Heavy-Metal Pollution and Its State in Algae in Kakehashi River and Godani River at the Foot of Ogoya Mine, Ishikawa Prefecture

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Alga as *Achnanthes minutissima* among diatoms is a widely adaptable taxon on the state of an aquatic environment. In this study, it was found that diatom had a specific tolerance to heavy metals (Cu, Zn, Pb, and Cd *etc*.) in river water samples, because the diatom assemblage consisted of almost only *Achnanthes minutissima* in Kakehashi river and Godani river, which were polluted with waste water from Ogoya copper mine. The relationship between the concentrations of heavy metals (Cu, Zn, Pb and Cd *etc*.) in river water and the attached substances (algae and silt *etc*.) and the relative abundances of diatom taxa were investigated in detail. The results indicated that the higher is the concentration of heavy metals in the river environment, the higher is only the relative abundances of *Achnanthes minutissima*. Thus, the taxon can be used as a bioindicator of heavy metal pollution. The relative rates of toxic chemical forms of copper in algae were 61 – 92% in the attached substances and 49 – 70% in the sediment on the river bed, respectively. Therefore, it was found that diatom as *Achnanthes minutissima* had a tolerance to heavy metals in river water, being able to live in such an environment. Since the water treated with calcium hydroxide from the deposition reservoir of Ogoya mine enters in Godani river, the river is polluted by heavy metals (Cu, Zn, Pb and Cd *etc*.). From the viewpoint of both biological and chemical analyses, Godani river is still polluted with heavy metals, because their concentrations in the river samples were very high. On the other hand, in Kakehashi river, the concentrations of heavy metals were very low and the distributions of some diatoms appeared in an unpolluted Nishimata river were observed. Therefore, Kakehashi river seems to be considerably recovered from heavy-metal pollution after closing the Ogoya mine.

Diatom is an influential bioindicator in the attached algae (epilithic algae), and has been widely employed as an environmental indicator for a long time.[1] This approach is based on the following facts: (1) a highly progressed classification of diatom taxa, (2) a wide distribution of various taxa in clean and/or polluted water areas, and (3) an easiness of counting of the number of diatom valves.

The attached diatom is useful as a bioindicator of organic and inorganic pollution. In regard to organic pollution, Sumita[2,3] and Watanabe *et al*.[4] estimated organic water pollution (DAIpo) by using the Diatom Assemblage Index, and Kobayashi *et al*.[5] studied the correlation between BOD and diatom assemblage.

Many studies have been carried out by using diatoms as an indicator of acidification (lower pH variation)[6-8] in an aquatic environment. By surveying diatoms in polluted rivers, it has been found that several kinds of diatoms can be used as the bioindicator of heavy-metal pollution.[9-12] In these biological studies, the distribution of the attached algae was investigated in detail, but the relation of the algae distribution with heavy metals evaluated by the chemical analysis was not examined very much. Therefore, it may be significant to discuss the relation between heavy metals and algae flora by both biological and chemical analyses.

In this research work, Godani river and Kakehashi river were selected as a model area in order to study the relationship between heavy-metal pollution and the attached algae distribution. Waste water containing heavy metals from Ogoya mine flowed into Godani river without any treatment before December, 1971, and after closing Ogoya mine in 1972, the water treated with calcium hydroxide from Kuratani deposition reservoir has flowed into the river until now. Since Godani river joined the main Kakehashi river stream, a partial area of the latter river was also polluted with heavy metals (Cu, Zn, Pb and Cd *etc*.). Various aquatic organisms have gradually entered into Kakehashi river along with the decrease in the concentrations of heavy metals in the river water after closing Ogoya mine.[9-12] In polluted and unpolluted areas, the concentrations of heavy metals (Cu, Zn, Pb and Cd) and that of alkaline earth metals (Ca and Mg) in the river water, the attached substances on the surface of psephite, and the sediment on the river bed were determined, and the taxa of the attached diatoms were identified in detail to clarify the effects of heavy metals on the distributions of the taxa. The toxic chemical forms of copper for algae in the attached substances and in the sediment on the river bed were also ascertained by means of sequential extraction methods.[23-25] The present state of Godani river and Kakehashi river was compared with the past state[9,11,21,22] by examining the attached algae in heavy metal-polluted rivers.

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Experimental

Apparatus

A pH meter (Model B-212; HORIBA, Japan) and an electric conductivity meter (Model B-173; HORIBA) were used to measure the pH and EC values of river waters, respectively. An aspiration pump (Model JP mini 5; Tokyo Deodoranto, Japan) was used to filtrate river water. For the pretreatment of samples, a centrifugation separator (Model LC-100; TOMY) and an electric drying oven (Model FS-32D; Toyo Seisakusho, Japan) were used. An electronic balance (Model AE163; Mettler, Switzerland) was used to weigh the sample mass. A quick digester (Model QD-01; Mitsubishi Chemical Industries, Japan) was used for wet ashing of the samples, and an ultrasonic cleaner (Model W-103T; HONDA, Japan) was used to leach metals from the residues after digestion. A flame atomic absorption spectrophotometer (FAAS, Model Z-6100; Hitachi) and a graphite furnace atomic absorption spectrophotometer (GFAAS, Model Z-8270; Hitachi) were used to determine the concentrations of metals in the samples. In the ashing experiment, an electric muffle furnace (Model OPM-28S; Toyo Seisakusho) was used. A scanning electron microscope (Model S-4500; Hitachi), an energy dispersion X-ray analyser (Model EMAX-5770W; HORIBA), and a X-ray fluorescence spectrometer (Model S-782XII; HORIBA) were used for SEM and XRF analyses.

Reagents

Nitric acid, perchloric acid, sulfuric acid, potassium permanganate, strontium nitrate, and formaldehyde solution were obtained from Katayama Chemicals (Osaka) and Wako Pure Chemicals (Tokyo), and were used without further purification. All of the reagents used were of analytical grade. Deionized water was used throughout this investigation. Stock solutions (1000 μg/mL) of copper, zinc, lead, cadmium, calcium and magnesium were obtained from Katayama Chemicals, Ishizu Seiyaku, and Kanto Chemicals. A working solution of metals was prepared by a suitable dilution of the stock solution with 0.1 M nitric acid. New M·X was obtained from Matsunami Glass Industries, and was used as a mounting medium of specimens.

Sampling

Sites and date. Two sites in Godani river (Site 1 and Site 2) and two sites in Kakehashi river (Site 3 and Site 4) of the polluted areas with heavy metals (Cu, Zn, Pb and Cd *etc*.), and one site in Nishimata river (Site 5) with no heavy metal pollution were selected as the sampling sites, as shown in Fig. 1. Sampling was carried out from May 1996 to December 1999, once a month or two months, except for the winter season. Sampling was carried out on fine-weather days. The temperature, pH and the electric conductivity of the water samples, and the surrounding temperature, were recorded. The river water, the attached substances and the sediment on the river bed were taken as the samples throughout this investigation, as reported previously.[9-11,22] Sampling for the specification of copper in the sediment on the river bed and in the attached substance was also carried out from October in 2002 to July in 2003.

River water. The river-water samples were taken from 20 – 30 cm in depth, and were filtered through a 0.45 μm membrane filter (Type HA; Millipore Corp.) with an aspiration pump soon after the sampling. A 1-ml volume of concentrated nitric acid was added to 100 mL of the filtrate. The water samples were stored in glass bottles, and were brought back to author's
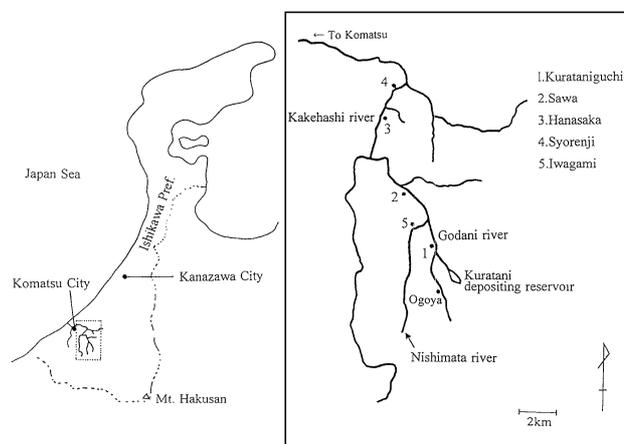

Fig. 1　Sampling sites.

laboratory. The samples were stored in a refrigerator (5°C) until analysis.

Attached substances. The attached substances consisted of algae (diatom, blue green alga, and green algae) and silt (clayey particles having *ca*. 10 μm in diameter). At the river bed of 30 – 40 cm in depth, psephite, which had an almost parallel surface to that of water, was taken. The upper surface of the psephite in a square (25 cm^2) was brushed down by using a nylon brush, and the attached substances obtained were treated with formaldehyde solution to identify diatom taxa. In order to determine their ignition loss, five sets of the attached substances in a square (25 cm^2) of the surface of psephite were taken at each site. The samples taken in polyethylene bottles were stored in a refrigerator prior to analysis.

Sediment on the river bed. The sediment on the river bed of 30 – 40 cm in depth was taken, and the relatively large stone and psephite in it were removed. Then, the residual sediment in the river waters was transferred into a polyethylene bottle. The samples were stored in a refrigerator prior to analysis.

Determination of the concentrations of metals in river water

The concentrations of metals were determined by FAAS for Cu, Zn, Ca and Mg, and by GFAAS for Pb and Cd, respectively. All of the working solutions of metals were prepared to be 0.1 M nitric acid solution. In the case of determining Ca, 1000 mg/L of Sr was added to the standard solution as an interference suppressor. The determination of metals was replicated at least three times, and the abnormal data were omitted by using Grubbs' test.

Determination of the concentrations of metals in the attached substances

The samples of the attached substances were transferred to a centrifugation tube, and were centrifuged (2000 rpm × 10 min) in order to remove the bulk water. The supernatant was discarded, and the precipitate was dried overnight at 80°C. A 10 – 20 mg portion of the dried sample was accurately weighed, and transferred into a glass test tube. After a small amount of water was added so as to wet the samples, 1 mL of concentrated nitric acid was added. The tube was set in a quick digester, and treated at the half-power mode, *i.e*. 350 W for 30 min. Then, 1 mL of the mixture of concentrated nitric acid and perchloric acid (3:1 v/v) was added to the sample. It was treated in the digester at 350 W for 30 min, and continually at 700 W for 20 min again. Most of the sample was dried up under this

condition, but the heating treatment at 700 W was continued, unless the content was dried up. After cooling the tube, 1 mL of 1 M nitric acid was added to the residue in the tube, and ultrasonicated to leach metals in aqueous solution. In order to remove white crystals of silicate, the suspension was filtered through a 0.45 μm membrane filter. The filtrate was transferred to a 25 or 50 mL volumetric flask. A proper amount of nitric acid was added, so that the final solution should be 0.1 M nitric acid solution; 1000 mg/L of Sr was also added to suppress any ionization interference. Then, the solution was diluted to 25 or 50 mL with distilled deionized water. The concentrations of metals in the thus-prepared solution were determined in the same way as that used for river water. The determination of metals was replicated at least three times, and any abnormal data were omitted by using Grubbs' test.

Determination of the concentration of metals in the sediment on the river bed

The sample of the sediment on the river bed was transferred to a 500 mL beaker. Deionized water was added to it, and the sample material was mixed with a glass rod. After leaving it for 3 min, psephites and fine sands were precipitated down quickly, while the floating particles were suspended in the mixture. The suspension was taken out by centrifugation (2000 rpm × 10 min), and dried overnight at 110°C. The dried sample was similarly digested as in the case of the attached substance. The concentrations of metals in the solution prepared was determined in the same way as that used for the river water.

Specification of heavy metals in the attached substances and in the sediment on the river bed

The sequential extraction method used for polluted soil and sediment[23-25] was adopted to elucidate the chemical species of copper, especially in toxic forms for algae in the attached substances and in the sediment on the river bed. The extraction of ion exchangeable and carbonate bound to copper, the toxic species of copper, was carried out by using a 0.11 M CH_3COOH solution for 16 h at room temperature (25°C).

Identification of diatom taxa in the attached substances

A proper amount of the attached substances was taken in an evaporating dish. The sample of the attached substance was treated with potassium permanganate and sulfuric acid, and then transferred to a centrifuging tube. After centrifugation (2000 rpm × 2 min), the supernatant was discarded. Deionized water was added to the precipitate, and the mixture was centrifuged again. This washing operation was repeated five times. The precipitate was suspended with deionized water, and mixed well. A small amount of suspension was dropped onto a cover glass, and dried at 100°C. A mounting medium was dropped on a slide glass, and a cover glass was mounted on it, as the attached side of the sample became below. In this way, a permanent preparation of diatom taxa was prepared. The taxa of diatom were identified by using an optical micrograph (×1500), referring them to the literature of diatom.[13–16] The number of valves of each taxon was counted in the preparation. The total number of individuals counted in every preparation was often in the range of 300 to 800. The relative abundance of each taxon, which was defined as the ratio of the number of each taxon to the total number of diatom, was calculated.

Identification of blue-green and green algae taxa in the attached substances

The taxa of the blue green and green algae were identified by using an optical micrograph (×1500), referring them to the literature of algae.[17,18] The abundance of each alga taxa was roughly expressed by five grades in the evaluation.

Determination of the amount of algae in the attached substances

Since the attached substances consisted of inorganic silt and algae living on the surface of psephite, the following study was carried out in order to determine the amount of algae in the attached substances.

First, the sample of the attached substances was heated at 110°C for 60 min in a porcelain crucible. After cooling the sample in a desiccator for 30 min, the weight was measured. This operation was repeated until its weight difference became smaller than 0.3 mg before and after heating. Then, the temperature was raised to 500°C, and the sample was treated similarly. The ignition loss (L) was regarded as the amount of contained organic matter originated from algae. The abnormal data were omitted by using Grubbs' test.

Electron microscope observation and X-ray fluorescence analysis of the attached substances

After treating the attached substances with carbon by vacuum evaporation, they were observed by a scanning electron microscope. The X-ray fluorescence spectrum of the valve surface of *Achnanthes minutissima* in the attached substances was also surveyed under the operating conditions of an accelerating voltage of 20 kV (10 mA and a measurement time of 300 s). The elements present on it were identified.

Results and Discussion

The water temperature (°C), pH, and EC (electric conductivity, μS/cm) of the river water at 5 sites during the sampling periods were as Site 1 (7.2 – 20.8, 5.8 – 7.2, and 100 – 410), Site 2 (6.9 – 21.5, 6.7 – 8.1, and 99 – 230), Site 3 (7.2 – 20.9, 6.9 – 8.7, and 74 – 140), Site 4 (7.7 – 22.8, 6.5 – 8.0, and 100 – 140), and Site 5 (11.1 – 18.8, 7.2 – 7.9, and 81 – 85), respectively.

Table 1 gives the analytical results for the concentrations of metals (Cu, Zn, Pb, Cd, Ca, and Mg) in the river water, the attached substances, and the sediment on the river bed at Sites 1 – 5. Each determination of metals was accomplished within ±10% error. In this investigation, 120 diatom taxa[9,11,22] were identified through all of the attached diatom assemblages surveyed. In every diatom assemblage at 4 sites (Sites 1 – 4), the first two taxa with the highest values of the relative abundance were taken. Those two taxa were labeled as 1st and 2nd grade taxa, according to the degree of the relative abundances. Of all the 62 assemblages surveyed, the 1st grade taxon was *Achnanthes minutissima*, which has a tolerance to copper ion in the river water,[19,20] and the 2nd grade taxon was *Surirella angusta* in the same polluted water area, as shown in Table 2, as well as in Fig. 2. In Site 5, the 1st grade taxon was *Gomphonema helveticum*, and the 2nd grade taxon was *Cocconesis placentula var. lineatal*. The range of the relative abundance of *Achnanthes minutissima and Surirella angusta* in Site 5 was 1.1 – 7.3% and 0 – 1.0%, respectively. In every table, the indicated values were the mean values during the sampling periods. The ranges of the concentrations of metals are also given in parentheses.

Figures 3 and 4 indicate the relationship between the concentrations of heavy metals (Cu, Zn, Pb and Cd) in the river water samples and the attached substances and the relative abundance of *Achnanthes minutissima* and *Surirella angusta*, respectively. Attention was paid to these two taxa, because they

Table 1 Concentrations of metals in the river water samples, attached substances and sediment on the river bed[a]

Sample	Site	Metal					
River water		Cu/mg L⁻¹	Zn/mg L⁻¹	Pb/μg L⁻¹	Cd/μg L⁻¹	Ca/mg L⁻¹	Mg/mg L⁻¹
	1	0.22 (0.11 – 0.37)	1.0 (0.29 – 2.7)	< 5	4.3 (1.0 – 8.8)	15 (3.7 – 33)	8.7 (2.9 – 14.6)
	2	0.08 (0.019 – 0.24)	0.35 (0.18 – 0.74)	< 5	1.5 (0.9 – 2.2)	6.9 (4.9 – 9.9)	4.4 (2.7 – 6.3)
	3	0.015 (0.008 – 0.025)	0.17 (0.11 – 0.27)	< 5	0.9 (< 0.5 – 1.5)	4.2 (2.8 – 5.8)	2.5 (1.8 – 3.4)
	4	0.013 (0.006 – 0.020)	0.15 (0.08 – 0.26)	< 5	0.8 (< 0.5 – 1.3)	4.2 (3.6 – 5.5)	2.7 (2.0 – 3.3)
	5	< 0.005	< 0.004	< 5	< 0.5	2.8 (2.3 – 3.1)	1.1 (1.0 – 1.2)
Attached substances[b]		Cu, %	Zn, %	Pb/μg g⁻¹	Cd/μg g⁻¹	Ca, %	Mg, %
	1	3.32 (1.42 – 5.20)	0.95 (0.24 – 2.10)	750 (240 – 1350)	14 (3.2 – 24)	0.31 (0.13 – 0.51)	0.53 (0.30 – 1.38)
	2	1.12 (0.83 – 1.37)	0.63 (0.27 – 1.10)	150 (66 – 240)	11 (5.7 – 26)	0.32 (0.14 – 0.58)	0.43 (0.30 – 0.56)
	3	0.13 (0.09 – 0.20)	0.36 (0.18 – 0.53)	180 (110 – 380)	16 (9.2 – 30)	0.35 (0.33 – 0.38)	0.44 (0.32 – 0.62)
	4	0.16 (0.11 – 0.20)	0.28 (0.20 – 0.36)	180 (110 – 330)	17 (8.7 – 30)	0.26 (0.18 – 0.30)	0.44 (0.35 – 0.54)
	5	0.02 (0.01 – 0.03)	0.03 (0.02 – 0.07)	80 (24 – 220)	0.8 (0.7 – 0.9)	0.14 (0.05 – 0.32)	0.46 (0.42 – 0.56)
Sediment on the river bed[b]		Cu, %	Zn, %	Pb/μg g⁻¹	Cd/μg g⁻¹	Ca, %	Mg, %
	1	1.05 (0.60 – 2.20)	0.26 (0.13 – 0.53)	310 (190 – 470)	3.5 (2.1 – 5.0)	0.26 (0.11 – 0.52)	0.71 (0.46 – 0.82)
	2	0.23 (0.077 – 0.37)	0.12 (0.083 – 0.17)	75 (52 – 88)	2.9 (1.7 – 5.2)	0.39 (0.25 – 0.69)	0.61 (0.46 – 0.68)
	3	0.10 (0.050 – 0.16)	0.11 (0.089 – 0.18)	110 (61 – 190)	5.1 (2.8 – 11.8)	0.43 (0.38 – 0.52)	0.69 (0.47 – 0.84)
	4	0.11 (0.067 – 0.13)	0.12 (0.080 – 0.19)	120 (63 – 160)	6.9 (2.7 – 11)	0.47 (0.39 – 0.61)	0.78 (0.73 – 0.85)
	5	0.004 (0.003 – 0.005)	0.022 (0.011 – 0.037)	18 (9 – 28)	0.5 (0.2 – 1.0)	0.11 (0.01 – 0.19)	0.41 (0.30 – 0.51)

a. Values in parentheses are the concentration ranges of metals determined. b. Expressed on the dry weight basis of the sediment on the river bed.
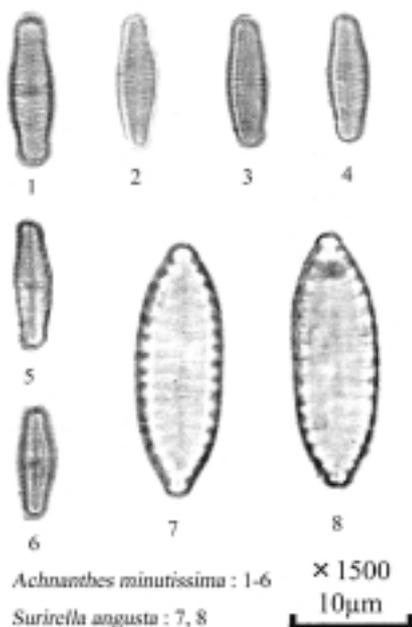

Fig. 2 *Achnanthes minutissima* and *Surirella angusta* of diatom in Godani river.

were always found as the first two taxa in the heavy-metal polluted area. In the river water, the higher was the concentrations of heavy metals, the higher was the relative abundances of *Achnanthes minutissima* alone. The concentration of lead in the river water was always less than the detection limit by FAAS and GFAAS at every site. A graphical plot of the results for lead was not given. Based on these results, it turned out that there was a good correlation between the relative abundance of *Achnanthes minutissima* and the concentrations of heavy metals in the river water. The results obtained here are in good agreement with those reported before.[9–12] On the other hand, the correlation between the concentrations of heavy metals in the attached substances and the abundances of *Achnanthes minutissima* is not as clear as in the case of the river water. Even if the concentration of cadmium in the attached substances was relatively high, the relative abundances of *Surirella angusta*, except for *Achnanthes minutissima*, were sometimes more than 50%. This may be due to the lower concentration of cadmium than those of other heavy metals, and hence the other taxa can endure it. The relative abundance of *Achnanthes minutissima* showed a tendency to increase when the concentrations of heavy metals (Cu, Zn, and Pb), except for cadmium, increased. The relative rate of the toxic species of copper, the ion exchangeable and carbonate bound to copper (Cu^{2+}, $CuOH^+$, $CuCl^+$, $CuHCO_3^+$, $CuCO_3 \cdot Cu(OH)_2$ etc.) obtained by means of the sequential extraction method,[23–25] was in the following order: Site 4 (61%) < Site 3 (64%) < Site 2 (92%) > Site 1 (84%) in the attached substances; Site 4 (56%) > Site 3 (49%) < Site 2 (70%) > Site 1 (69%) in the sediment on the river bed. Then, the relative abundance of *Achnanthes minutissima* tended to increase in the order of Site 4 (36%) < Site 3 (56%) < Site 2 (83%) < Site 1 (97%). These results demonstrated that *Achnanthes minutissima* has a tolerance to heavy metals (Cu, Zn, Cd and Pb etc.), and that the taxon can be used as a bioindicator of heavy-metal pollution.

The taxa of blue green and green algae are stated as follows: blue green algae, *Xenococcus kerneri,* was distributed at Site 1 and Site 2. *Phormidium tenue* and *Lyngbya martensiana* were widely distributed at all sites. *Oscillatoria limosa* and *O. rubesens* were distributed at Site 3 and Site 4. *Homoeothrix janthina* was slightly found at Site 3 and Site 4. Green algae, *Hormidium klebsii*, *Ulothrix tenuissima*, *Spirogyra* sp., and *Mesotaenium* macrococcum were distributed at Site 3 and Site 4.

All amounts of heavy metals in the attached substances may not be contained in algae, because the attached substances consisted of attached algae and silt. The silt has been postulated to contain the same components as the floating particles in the sediment on the river bed, and the results of its concentrations of heavy metals, especially copper and zinc, in the attached substances were compared with those in the sediment on the river bed (Fig. 5). At Site 1, the concentrations of heavy metals, particularly copper, in the attached substances were higher than those in the sediment on the river bed, and the attached algae

Table 2   First and second grade taxa in the attached diatom assemblages

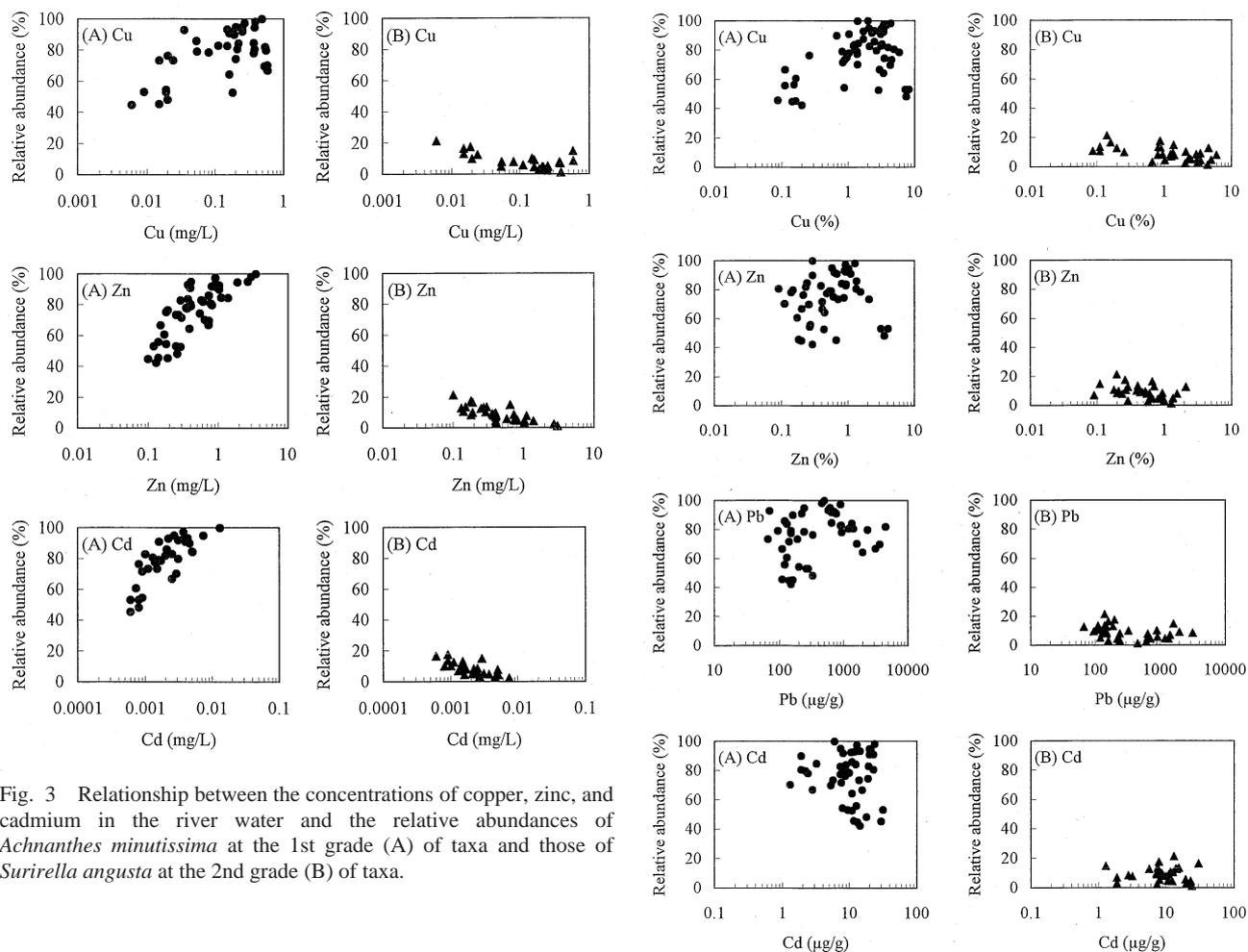| Site | 1st grade | | 2nd grade | |
	Taxa	Range of relative abundance	Taxa	Range of relative abundance
1	*Achnanthes minutissima*	63.6 – 97.3	*Surirella angusta*	0.0 – 27.2
2	*Achnanthes minutissima*	54.3 – 92.9	*Surirella angusta*	1.7 – 17.4
3	*Achnanthes minutissima*	14.6 – 73.3	*Surirella angusta*	10.6 – 61.9
4	*Achnanthes minutissima*	13.4 – 79.7	*Surirella angusta*	10.0 – 65.1
5	*Gomphonema helveticum*	0.0 – 53.8	*Cocconesis placentula var. lineata*	1.7 – 33.9

Fig. 3   Relationship between the concentrations of copper, zinc, and cadmium in the river water and the relative abundances of *Achnanthes minutissima* at the 1st grade (A) of taxa and those of *Surirella angusta* at the 2nd grade (B) of taxa.

Fig. 4   Relationship between the concentrations of copper in the attached substances and the relative abundances of *Achnanthes minutissima* at the 1st grade (A) of taxa and those of *Surirella angusta* at the 2nd grade (B) of taxa.

seems to retain considerable amounts of heavy metals (Cu and Zn *etc*.).   In the X-ray fluorescence spectra of *Achnanthes minutissima* and another algae in the attached substances, a small peak of copper was found in *Achnanthes minutissima*, but not in another algae.   Therefore, a considerable amount of copper was laid on the valve surface of *Achnanthes minutissima*.   Although the mechanism of the biological accumulation of heavy metals by *Achnanthes minutissima* is not clear, diatoms may take heavy metals through complex formation with some kinds of polysaccharides and amino acids,[19] of which the valve walls of alga are composed.

In order to ascertain the amount of algae in the attached substances, an ashing study (ignition loss) was carried out. The results for ignition loss indicated that the attached substances contained 25 – 55% of algae.

Finally, the authors tried to analyze the present situation of heavy metals pollution in Godani river and Kakehashi river. At Site 1 and Site 4, the results of the present research were compared with those of past research.[9,11,21,22]   Figure 6 shows

that annual change of the concentrations of copper and zinc in river water for the last 50 years.   The concentrations of these metals has decreased remarkably from the middle of the 1950s (the working time of Ogoya mine) to December 1971 (the closing time of Ogoya mine).   However, the concentrations of copper and zinc in the river water has approximately levelled off up to the present day after the closure of Ogoya mine.   On the contrary, the concentrations of heavy metals in the attached substances at present was not very much changed at Site 1, while it dramatically decreased at Site 4 (Table 3), when compared with the results from 1975.   The concentration of copper in the attached substances was 3.80% at Site 1 and
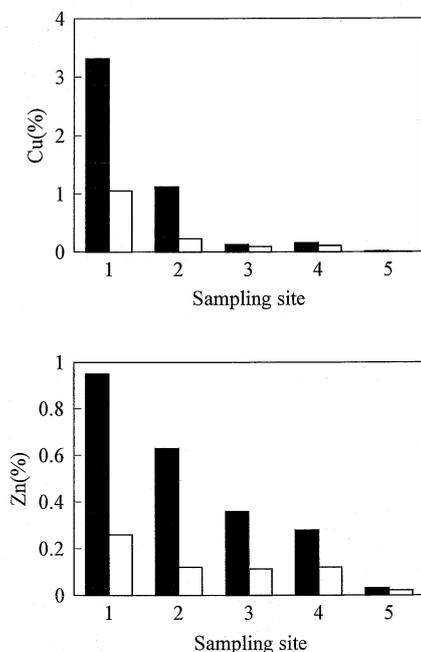
Fig. 5   Comparison of the concentrations of heavy metals in the attached substances and the sediment on the river bed. ■, Attached substances; □, sediment.
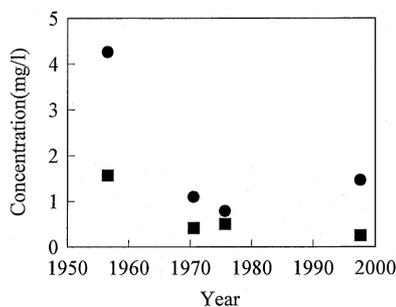

Fig. 6   Annual change in the concentrations of heavy metals in the river water at Site 4. ■, Cu; ●, Zn.

Table 3   Change in the concentrations of heavy metals in the attached substances at Site 1 and Site 4

Site	Date	Cu, %	Zn, %	Pb/μg g^{-1}	Cd/μg g^{-1}
1	1975/ 1/16	2.06	0.503	1980	49.1
	1998/12/21	3.25	0.807	1350	12.5
4	1975/ 7/ 9	2.61	1.25	316	21.6
	1996/ 6/14	0.14	0.20	140	13

were very low, and the distribution of some diatom taxa appearing in the unpolluted Nishimata river (Site 5) was observed in the Kakehashi river area.

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0.13% at Site 4 on April 15 in 2003. Therefore, it seems that Godani river is still polluted with copper, while Kakehashi river has been considerably recovered from its pollution.

The number of kinds of diatoms observed was 26 species, but the relative abundance of *Achnanthes minutissima* was very high (54 – 97%) in the area of the upper stream (Site 1 and Site 2) in Godani river, where the treated water with calcium hydroxide from the deposition reservoir of Ogoya mine had been directly discharged. The same result was obtained at the tributary from the reservoir to Godani river. All of river water samples, the attached substances, and the sediment on the river bed contained highly concentrations of heavy metals at the upper stream area (Site 1 and Site 2) compared with the state of an unpolluted area (Site 5). Hence, Godani river would be still polluted with heavy metals. On the other hand, the down stream area (Site 3 and Site 4) in Kakehashi river has recovered from heavy-metal pollution, because the concentrations of both heavy metals in the river water and in the attached substances