°C of Sample 7 to 21°C of Sample 2 and 9. *Navicula seminulum* was in the waters ranging from 9°C in Sample 8 to 14°C in Sample 3 and *Navicula atomus* was only in Sample 4 collected from 7°C water. Therefore, water temperature appears to be one of the important factors even in excessively polluted biotopes.

After careful examination, a number of species not listed in LANGE-BERTALOT'S diatom groups were added. Each of these species has been empirically evaluated on the basis of its presence or absence in our considerable number of field studies according to the procedure suggested by LANGE-BERTALOT (1979a, b) and allocated to a relevant group, as follws:

Achnanthes minutissima KUETZ. var. *saprophila* var. nov. (Fig. 2) and *Pinnularia braunii* (GRUN.) CL. var. *amphicephala* (MAYER) HUST. (Fig. 11) were placed in group 1, *Amphora fontinalis* HUST., *Fragilaria intermedia* (GRUN.) GRUN., *Gomphonema angustatum* (KUETZ.) RABH. var. *productum* GRUN., *Gomphonema angustatum* var. *undulatum* GRUN., *Gomphonema quadripunctatum* (OESTR.) WISL., *Navicula acceptata* HUST., *Navicula excelsa* KRASSKE, *Navicula ventralis* KRASSKE and *Nitzschia intermedia* CL. and GRUN. in group 2, and *Fragilaria crotonensis* KITTON, *Navicula contenta* GRUN. var. *biceps* (ARNOTT) CL., *Nitzschia minuta* BLEISCH and *Nitzschia pusilla* (KUETZ.) GRUN. emend. LANGE-B. in group 3.

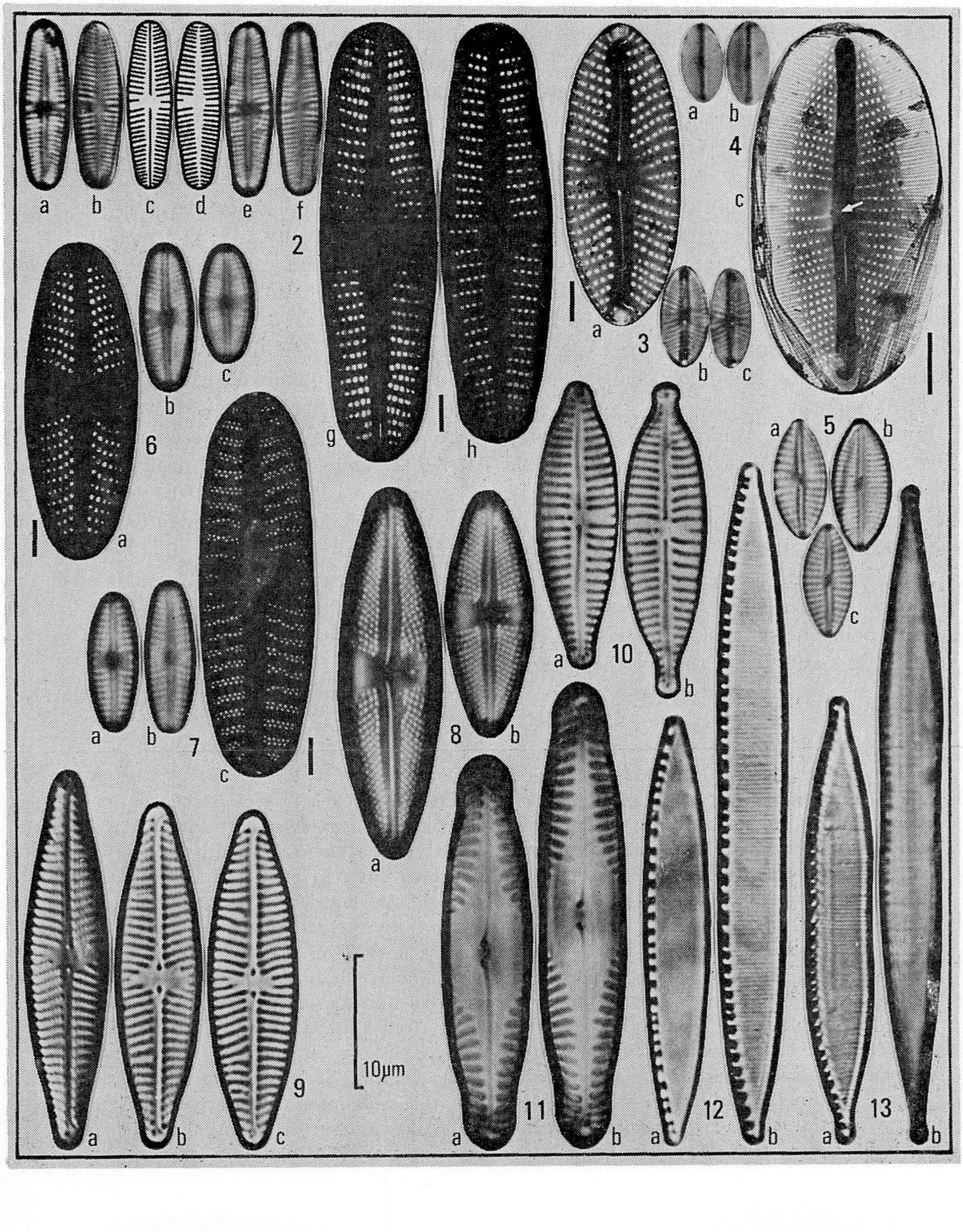
Achnanthes minutissima var. *saprophila* was observed in all samples (Table 3). However, its relative densitiy in each sample was not very high. Maximum rates of 6.1 and 6.0 were only recorded at the Yanase-gawa River and the Minamiasa-kawa River. However, it was commonly found not only within the present samples but also in the samples collected from the other excessively polluted rivers. Recent LANGE-BERTALOT & RUPPEL'S examinations (1980) dealing with a wide range of typematerials of the infra taxa of *Achnanthes minutissima* var. *minutissima* have clearly realized the circumscription of this species. However, in addition to the above mentioned ecological difference, valves of var. *saprophila* have broadly rounded ends and less radiate terminal striae composed of densely arranged square shaped, often apically elongated puncta. It seems to be a new taxon as far as examined by transmission and scanning electron microscopy. We could

Table 3. Diatom population dynamics.

Diatoms observed	Lange-Bertalot's grouping	Newly proposed grouping	Samples: Karabori-gawa 1	Karabori-gawa 2	Karabori-gawa 3	Minamiasa-kawa 4	Minamiasa-kawa 5	Onda-gawa 6	Sen-gawa 7	Yanase-gawa 8	Yanase-gawa 9	Yanase-gawa 10
Achnanthes												
lanceolata (Breb.) Grun.	2						0.3					
minutissima Kuetz. var. *saprophila* var. nov.		1	2.4	2.2	1.5	6.0	1.3	0.4	0.6	6.1	2.1	2.1
Amphora												
fontinalis Hust.		2							0.5			
Fragilaria												
crotonensis Kitton		3							1.3			
intermedia (Grun.) Grun.		2				0.3						
Gomphonema												
angustatum (Kuetz.) Rabh. var. *productum* Grun.		2	0.3	0.4				0.1				
angustatum (Kuetz.) Rabh. var. *undulatum* Grun.		2								0.1	0.3	
parvulum (Kuetz.) Kuetz.	1		0.3		0.2	0.8	6.1	0.7	0.1	5.6	0.3	3.3
pseudoaugur Lange-B.	2				0.3		0.3	0.1		0.9	1.4	
quadripunctatum (Öster.) Wislouch		2				0.3						
truncatum Ehr.	3				0.3							
Navicula												
acceptata Hust.		2				0.2						
atomus (Kuetz.) Grun.	1			0.4		67.7	3.5	0.6	0.6	0.9	0.8	
contenta Grun. var. *biceps* (Arnott) Cl.		3			0.2							
cryptocephala Kuetz.	3							0.1		0.1		
excelsa Krasske		2				0.3			0.6			
exilis Kuetz.	3										0.3	
frugalis Hust.	1					0.4	0.2			0.1	0.1	
goeppertiana (Bleisch) Grun.	1			6.8	0.6							
gregaria Donkin	2					1.0						
minima Grun.	1		0.3			11.7	11.7	2.2		0.9		0.8
pupula Kuetz.	2			0.1					0.1		0.4	
saprophila Lange-B. & Bonik	1					2.2	5.4				0.3	
seminulum Grun.	1		89.6	3.8	51.5	2.7	1.6	0.3	0.1	52.4	2.0	92.5
trivialis Lange-B.	2			0.1	0.2					0.2		
veneta Kuetz.	1			0.1	0.2	0.1		3.2	0.1	0.1	0.3	
ventralis Krasske		2								0.1		
Nitzschia												
amphibia Grun.	2							0.1	0.3			
dissipata (Kuetz.) Grun.	3								0.9	1.2		
frustulum (Kuetz.) Grun.	3					1.2	0.7					
gandersheimiensis Krasske	1		0.4			0.3	0.9			5.0		
intermedia Cl. *et* Grun.		2					1.2			0.5		
minuta Bleisch		3								1.1		
palea (Kuetz.) W. Sm.	1		6.4	76.3	36.9	2.4	66.6	92.0	94.8	23.6	91.5	1.3
paleacea Grun.	2					1.5						
pusilla (Kuetz.) Grun.		3		0.3						0.5		
romana Grun.	3					0.9	0.2					
Pinnularia												
braunii (Grun.) Cl. var. *amphicephala* (Mayer) Hust.		1	0.3	9.5	8.1			0.1		0.5	0.1	
Surirella												
angusta Kuetz.	2							0.1		0.1	0.1	
Relative abundance: Group 1 (most torelant taxa)			99.7	99.1	99.0	94.3	97.3	99.5	96.3	95.2	97.5	100
Relative abundance: Group 2 (less torelant taxa)			0.3	0.6	0.5	3.6	1.8	0.4	1.5	1.9	2.2	0
Relative abundance: Group 3 (sensitive taxa)			0	0.3	0.5	2.1	0.9	0.1	2.2	2.9	0.3	0

Table 4. Saprobic index estimated for the samples studied and corresponding water quality class; according to saprobic value of diatoms as far as proposed by SLÁDEČEK (1973, tab. 64).

	Sample No.									
	1	2	3	4	5	6	7	8	9	10
Saprobic index	2.0	2.6	2.6	2.3	2.2	2.6	2.3	2.2	2.5	2.4
Water quality class	II	II/III	II/III	II/III	II	II/III	II/III	II	II/III	II/III



find specimens to be identified as var. *saprophila* which were collected from "Kläranlage Frankfurt-Bad Vilbel" by LANGE-BERTALOT and RUPPEL (1980, figs. 312, 313).

Pinnularia braunii (GRUN.) CL. var. *amphicephala* (MAYER) HUST., occurring characteristically in the acidic waters in Japan (NEGORO 1942, 1962), was found in six of ten samples, in relatively higher rates than *Achnanthes minutissima* var. *saprophila* being 9.5% and 8.1% in sample 2 and 3, which were collected from the severely polluted Karabori-gawa River. After careful examination with scanning electron microscopy, we could not find any difference between the valves of the present samples and those collected from other highly acidic biotopes such as Kusatsu Hotspring, Bokke-numa Hotspring on the shore of Akan-ko Lake, Ohwaku-dani Hotspring, Shiobara Hotspring, Naruko Hotspring and Jigokudani Hotspring in Mt. Osore-san.

With regards to *Navicula frugalis*, though this taxon occurred in four samples (Table 3), its relative densities were all less than 1% in the present samples. However, our observation on the other samples showed relatively higher percentages, more than 10%, in α-meso/polysaprobic waters (very heavily polluted) of which water qualities were little better than that of the present ones.

As stated by SCHOEMAN (1979), although the species composition may change seasonally, the taxa forming the association remain indicative of a particular environmental condition. The present results, using LANGE-BERTALOT's method, as mentioned above, consistently showed the same pollution level as that obtained by chemical analysis. Though the dominant species or the species composition of the association varied from sample to sample, even at the same sampling station, the abundance of the group 1 species remained constant and that of group 2 and 3 also remained at the level of less than 10%, as summarized in Table 2.

On the other hand, the saprobic index (PANTLE and BUCK 1955) calculated from the present data remained quite low, being 2.0 in Sample 1 to 2.6 in Sample 6 (Table 4). These values can only indicate β or β/α-mesosaprobic conditions. Since the saprobic index was originally devised using all aquatic organisms, saprobic values were not given to all diatom taxa as seen in SLÁDEČEK (1973), and therefore unsuitable results seemed to be obtained in the present study using only diatoms. In addition, the second reason may be the fact that the saprobic values have been generally estimated on the basis of pollution affinities of the individual species rather than their tolerances to pollution.

In conclusion, it may be said that LANGE-

Figs 2–13. Most pollution-tolerant taxa. 2. *Achnanthes minutissima* var. *saprophila* var. nov. a-d. Photomicrographs and figures of the holotype specimen. e, f. Specimens from the type slide. g, h. Raphe valve and rapheless valve showing poroids of the striae (TEM), from Minamiasa-kawa River; 3. *Navicula atomus*. a. Valve view showing striation (TEM), from Minamiasa-kawa River. b, c. Specimens from Minamiasa-kawa River; 4. *N. saprophila*. a, b. Specimens from Minamiasa-kawa River. c. Valve view showing densely striated marginal zone and a stigma (arrow) (TEM), from Minamiasa-kawa River; 5. *N. frugalis*. a-c. Variation in valve shape and density of striae, from Minamiasa-kawa River; 6. *N. minima*. a. Valve view showing striation (TEM), from Yanase-gawa River. b, c. Specimens from Minamiasa-kawa River; 7. *N. seminulum*. a, b. Specimens from Karabori-gawa River. c. Valve view showing double row of poroids of the striae (TEM), from Karabori-gawa River; 8. *N. goeppertiana*. a, b. Specimens from Karabori-gawa River; 9. *N. veneta*. a-c. Specimens from Minamiasa- kawa River; 10. *Gomphonema parvulum*. a, b. Specimens from Minamiasa-kawa River; 11. *Pinnularia braunii* var. *amphicephala*. a, b. Specimens from Karabori-gawa River; 12. *Nitzschia palea*. a, b. Specimens from Minamiasa-kawa River; 13. *N. gandersheimiensis*. a, b. Specimens from Minamiasa-kawa River. Bars for each TEM phot. = 1 μm.

BERTALOT's method is highly reliable and is practical for routine use in applied hydrobiology. However, a slight revision is needed in the species composition within the groups for useful application in the Japanese waters.

Description of a New Taxon

Achnanthes minutissima KUETZ. var. **saprophila** var. nov. (**Fig. 2**)

Valvae lineares marginibus subconvexis et apicibus latissime rotundatis et interdam leviter rostratis vel capitatis, 8-14.5 μm longae, 3-3.5 μm latae. Areovalva area axiali lanceolata et dilatata, striis transapicalibus leniter radiantibus, circiter 28 in 10 μm, paulo distantiores in media parte valvae et leniter densioribus ad apices versus, circiter 32 in 10 μm, tenuissime punctatis. Rhaphovalva rhaphe recta, area axiali lanceolata et dilatata, area centrali parva et transapicaliter dilatata, striis transapicalibus leniter radiantibus, circiter 28 in 10 μm, clare abbreviatis et distantibus in media parte valvae et leniter, densioribus ad apices versus, circiter 32 in 10 μm, tenuissime punctatis.

Valves linear with slightly convex margins and very broadly rounded and sometimes slightly rostrate or capitate ends, 8-14.5 μm long and 3-3.5 μm broad. Rapheless valve with lanceolately broadened axial area and slightly radiate transapical striae, about 28 in 10 μm, somewhat more distant in the center, and more dense about 32 in 10 μm near the ends, and delicately punctate. Raphe valve with straight raphe, lanceolately broadened axial area and transapically dilated small central area. Transapical striae slightly radiate, clearly distant and shortened in the middle, about 28 in 10 μm, more dense near the ends, about 32 in 10 μm, and delicately punctate.

This variety is very small. Therefore, it can only be clearly distinguished structurally by electron microscopy. However, its autecological feature is conspicuous. It was found restricted to severely polluted river stretches. It differs from the nominate variety by its rectangular puncta elongated in the apical direction, less radiate striation at the valve ends and conspicuously widened axial area of the rapheless valve.

Holotype: H. K. T.-78 in coll. H. KOBAYASI

Iconotype: Fig. 2. a-d, photomicrographs and figures of the holotype specimen.

Type locality: Minamiasa-kawa River, tributary of the Tama-gawa River.

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小林　弘・真山茂樹：東京近郊の強腐水河川に見られるケイソウの耐性種群について

東京近郊にある著しく汚濁された5河川から得た10試料を選び，LANGE-BERTALOT 法による水質判定法の検討を行った。同じ地点からの試料でも，採取時期が異ると，その優占種も群落の種構成も異った。しかし識別ケイソウ群という観点からまとめると，いずれの地点においても極めて共通する値が得られた。このことは LANGE-BERTALOT 法の高い信頼性を示したものといえる。今回の試料からは LANG-BERTALOT の識別ケイソウ群-1にあげられている18分類群のうちの10分類群が得られた。また本邦の河川でこの群に加えるべき特徴的な分類群として，*Achnanthes minutissima* var. *saprophila* var. nov. と *Pinnularia braunii* var. *amphicephala* を提案した。（184 小金井市貫井北町4-1-1 東京学芸大学生物学教室）