Major and minor elemental compositions of streambed biofilms and its implications of riverine biogeochemical cycles

Short title: Biofilm geochemistry

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Abstract

Chemical compositions of streambed biofilms from a major river of central Japan (the Kushida River) were obtained, with data of associated sediments (fine-grained fractions < 63 μm) and dissolved components of waters, in order to provide preliminary information about biogeochemical significance of streambed biofilms. During the sampling period (July 31st to August 3rd, 2013), dissolved components of the river waters were influenced by the dam reservoir. Concentrations of NO_3^-, silica (as Si), SO_4^{2-}, PO_4^{3-} and Ca^{2+} decreased across the dam, whereas Fe and Mn increased across the dam, and then decreased downstream rapidly. Streambed biofilms contain significant amount of non-nutrient elements such as Al (up to 21% as Al_2O_3 on water and others-free basis), indicating that they are contaminated as siliciclatic (silt and clay) materials. Siliciclastic materials in the biofilms are basically compositionally similar to fine-grained (< 63 μm) fractions of streambed sediments. However, some elements such as Ca, P, Mn, and Zn are markedly enriched in the biofilms. Particularly, Mn concentrations in the biofilm samples collected just below the dam reservoir are very high (~4.0 wt %), probably due to accumulation from the discharged water. Concentrations of trace elements such as P, Cr, Cu, Zn and V appear to be controlled by amounts of Fe-oxides and/or Mn-oxides in biofilms. Numbers of factors are involved in controlling chemical compositions of streambed biofilms, including amount of contaminated siliciclastics, authigenic mineral formation, adsorption of dissolved materials and microbial metabolisms. As demonstrated by this study, systematic analyses including major elements and comparison with associated sediments and waters could reveal biogeochemistry of this complex system.

Capsule:

Chemical compositions of streambed biofilms can be interpreted by contaminated silts and clays, bioaccumulation and adsorption onto Fe- and Mn-oxides.

1. Introduction

Streambed biofilms composed mainly of microbes and extracellular polymeric substances (EPS) are quite complex systems. They have been studied extensively in the context of microbial community compositions, and primary production and nutrient cycles of river ecosystem, and metal accumulation mechanisms (e.g, Battin et al., 2003; Besemer et al., 2009; Dranguet et al., 2017; Drahota et al., 2014; Konhauser et al., 1994; Tani et al., 2003). Biofilms can be regarded to represent consortia of primary producers in river ecosystem, and are fed directly or indirectly as POM (Particulate Organic Matter) by aquatic organisms such as grazers and collectors. Thus these primary consumers and those of higher trophic levels could be affected by chemistry of biofilms (e.g., Ancion et al., 2013; Farag et al., 2007; Rhea et al., 2006). For example, Farag et al. (2007) demonstrated that metal concentrations in biofilms and in aquatic invertebrates are closely related with each other. Also, Ancion et al. (2013) reported that three trace metals (Zn, Cu & Pb) in biofilms explained the community variations in bacteria and ciliate protozoa more than those in sediments. Significance of inorganic geochemistry of streambed biofilms has been well demonstrated by these studies and others (e.g., Haack and Warren, 2003; Kamjunke et al., 2015). To our knowledge, however, these and other previous geochemical studies of streambed biofilms did not provide dataset of major elements as Si, Al, Ti, Fe, Mn, Mg, Ca, Na, K and P, despite concentrations of these elements provide basic information about what components contribute to biofilm chemical compositions and are expected to give insights into dynamics of trace metals in biofilms. Thus, the purposes of this study are to present the first comprehensive dataset of major and minor elemental compositions of streambed biofilms, providing basis on how biofilms contribute to biogeochemical cycles in the stream ecosystem.

2. Materials and Methods

2.1. Sampling area

The Kushida River originates from the Mount Takami (alt. 1, 249m) at the border between Mie Prefecture and Nara Prefecture in central Japan. It flows 87km into the Ise Bay and its basin area is 436km^2 (Fig. 1). The basement of the northern part is composed mainly of granitic rocks, whereas that of the southern part consists of schists (Geological Survey of Japan, 2014). Population at the upper and the middle reaches is 15, 000 persons and that at the lower reaches is 16, 000 persons. The basin of the Kushida River is occupied largely with forests, paddy and tea fields, and residential lands. Industries are also present but of small scale. The Kushia River water during the sampling season can be assumed to be pristine and its trophic level is very low, as demonstrated by low BOD concentration (<1mg/l) (Chubu Regional Bureau, 2013a). The Kushida River has many tributaries, most of which, except for the Hachisu River and the Sana River, are very small. The Hachisu River is located at the uppermost reaches and has a large dam (the Hachisu Dam) with height of 78 m, effective water depth of 41m, and effective storage capacity of 29,400,000m^3 (Chubu Regional Bureau, 2014). The residence time of water in the dam reservoir is calculated to be 78 days, based on an average inflow rate (4.30 m^3/S) in 2009 and the effective storage capacity.

2.2. Sampling and sample treatment

The samplings were performed from July 31st to August 3rd, 2013. We collected 31 water samples including 6 samples from the Hachisu River and 5 reference samples from drainages and small tributary, which were not used for this study (Fig. 1). Although we intended to collect biofilms and sediments at the same localities for the

water samples, actual collected number of biofilms are 26, among which only 17 samples were enough in amount for analyses of major and minor elements. Sediment samples used for chemical analyses of fine fractions (< 63μm) were collected mainly from dips of exposed boulders in stream and shores of the river. We collected and analyzed 21 sediment samples, among which 17 samples corresponding to biofilm samples were used in this study.

Water samples were filtered on site (< 0.45 μm) and stored pre-cleaned polyethylene bottles (100ml). For samples subjected to heavy metal analyses, polyethylene bottles were pre-washed with HNO_3 and 0.5ml HNO_3 were added to the samples immediately back to the laboratory. Streambed biofilms were removed from gravels using a plastic brush and suspended in a plastic bowl to remove coarse fractions. Suspended fractions were pipetted and transferred into plastic test tubes. The tubes were manually centrifuged on-site, and supernatants were removed. This procedure was repeated until sufficient quantities (approximately 7ml) of streambed biofilms were collected, although as described earlier some samples were not enough for chemical analyses. The biofilm samples were stored in an icebox and transported to the laboratory, where we disentangled biofilm with distilled water and removed aquatic insects and large detritus. The samples were subsequently dried at 110°C for 48 hours, and then grinded using an agate mill. Sediment samples were dried at room temperature for several days and then at 110°C for 48 hours. After being dried, they were sieved and fractions less than 63μm were retrieved for analyses.

2.3. Analyses

pH, DO and temperature were analyzed on-site using glass electrode pH analyzer and fluorometric DO analyzer. Cations (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}) and anions (Cl^-, NO_3^-, SO_4^{2-}) were analyzed using ion chromatography. Dissolved metals (Fe, Mn), silica (as Si), and phosphorous were analyzed by Inductively coupled plasma

atomic-emission spectroscopy (ICP-AES) (IRIS ICAP, Nippon Jarrell Ash). Streambed biofilms and sediments (<63 μm) were combusted at 500°C and then mixed with lithium tetraborate in the ratio of 1:7. This mixed powder was melted in a high frequency muffle furnace at 1100 °C to be glass bead, which was subjected to X-ray fluorescence spectroscopy (XRF)(Axios-N system, PANalytical). Ten major elements (Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P) and 7 minor elements (Ba, Cr, Cu Ni, V, Y, Zn) were analyzed. For some samples whose amounts were not enough for XRF analyses, sample powders were admixed with standard rock sample powder (JG1a) and the data were recalculated to obtain chemical compositions of biofilms. Organic elements (C, H, N) of dried samples of biofilms were analyzed using an elemental analyzer (Frash 2000, Thermo Scientific). Isotopic compositions of C and N in biofilms were analyzed using a mass spectrometer (EA-Mass Spectrometr, Thermo Scientific). Original chemical data of streambed sediments and water are available upon request.

3. Results and Discussion

3.1. Influence of the dam reservoir to water qualities

Concentrations of NO_3^-, SO_4^{2-}, PO_4^{3-}, silica and Ca decrease to variously degree from 10 % to nearly 100% of the upstream levels across the Hachisu Dam reservoir, whereas Cl^- and K^+ do not change (Fig. 2). Though not shown in this figure, Mg^{2+} and Na^+ show a similar trend as K^+. Concentrations of Fe and Mn increase drastically through the dam reservoir, from less than 5ppb to nearly 40 ppb and more than 70 ppb, respectively (Fig. 2). While concentrations of the other components remain nearly constant in the downstream from the dam, those of Fe and Mn exponentially decrease. Inflow of such the Hachisu River water appears to influence some component of the mainstream water. Concentrations of NO_3^- and silica of the mainstream water are likely to be lowered by mixing of the Hachisu River water.

The above-described changes of dissolved components through the Hachisu Dam reservoir may be explained by the results of previous studies (Beaulieu et al., 2014; Hotta and Sugitani, 2007; Némery et al., 2016). Hotta and Sugitani (2007) reported depletion of dissolved NO_3^- and SiO_2 in a small oligotrophic dam reservoir in central Japan (Iwamura Dam, Gifu Prefecture) with much shorter retention time (ca. 10 days) of water than the Hachisu Dam (ca. 80 days); the dam surface and outflow waters from ca. 8m depth show depletion of these nutrients relative to inflow waters during summer. Hotta and Sugitani (2007) also demonstrated high concentrations of dissolved Fe and Mn in the surface and outflow waters during the autumn and winter, contrastive to their very low concentrations during summer and inflow waters. Temperature data of water column of this dam indicate the development of thermal stratification during summer and its disappearance in autumn. This thermal stratification was in harmony with DO profile; the bottom water is anoxic during the stratification. Thus, Hotta and Sugitani (2007) attributed high concentrations of Fe and Mn of the surface and outflow waters of the Iwamura Dam in autumn to mixing of surface and bottom waters caused by destruction of thermal stratification; the bottom water has been enriched in Fe and Mn due to elution from the sediment under anoxic condition.

In the present study, causes for decrease in NO_3^- and SiO_2 and increase in Fe and Mn across the dam reservoir are less constrained, because the sample collection was performed only in the summer. Also, the Hachisu Dam has three different discharge gates, one of which is changeable with its height, making it difficult to specify the depth from which outflow waters were discharged. Additionally, retention of the discharged water in the shallow pool just below the dam, from which the St. 3 sample was collected, possibly influenced the water quality, considering luxuriance of green algae. Despite such ambiguities, we may suggest the possibility that uptake by phytoplankton in the dam reservoir can be one of promising causes for

189 decrease in NO_3^- and SiO_2, because other nutrients such as PO_4^{3-}, SO_4^{2-} and Ca^{2+}
190 also show decrease across the dam, while Cl^- and K^+ do not. As the sampling was
191 demonstrated from the end of July to August, the water stratification is assumed have
192 already been established in the dam reservoir. In fact, a minimum value of DO for the
193 bottom water recorded from 2003 to 2013 at the monitoring point is 4.8 mg/l, nearly
194 half of minimum values of middle and surface waters (9.5 and 9.0 mg/l, respectively)
195 (Chubu Regional Bureau, 2013b). Therefore denitrification should be considered as
196 another or additional cause for decrease in NO_3^- across the dam (e.g., Beaulieu et al.,
197 2014). As DO in the water sample collected from the shallow pool (St. 3) (6.19 ppm)
198 was higher than levels for anoxic or dysoxic conditions, it is difficult to attribute Fe and
199 Mn enrichment to in-situ reduction of Fe- and Mn-oxides. Enrichment in dissolved Fe
200 and Mn is therefore more likely explained by contribution of anoxic bottom water of the
201 dam reservoir where release of Fe and Mn from sediment occurred by abiogenic
202 reduction due to lowering of redox potential and related biological respiratory
203 reduction of Fe- and Mn-oxides (Beutel et al., 2008; Davison, 1993; Ito et al., 2008;
204 Munger et al., 2017).
205
206 *3.2. Geochemical characteristics of biofilms*
207 The streambed biofilms have various values of ignition loss from 18.1 % to 59.8 %
208 (Table 1). Concentrations of carbon and nitrogen known as bioessential elements also
209 range widely from 6.1 to 29.3 % and from 0.9 to 4.1 %, respectively. Significant
210 amounts of Al and Ti, which in general are not identified as bioessential (e.g., Maret,
211 2016), are also detected. Al and Ti concentrations range from 1.64 % to 7.32 % and
212 from 0.18 % to 0.5 %, respectively. Total Si concentrations range from 10.13 % to
213 17.87 %, showing a positive correlation with Al (c.c. = 0.61). Al and Ti in biofilms are
214 thought be contained exclusively in siliciclastic fractions (clays and silts), products of
215 weathering of basement rocks of tributaries, because these two elements are

basically stable during weathering and retained as solid phases (e.g., Young and Nesbitt, 1998; Wei et al., 2003). This indicates that siliciclastic materials, in addition to organic materials, comprise significant proportion of streambed biofilms. Silts and clays suspended in river waters can be adsorbed by adhesive EPS (Extracellular Polymeric Substances) of biofilms, particularly during the period of subsidence of water level after flooding. On the other hand, the dominance of H, O, C, and N in organic fractions of biofilms could obscure substantial features of geochemistry of biofilms, because proportions of organic matter to siliciclastic material in biofilm are various as indicated by data of ignition loss. Therefore, in order to examine precisely geochemical features such as downstream trend and make comparison with streambed sediments, the raw data of major elements are once recalculated to 100% (water and others free basis) as oxide forms. Oxide-form concentrations of major elements of the biofilms are basically similar to fine-grained sediments (< 63 μm) and the Japanese Upper Crustal Composition representing averaged compositions of surface rocks of Japanese Islands (Table 2). This may give impressions that inorganic chemical compositions of streambed biofilms are controlled largely by clays and silts settled down at each site. This is correct; however as inferred from e.g., unusually high Mn concentration in biofilm (Table 1), more complex processes are involved in geochemistry of biofilms.

Comparisons of Al-normalized values, often used to assess enrichment of elements in sediments (e.g., Din, 1992), provide further insights into chemical differences between biofilms and sediments at the same localities (Figs 3 and 4). The distribution patterns indicate that Mg and K in the biofilms are derived largely from contaminated silt and clay, whereas Ca and P, and to lesser extent Si appear to be enriched in the biofilms, relative to the associated sediments. Fe, Mn, Cr, Cu, V and Zn also show relative enrichment. Na and Co tend to be depleted in biofilms. Enrichment or depletion of concentration of certain element in biofilm relative to

sediment could be interpreted in the context of bioaccumulation, microbial metabolic reduction and resultant removal from biofilm, inorganic oxidation and reduction, and adsorption on other phases such as Fe-oxide and organic matter (e.g., Carpentier et al., 2003; Du Laing et al., 2009; Gounot, 1994; Weber et al., 2006). For example, enrichment of Ca and P can be simply interpreted to reflect biomass in biofilms, because they are representative bio-essential elements. Si-enrichment is also attributed to populations of diatoms, which are the most dominant unicellular eukaryotic organisms with silica frustule in the biofilm. As discussed later, enrichment of the other elements could be interpreted adsorption on Fe- or Mn-bearing phases, although detailed processes require further studies.

In biofilms from the Kushida River, carbon isotopic compositions ($\delta^{13}C$) range significantly from -10.8 to -25.8 ‰, while nitrogen isotopic compositions ($\delta^{15}N$) do from -0.4 to 5.3 ‰, respectively (Table 1). Nitorgen isotopic compositions show a trend of becoming heavier downstream. This trend is consistent with the downstream increasing trend of concentration of NO_3^-, Cl^- and PO_4^{3-} in river water (Fig. 2). As populations of inhabitants increase downstream, these trends of dissolved components in the river water are likely explained by influx of domestic wastewater. Considering that domestic wastewaters enriched in organic matter generally have high $\delta^{15}N$ (e.g., Wada, 2009; Sweeney et al., 1980), the downstream trend of $\delta^{15}N$ in biofilm could be a consequence of assimilation of NO_3^- with relatively higher $\delta^{15}N$ values. Although denitrification, which could occur within biofilms and leave heavy NO_3^-, may need to be considered as another or additional mechanism for the N-isotopic trend (e.g., Cline and Kaplan, 1975; Maritotti et al., 1988), the clear downstream trend favors the former interpretation.

3.3. Downstream trends of biofilm geochemistry

Downstream chemical trends of streambed biofilms could provide some constraints

on the cause of enrichment in Fe, Mn and other metals in the streambed biofilms relative to sediments (Figs 3 and 4). As shown in Fig. 5, concentrations of these elements in the streambed biofilms are markedly higher in the Hachisu River samples. In particular, Mn concentrations of the Hachisu River samples range from 5.5 to 1.2% (as water and others free basis), which are much higher than Japanese Upper Crustal (JUC) value (0.085 % as Mn)(Togashi et al., 2000). Additionally, it rapidly decreases downstream. A similar trend can be seen also for Fe, Ba, Co, and Zn. Although dissolved metals in river waters we analyzed were only Mn and Fe, their trends shown in Fig. 2 could give some constraints on mechanism responsible for their and others' enrichments. Mn- and Fe-enrichment in the biofilms from the Hachisu River is best explained by accumulation from the discharged water enriched in dissolved in Mn and Fe. Several pathways are potentially involved in this enrichment, including 1) formation of fine metalliferous particles by oxidation of dissolved Fe and Mn and their adhesion onto EPS of biofilms, 2) precipitation onto biofilm surface enriched in functional groups, 3) precipitation as results of bacterial metabolisms (Fe- and Mn-oxidizing bacteria), and 4) bioaccumulation (e.g., Carmichael et al., 2013; Clarke et al., 1997; Gounot, 1994; Konhauser et al., 1994a; Weber et al., 2006). In either case, like Fe and Mn, the other trace elements were probably enriched in the discharged water and co-precipitated or adsorbed onto Fe- and Mn-oxides, which will be discussed in the next section.

3.4. Inter-element relationships in biofilms

As mentioned above, mechanisms of enrichments of constituent elements of biofilms are complex, but could be partially inferred from inter-element relationship. Relationships of Al-normalized values of some selected elements to Fe/Al are shown in Fig. 6, where biofilm and sediment samples from the Kushida and the Hachisu rivers are discriminated.

297 Notably, P/Al, showing excess P relative to siliciclastic components,
298 positively correlates with the excess Fe (Fe/Al). Correlation coefficient between P/Al
299 and Fe/Al is 0.82, which is higher than those between P/Al and Ca/Al (0.6) and
300 between P and C (0.56). It is thus implied that significant fraction of the excess P in
301 the riverbed biofilms is bound to Fe-facies, probably Fe-oxides/hydroxides or
302 comprises ferric-phosphate (e.g., Fang et al., 2017; Konhauser et al., 1994b;
303 Ueshima et al., 2004). Some other elements such as Zn and V showing clear positive
304 correlations with Fe/Al are also considered to be associated with Fe- facies (e.g.,
305 Trivedi et al., 2001; Martinez and McBride, 1998; Wällstedt et al., 2010). On the other
306 hand, as can be seen from the relationship between Fe/Al and Mn/Al, the excess Mn
307 likely comprises a different facies such as Mn-oxides (Tani et al., 2003). Mn is
308 especially enriched in the biofilms collected from the lower reaches of the Hachisu
309 River. Although number of samples is limited, inter-elements relationships are
310 different from those of the Kushida River biofilms (Fig. 7). It is likely that Ba and Co
311 are incorporated into Mn-facies in the Hachisu lower reaches biofilms (e.g., Nicholson
312 and Eley, 1997; Gray and Malati, 1979).
313
314 ## 4. Conclusions
315
316 A major river of central Japan (the Kushida River, Mie Prefecture) was studied for
317 chemical and isotopic (C and N) compositions of streambed biofilms, with waters and
318 riverbed sediments. Obtained results are as follows.
319 1) During the sampling period (July 31st to August 3rd, 2013), NO_3^-, silica, SO_4^{2-}, PO_4^{3-}
320 and Ca^{2+} concentrations markedly decreased across the dam constructed at the
321 major tributary, the Hachisu River. Discharged water from the reservoir was
322 characterized by high concentrations of Fe and Mn.

2) Streambed biofilms contain significant amount of inorganic components. Inorganic components involved in the biofilms are derived largely from siliciclastic materials (silt and clay) whose compositions are basically identical to those of fine-grained (< 63 μm) fractions of sediments.
3) However, some elements such as Si, Ca, P, Mn, Fe, Mn, Ba, Cr, Cu, V, and Zn are enriched in the biofilms to various degrees on Al-normalization basis. Enrichment of Fe and Mn in the biofilm samples collected just below the dam is attributed to oxidation and accumulation of these elements (dissolved form) in the discharged waters, and Fe- and/or Mn-oxides may have played a significant role for accumulation of some elements.

Declarations

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

KS proposed the topic, conceived and designed the study. NM carried out the sampling and experimental studies conducted in Nagoya University, in collaboration with MY and RT. MS and NH performed isotopic analyses, with NM.

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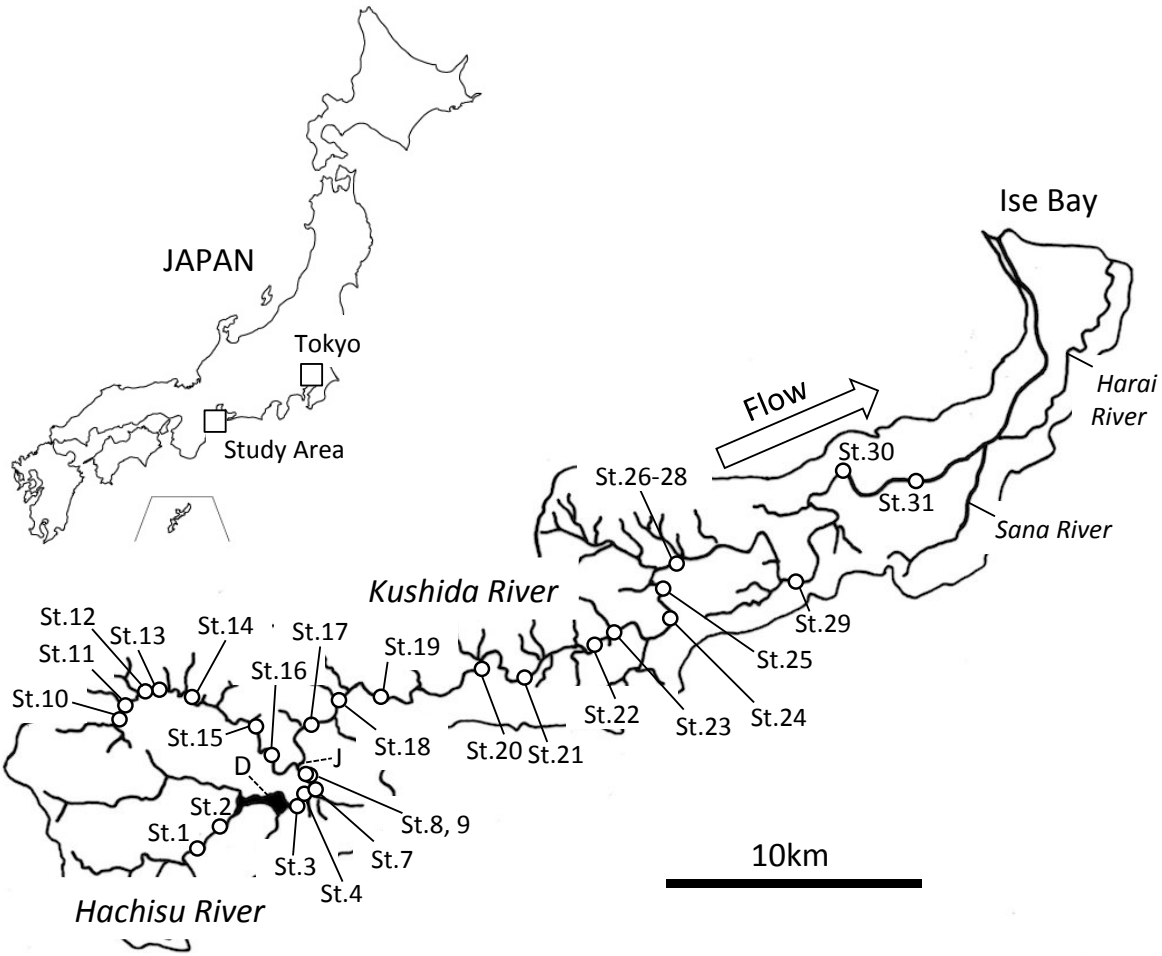

Figure 1. Sampling Localities. D and J show the Hachisu dam and the junction, respectively. Stations 5 & 6 are not shown, because these correspond to sampling sites at the small tributary of Hachisu River and are not used in this work.

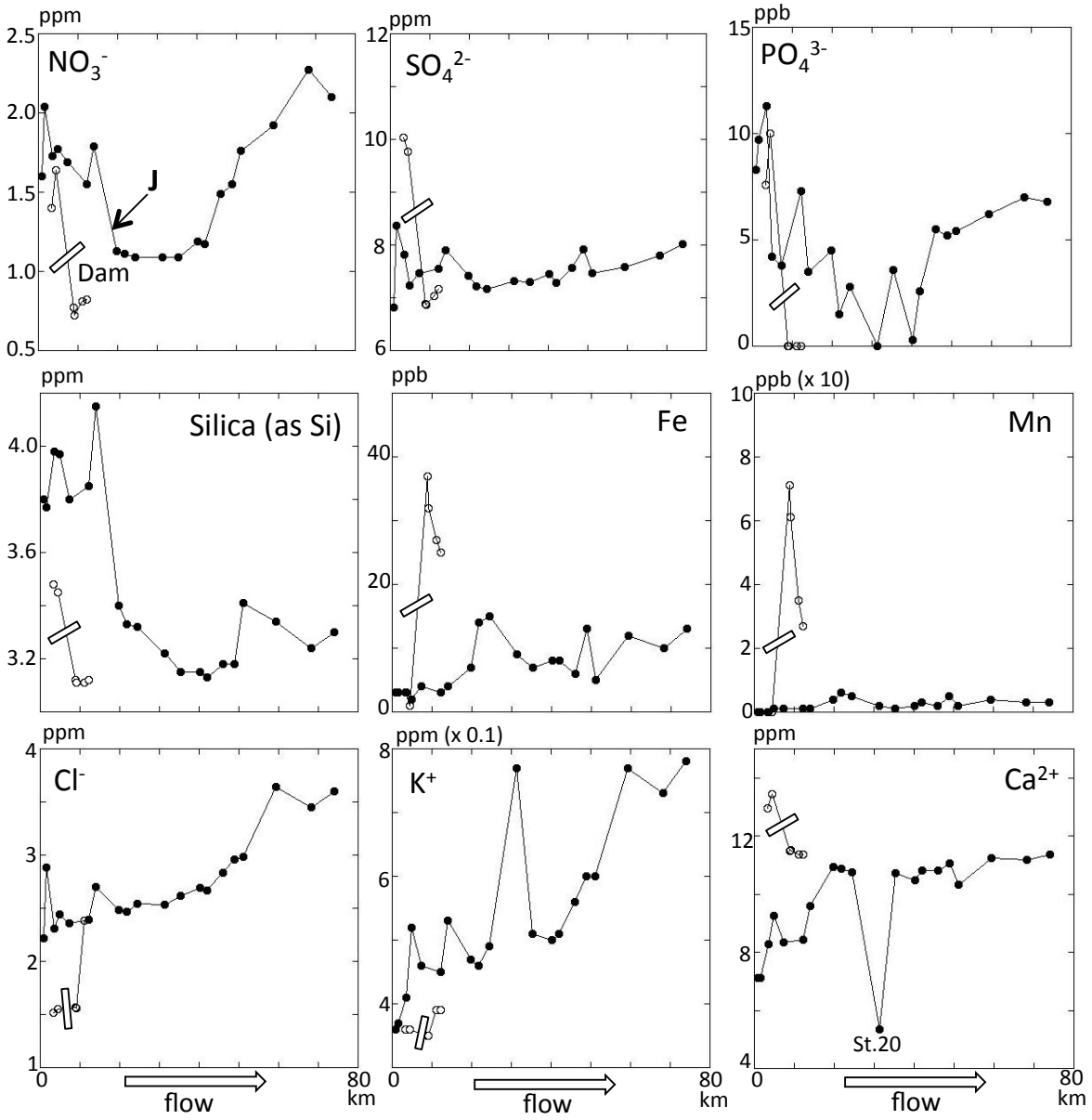

Figure 2. Downstream trends of NO_3^-, SO_4^{2-}, PO_4^{3-}, silica, Fe, Mn, Cl^-, K^+, Ca^{2+} in water samples. Closed and open circles show the Kushida River samples and the Hachisu River ones, respectively. The junction of the Hachisu River and the Kushida River is representatively shown as J in the NO_3^- diagram.

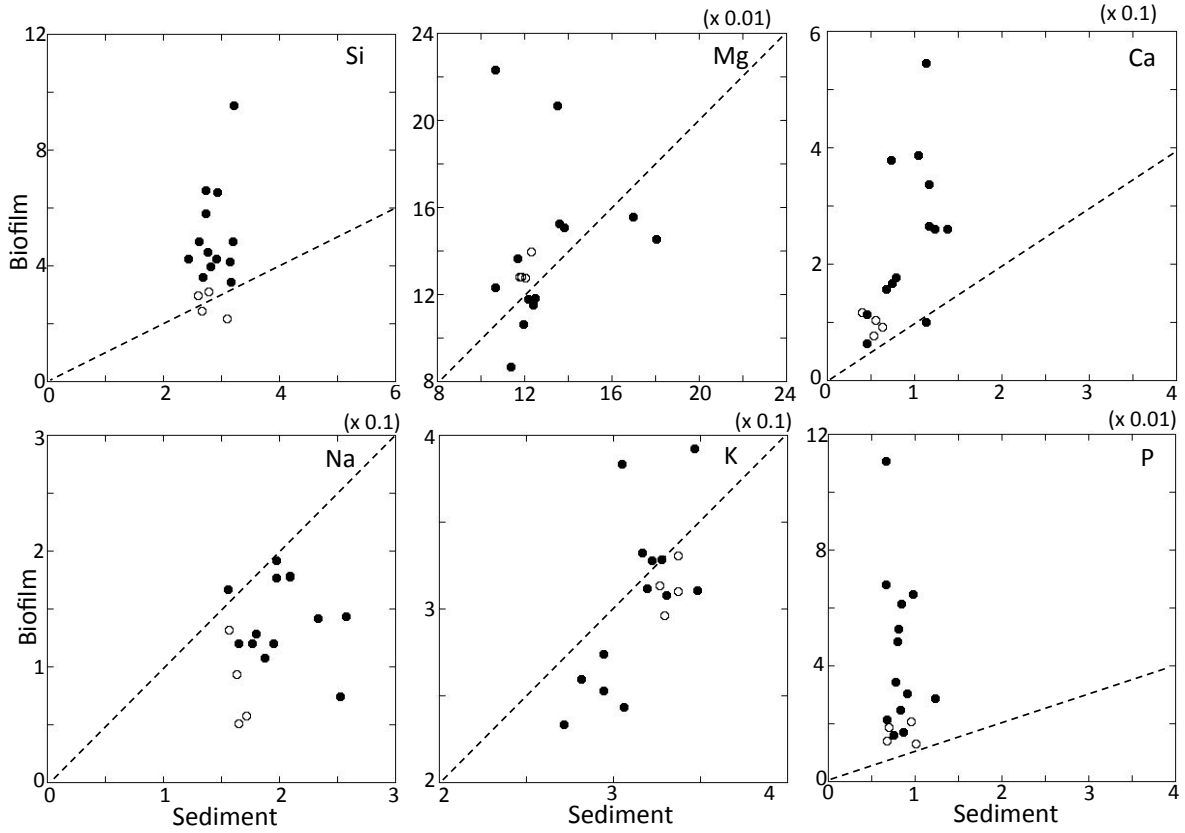

Figure 3. Relationships of Al-normalized values of elements (Si, Mg, Ca, Na, K, P) between biofilms and sediments. Plot on the dashed line indicates that at the sampling point biofilm and sediment have the same Al-normalized values. Closed and open circles show the Kushida River samples and the Hachisu River ones, respectively.

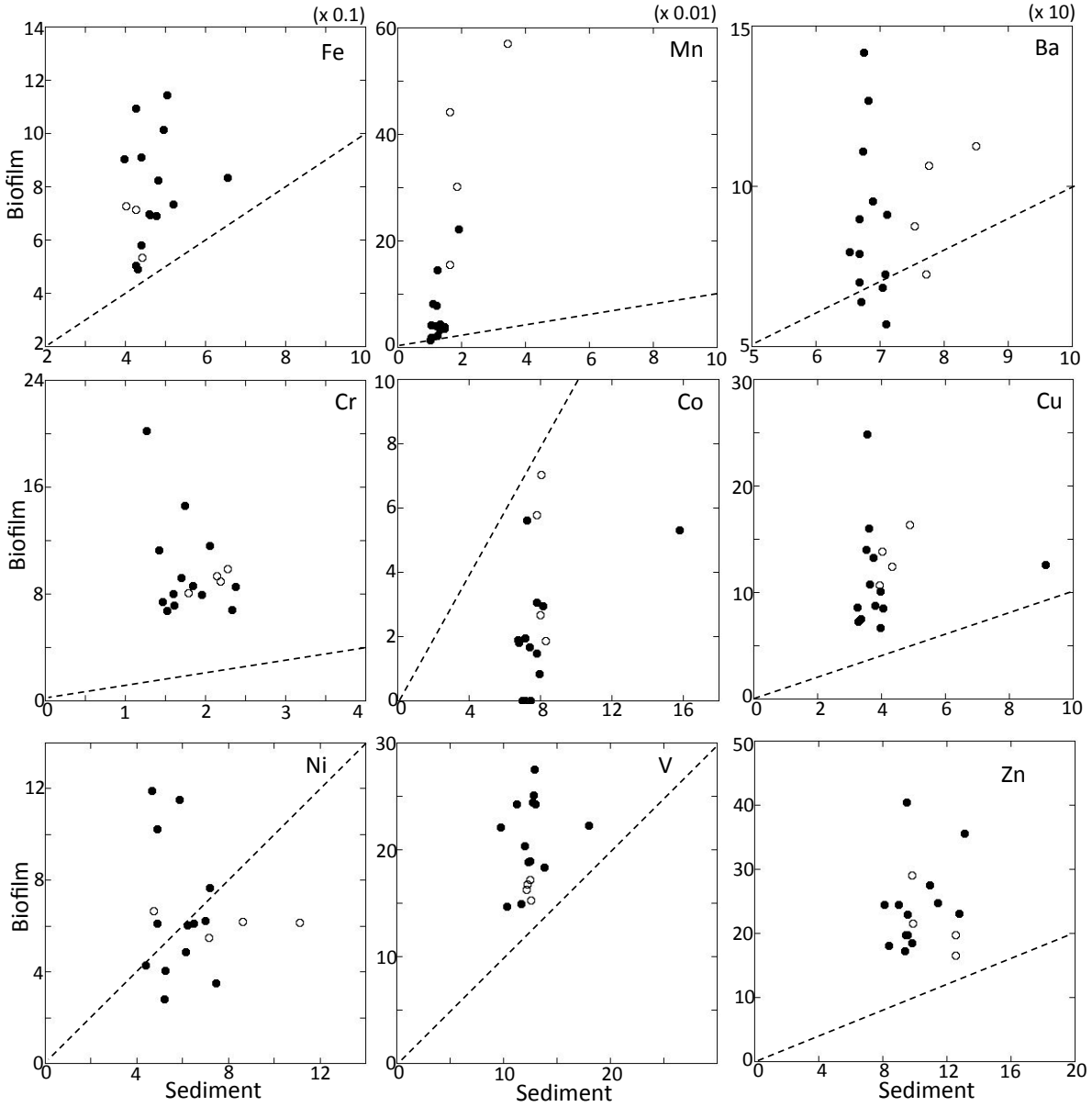

Figure 4. Relationships of Al-normalized values of elements (Fe, Mn, Ba, Cr, Co, Cu, Ni, V, Zn) between biofilms and sediments. Plot on the dashed line indicates that at the sampling point biofilm and sediment have the same Al-normalized values. Closed and open circles show the Kushida River samples and the Hachisu River ones, respectively.

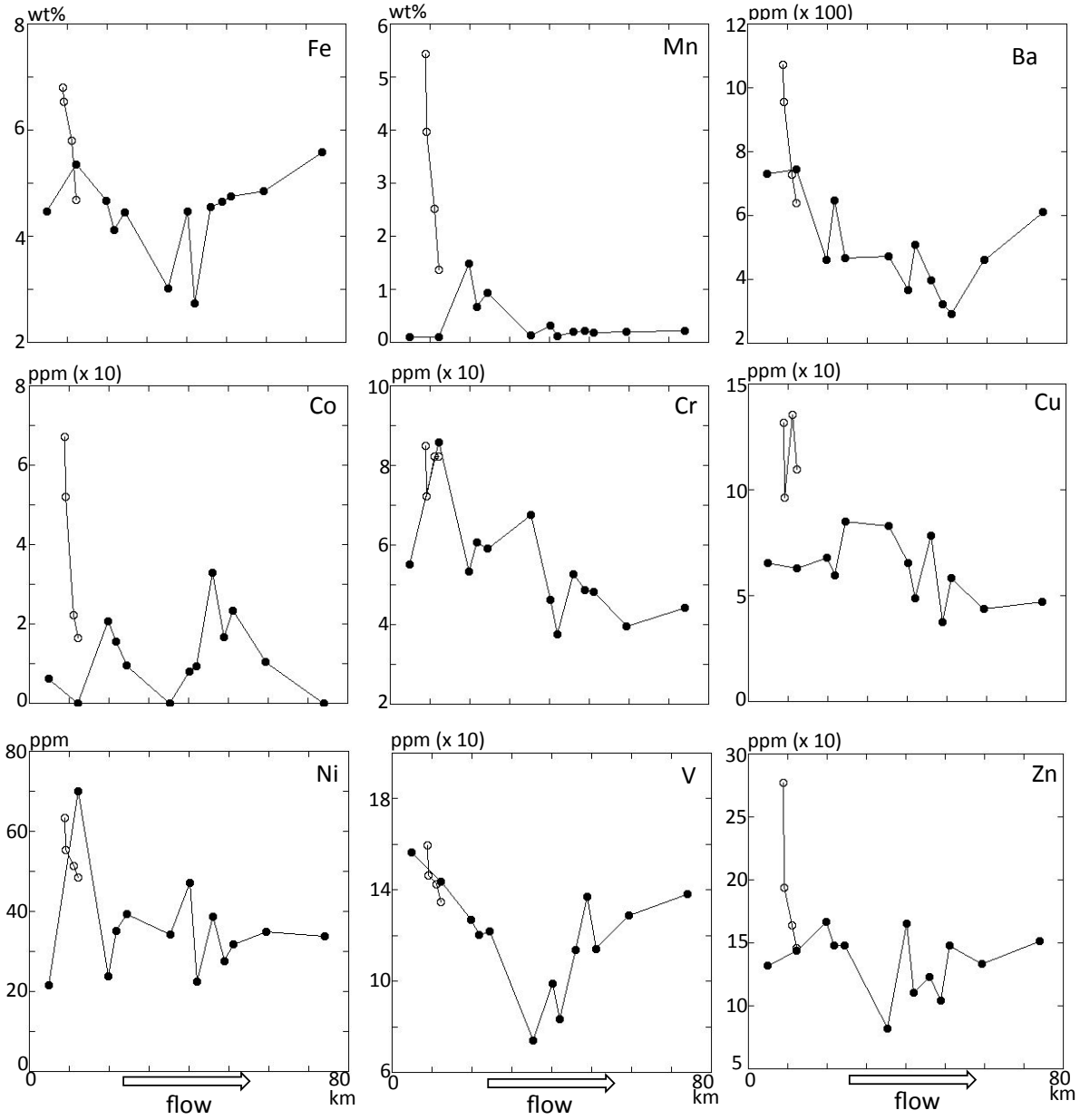

Figure 5. Downstream trends of Fe, Mn, Ba, Co, Cr, Cu, Ni, V and Zn (water- and others-free basis) in biofilm samples. Closed and open circles show the Kushida River samples and the Hachisu River ones, respectively.

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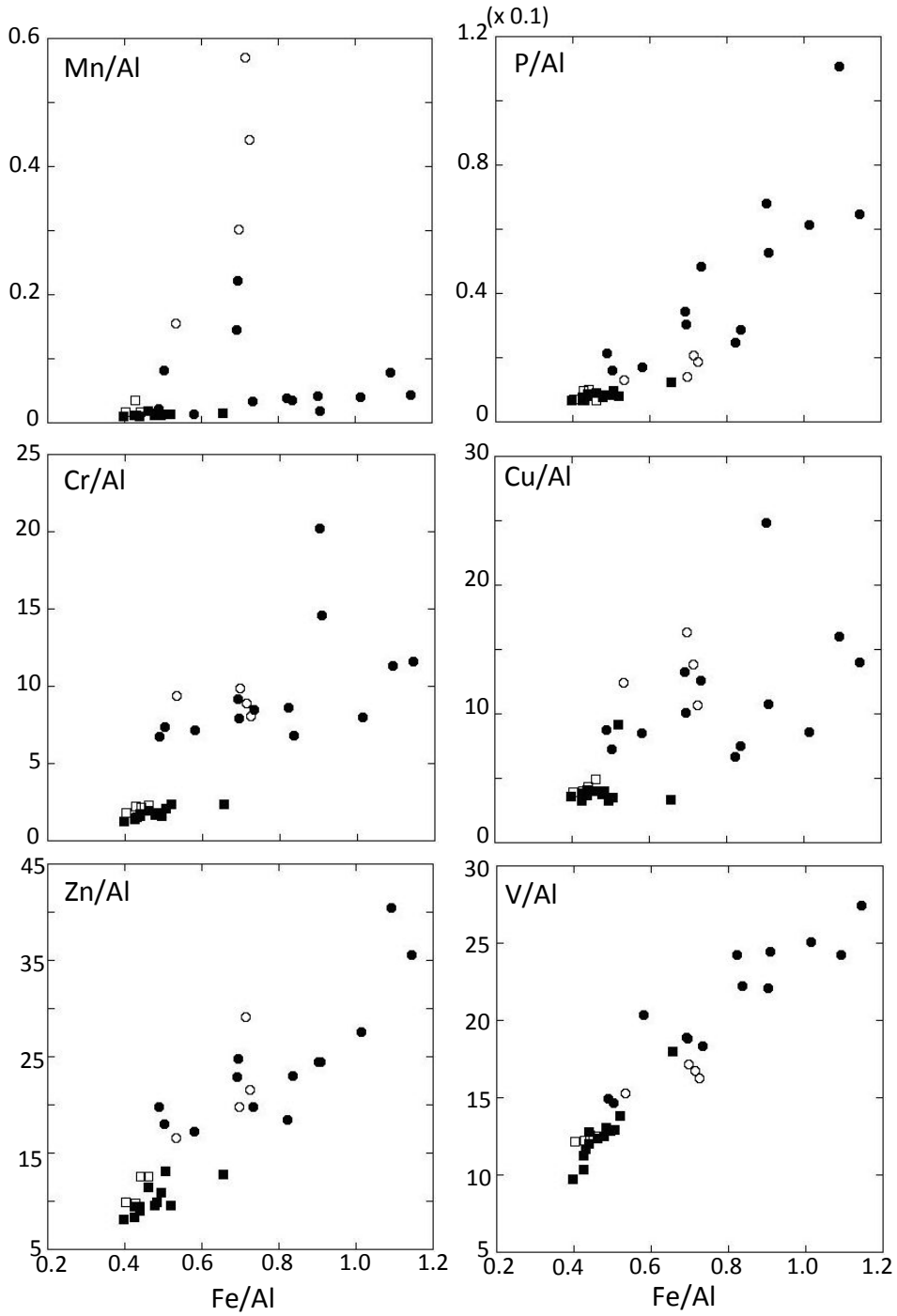

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610 Figure 6. Relationship between Fe/Al and Al-normalized values of some elements (Mn,
611 P, Cr, Cu, Zn, and V). Solid squares and open ones show the mainstream and the
612 Hachisu River sediment samples, respectively. Solid circles and open one show the

613 Kushida mainstream and the Hachisu biofilm samples, respectively.
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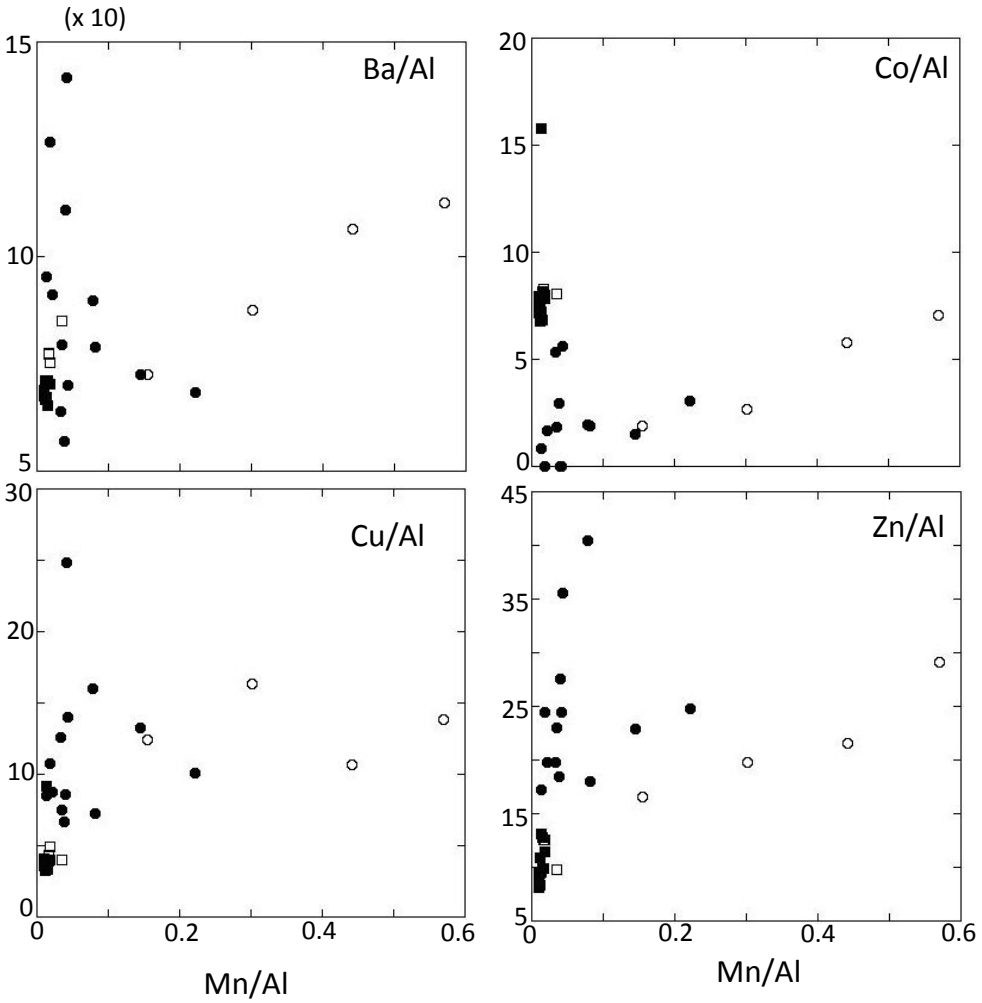

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617 Figure 7. Relationship between Mn/Al and Al-normalized values of some selected
618 elements (Ba, Co, Cu, and Zn). Solid squares and open ones show the mainstream
619 and the Hachisu River sediment samples, respectively. Solid circles and open one
620 show the Kushida mainstream and the Hachisu biofilm samples, respectively.
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Table 1 Chemical compositions of biofilms

Sample No.	3	4	8	9	13	15	17	18	19	21	22
Si (wt%)	15.66	14.43	19.41	22.43	15.63	20.07	19.81	22.97	17.98	17.13	10.74
Ti	0.455	0.409	0.417	0.45	0.48	0.343	0.358	0.414	0.33	0.176	0.214
Al	7.18	5.92	6.53	7.21	4.35	4.73	4.97	6.67	4.25	1.8	1.64
Fe	5.12	4.3	4.56	3.84	2.53	4.3	3.45	3.35	2.94	1.62	1.8
Mn	4.09	2.61	1.97	1.12	0.056	0.084	1.1	0.539	0.615	0.075	0.13
Mg	0.917	0.757	0.835	1.01	0.376	0.556	0.529	0.822	0.49	0.401	0.224
Ca	0.738	0.544	0.764	0.556	0.279	0.535	0.776	0.671	0.747	0.978	0.623
Na	0.411	0.301	0.607	0.95	0.769	0.786	0.637	0.945	0.508	0.258	0.122
K	2.25	1.95	2.02	2.13	1.45	1.45	1.55	2.07	1.39	0.705	0.631
P	0.149	0.11	0.092	0.094	0.074	0.249	0.15	0.107	0.146	0.122	0.182
Ig. Loss	24.7	34.2	21.4	18.1	43.4	19.6	26.2	18.8	34	46.2	59.8
N	1.4	1.8	2.3	0.9	0.9	3.0	1.9	1.6	2.3	2.9	4.1
C	11.4	15.9	16.8	8.3	6.1	23.5	13.8	10.0	16.8	22.5	29.3
$\delta^{15}N$ (‰)	1.7	0.9	1.2	1.3	0.5	-0.4	1.5	1.3	1.5	1.6	2.1
$\delta^{13}C$ (‰)	-25.8	-20.3	-21.0	-19.2	-17.8	-18.1	-17.4	-15.9	-15.4	-10.8	-13.3
Ba	807	629	571	523	414	598	340	526	308	254	147
Cr	63	47	64	67	31	68	39	49	39	36	18
Co	50	34	17	13	3	0	15	12	6	0	3
Cu	99	63	106	89	37	50	50	48	56	44	26
Ni	47	36	40	39	12	56	17	28	25	18	18
V	120	96	111	110	88	115	93	97	80	39	39
Y	22	16	18	20	13	10	9.7	18	15	0.5	4.7
Zn	208	127	128	119	74	115	122	120	97	44	66

	23	24	25	26	29	31	average	stdev.	min.	max.
Si (wt%)	21.53	14.16	15.1	13.25	17.49	10.69	16.97	3.75	10.69	22.97
Ti	0.274	0.325	0.402	0.237	0.381	0.327	0.35	0.09	0.18	0.48
Al	3.71	3.42	3.11	2.01	3.62	2.4	4.32	1.87	1.64	7.21
Fe	1.81	2.51	2.56	2.29	3.02	2.43	3.08	1.04	1.62	5.12
Mn	0.08	0.115	0.118	0.086	0.126	0.096	0.76	1.14	0.06	4.09
Mg	0.438	0.497	0.643	0.302	0.564	0.366	0.57	0.23	0.22	1.01
Ca	0.616	0.89	1.2	0.676	0.939	0.634	0.71	0.21	0.28	1.20
Na	0.713	0.611	0.553	0.216	0.434	0.288	0.53	0.25	0.12	0.95
K	1.22	0.863	0.758	0.521	0.845	0.656	1.32	0.6	0.52	2.25
P	0.08	0.165	0.077	0.13	0.104	0.147	0.13	0.04	0.07	0.25
Ig. Loss	33.5	44.8	44.9	51.7	37.6	56.5	36.2	13.4	18.1	59.8
N	2.0	3.9	2.3	3.8	2.1	4.1	2.4	1.1	0.9	4.1
C	15.8	22.9	20.6	25.9	17.9	27.2	17.9	6.7	6.1	29.3
$\delta^{15}N$ (‰)	2.1	3.4	2.7	3.6	3.8	5.3	2.0	1.38	-0.4	5.3
$\delta^{13}C$ (‰)	-11.8	-15.4	-12.1	-13.7	-13.4	-13.6	-16.1	3.9	-25.8	-10.8
Ba	338	219	177	140	287	265	384.9	193.0	140	807
Cr	24	29	26	23	24	19	39.2	17.5	18	169
Co	6	18	9	11	6	0	11.9	13.0	0	50
Cu	32	43	20	28	27	20	49.3	26.5	20	106
Ni	14	21	15	15	21	14	25.6	13.2	12	84
V	55	62	75	55	80	60	80.9	26.0	39	120
Y	11	9.9	11	2.2	7.8	5.5	11.4	6.2	0.5	22
Zn	73	67	57	71	83	65	96.2	39.8	44	208

627 Note: Sample No. corresponds to the number of sampling locality.

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Table 2 Comparison of major elements between sediments and biofilms (water and other free-basis)

	Riverbed Sediments (< 63 μm)			Biofilm			JUC
	average	min.	max.	average	min.	max.	
SiO_2	62.25±1.83	58.30	65.28	66.50±6.59	50.31	79.34	67.53
TiO_2	0.91±0.11	0.65	1.17	0.72±0.15	0.34	0.91	0.62
Al_2O_3	19.67±1.14	17.82	21.67	14.88±3.49	7.35	20.37	14.67
Fe_2O_3	6.86±0.85	5.39	9.75	8.21±1.72	4.42	11.30	5.39
MnO	0.196±0.069	0.129	0.434	1.32±2.08	0.145	7.93	0.11
MgO	2.17±0.29	1.68	2.93	1.70±0.43	0.96	2.28	2.53
CaO	1.09±0.43	0.59	2.00	1.78±0.81	0.69	3.58	3.9
Na_2O	2.65±0.31	2.23	3.44	1.38±0.46	0.52	2.08	2.72
K_2O	3.99±0.37	3.33	4.55	2.93±0.76	1.62	4.10	2.42
P_2O_5	0.194±0.034	0.145	0.294	0.584±0.311	0.283	1.32	0.12

640 Note: JUC is Japanese Upper Continental Crustal values (Togashi et al., 2000)
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