Si and Fe biomineralization by microorganisms in bottom sediments of Delta Selenga River, tributary of Lake Baikal, Russia

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Abstract : Bottom sediment samples, collected from Delta Selenga River, tributary of Lake Baikal, Russia, were analyzed with methods of elemental chemical analysis and optical microscopy. Chemical composition of sediments was studied with energy dispersive X-ray fluorescence spectrometer, which revealed presence of Si, Fe, Al, Ca and K as major elements, associated with traces of Mn, Mg, Ti, and Sr in the bottom sediment samples. Diatoms and bacteria were observed with light and epifluorescence optical microscopy, showing formation process of organic substances with mineralogical aggregates. Si and Fe biomineralization on the surface of diatoms has been observed with light microscopy. Fe-oxidizing bacteria with ability to metal accumulation were isolated on selective medium. The results suggest that Si and Fe biomineralization of diatoms with Fe-oxidizing bacteria indicate deoxidized ecological environment to form pyrite in/on the cell crust.

Key words : bottom sediments, ED-XRF analysis, diatoms, iron-oxidizing bacteria, metal accumulation, Si-Fe biomineralization.

1. Introduction

Recently, biomineralization in natural environments is one of the main interesting and key point in the study of the interactions between microorganisms and minerals. The process could be visualized not only in the deep core samples, but also in modern habitant, such as hot and mineral springs (Fouke et al., 2000), marine and freshwater sediments (Aguilar and Nealson, 1998). Diatoms, bacteria and fungi usually involved in these processes. Microbial communities are thought to play a significant role in both the oxidation and reduction of iron and manganese in freshwater sediments (Stein et al., 2001). Diatoms and algae create highly complex and intricate silica structures under physiological conditions, during Si-cycles in marine and freshwater sediments (Cox, 1996 ; Asada and Tazaki, 2001). Microbial reduced silicon is removed from the sediments to the water column.

Delta Selenga River has been actively sedimented to form extensive delta (Votintsev et al., 1963). Chemistry and microbiology of bottom sediments of Selenga River have been reported (Goldurev et al., 1971 ; Mladova, 1971). Their results show Si, Fe, Mn, and Al were major elements in the river sediments (Votintsev et al., 1963 ; Goldurev et al., 1971). River sediments counting of total number of bacteria revealed a large number of microorganisms to compare with sediments of Lake Baikal (Mladova, 1971). Cultivation of heterothrophic bacteria was carried, and microorganisms belong to *Pseudomonas, Arthrobacter, Flexibacter, Bacillus, Micrococcus, Desulfotomaculum, Cytophaga*, and *Clostridium* genera were identified in Selenga River sediments (Namsarayev et al., 1994). Diatoms, collected from Selenga River and Lake Baikal, were identified, as follows, *Aulacoseira* spp., *Synedra* spp., *Stephanodiscus* spp., *Cyclotella* spp., *Melosira* spp., and *Novicula* spp. (Likhoshway et al., 1996).

In this study the bottom sediment of Delta Selenga River were microbiologically and chemically discribed. The role of microorganisms in silicon cycles is discussed.

2. Materials and Methods

Bottom sediment samples were collected from 13 points of 10 cross-sections, as in the aquatory of open Baikal (cross-section 1 p.106, cross-section 10 p.102), as in the delta of Selenga River (points of cross-sections 2-9) (Fig. 1). Upper layer of sediments were freezed immediately after collecting samples and stored at temperature -20℃ before analysis. Energy dispersive X-ray fluorescent analysis was carried on JEOL Element Analyzer JSX-3201 with Rh Kα, at 30 kV in vacuum condition. Sediment samples were airdried and grounded to fine powder. Optical micrographs were taken with Nikon EFD 3 microscope. Samples were also stained with 4', 6-diamidino-2-phenilindole solution (DAPI, 50 μg/ml) during 3 min and visualized with UV-1 A filter set for epifluorescent microscopy. Fe-oxidizing bacteria were cultivated on selective medium with agar at 25℃ during 2 - 8 weeks. Medium contained, as follows, $(NH_4)_2SO_4$ (0.5 g/l), $NaNO_3$ (0.5 g/l), K_2HPO_4 (0.5 g/l), $MgSO_4$ (0.5 g/l), $FeSO_4$ (5.9 g/l), triptone (1 g/l), agar (15-20 g/l), pH 6.8.

3. Results

Chemical analysis of bottom sediments

Chemical composition of the bottom sediments of Delta Selenga River is shown in Table 1. The results revealed presence of Si, Fe, Al, Ca and K as major elements, associated with traces of Mn, Mg, Ti, and Sr. In Selenga River sediments Si was found in higher concentration than in lake sediments. Si concentration ranges from 53.61 to 66.07 wt % in river sediments, whereas from 39.05 - 46.44 wt % of Si are in lake sediments. Fe concentration ranges from 3.85 to 12.90 wt % in river sediments, whereas 25.87 - 29.25 wt % of Fe are in lake sediments. Sr concentration varies in from 0.17 to 0.33 wt % without any dependence to sampling points. Rb was detected only in few river samples. Cr was detected

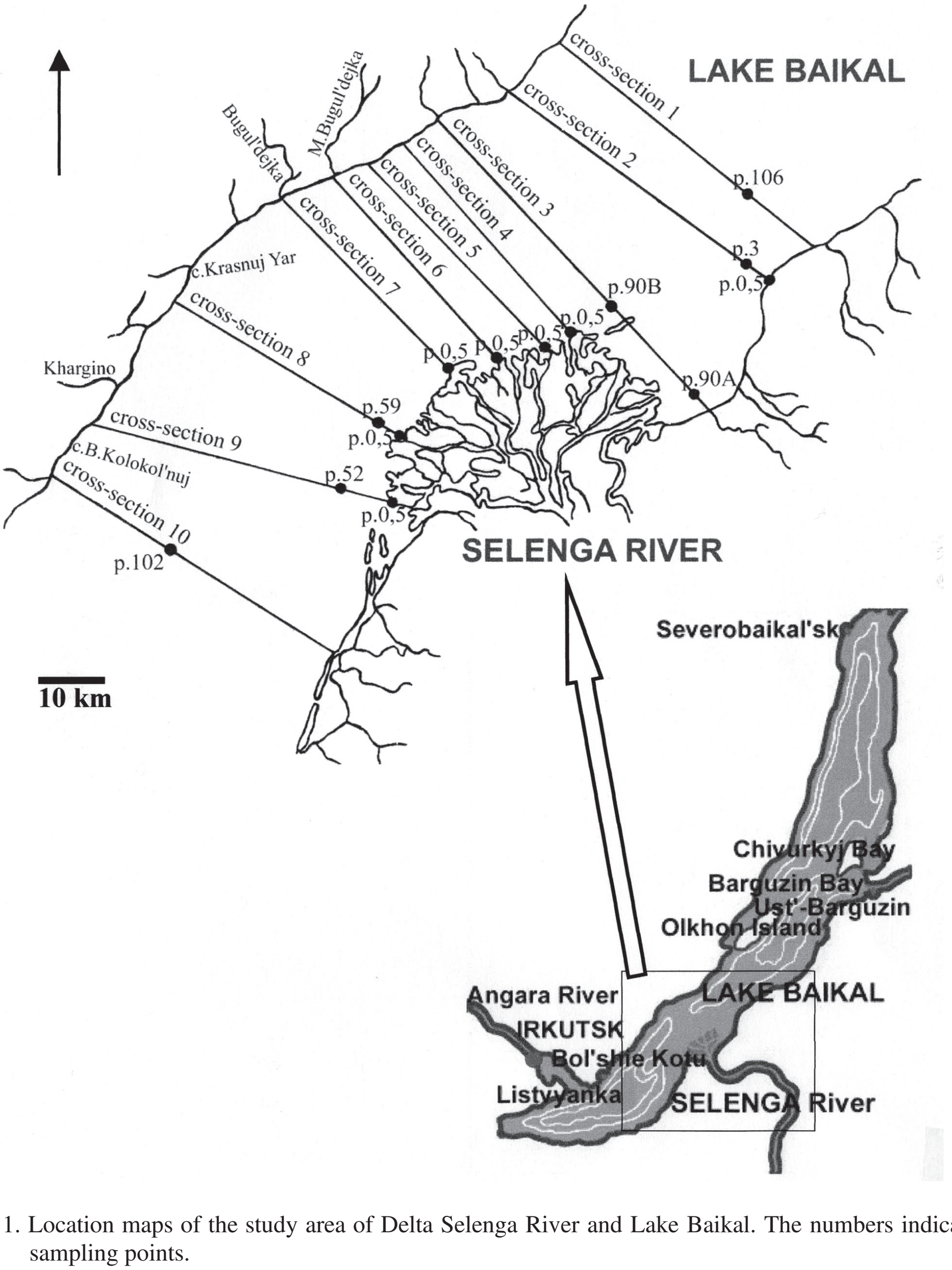

Fig. 1. Location maps of the study area of Delta Selenga River and Lake Baikal. The numbers indicate sampling points.

in one sample and the concentration was 0.10 wt % in the region of open Baikal−c-s 10 p.102.

Table 1. Energy dispersive X-ray fluorescent analysis of bottom sediment samples from Delta Selenga River and Lake Baikal, Russia.

wt %

Sample	Na	Mg	Al	Si	P	K	Ca	Ti	Cr	Mn	Fe	Rb	Sr	Zr
c-s1 p.106	Nd	1.96	8.39	46.44	1.33	4.42	8.78	1.96	Nd	0.61	25.87	Nd	0.17	0.07
c-s2 p.3	1.43	0.32	9.28	66.07	0.58	9.97	6.40	0.76	Nd	0.14	4.73	Nd	0.27	0.05
c-s2 p.0.5	1.72	0.52	9.33	57.43	0.67	8.73	8.11	1.96	Nd	0.35	10.59	Nd	0.33	0.26
c-s3 p.90A	Nd	1.36	10.86	53.61	0.94	7.52	6.75	1.47	Nd	0.58	16.58	0.06	0.20	0.07
c-s3 p.90B	Nd	Nd	9.26	61.60	1.01	9.03	7.56	1.62	Nd	0.33	9.29	Nd	0.22	0.08
c-s4 p.0.5	1.97	Nd	10.93	63.04	0.92	12.56	5.85	0.55	Nd	Nd	3.85	0.06	0.24	0.03
c-s5 p.0.5	1.70	Nd	10.59	62.17	0.98	11.12	6.58	0.84	Nd	0.17	5.54	Nd	0.27	0.04
c-s6 p.0.5	Nd	0.77	10.05	56.72	1.11	8.80	8.45	2.11	Nd	0.40	11.11	Nd	0.24	0.24
c-s7 p.0.5	1.63	0.42	10.44	59.29	1.08	10.76	8.14	1.11	Nd	0.22	6.50	0.06	0.27	0.08
c-s8 p.0.5	Nd	0.97	10.42	57.57	1.12	8.95	7.13	1.53	Nd	0.35	11.63	Nd	0.24	0.09
c-s8 p.59	Nd	1.13	10.03	54.52	1.16	8.54	8.53	2.14	Nd	0.48	12.90	Nd	0.28	0.29
c-s9 p.52	1.62	Nd	9.86	62.22	1.23	11.60	6.72	0.92	Nd	0.16	5.35	Nd	0.27	0.05
c-s10 p.102	Nd	3.44	9.42	39.05	0.61	4.37	10.52	2.36	0.10	0.57	29.25	Nd	0.23	0.08

Nd – not detected

Optical microscopy of bottom sediment samples

With optical microscopy of bottom sediments large amount of diatoms with variety of species were revealed. Fig. 2 A shows common view of the bottom sediment samples. More than 4 or 5 different species of diatoms were visualized in each sampling points. The diatoms belong to *Aulacosira, Cyclotella, Cymbella*, and *Novicula* genera were found commonly in sediments (Fig. 2 A, B, C). Single diatom cells with clearly distinguished internal structure were visualized in bottom samples, showing attached bacterial cells (Fig. 2 D, E). The diatom cell was covered on whole surface by mineral particles (Fig. 2 F). Spherical aggregates formed with mineral particles, diatoms and bacterial cells (Fig. 2 G).

Chemical composition of bottom sediments

High concentration of silicon was found in the samples c-s 9, p.52 - c-s 2, p.3. In the regions of open Baikal c-s 1, p.106 ; c-s 10, p.102, Si content is quite low (Fig.3 A).

Cultivation and isolation of Fe-oxidizing bacteria

Fe-oxidizing bacteria were isolated on selective medium. Mixture of microorganisms was initially cultivated at 25℃ during 3-8 weeks (Fig. 4 A). Colonies with different color and growth ability were observed. Microorganisms were purified and grown on the same medium after 3 or 4 passages (Fig. 4 B, C). The size of single colony was varied from 1 to 10 mm in diameter. Colonies of microorganisms have metallic color, white and transparent (Fig. 4 B, C). The colonies have various shapes. Rounded shape in metallic color (B) and star-typed shape colony (C) grow through agar.

4. Discussion

According to the previously investigations, the total amount of diatoms in the upper layer of Selenga River water is higher than in Lake Baikal water (Likhoshway et al., 1996). The sedimentation progress actively in river sediments than in lake sediments (Votintsev et al., 1963). Two processes of Si biomineralization were observed in the sediments by optical microscopy, as follows ; one is concerning to accumulation of silicon by diatoms, and other one is by microorganisms on the cell walls (Fig. 2 F, G). Digestion of diatom cells by microorganisms are shown in Fig. 2D, E. Because of extreme conditions in Lake Baikal

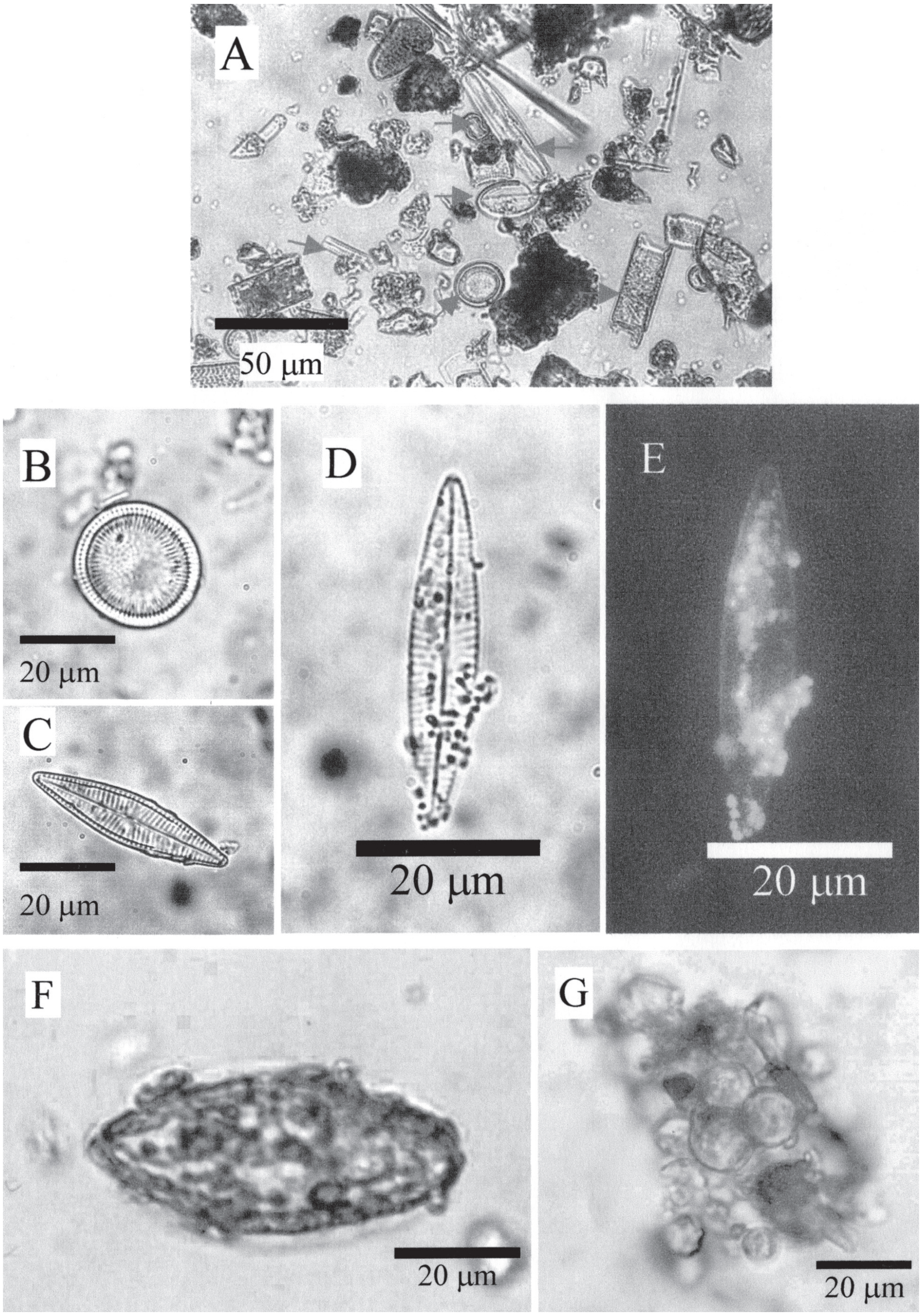

Fig. 2. Optical micrographs of diatoms in the bottom sediments collected from Selenga River. Common view of the sample : diatoms are marked with arrows (A). Diatoms commonly visualized in river sediment samples : *Cyclotella* sp. (B) and *Navicula radiosa* (C). Light (D) and epifluorescence (E) micrographs of *Cymbella* sp. with attached bacteria cells. Diatoms cell covered with attached bacteria and mineral particles (F). Aggregates formed with diatoms, microorganisms and mineral particles (G).

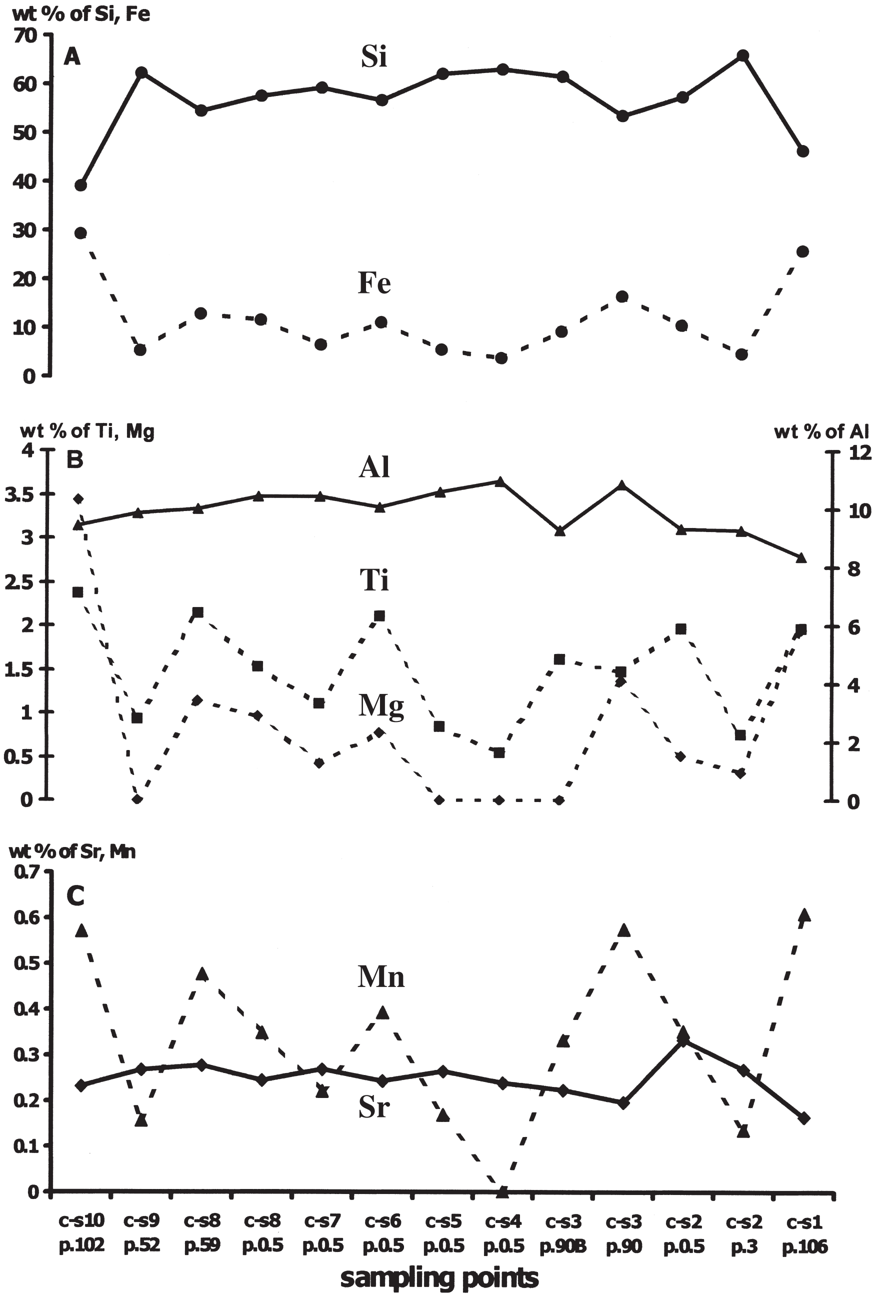

Fig. 3. Chemical composition of bottom sediments of Delta Selenga River and Lake Baikal. Si and Fe concentrations (A) ; Al, Ti and Mg concentrations (B) ; Mn and Sr concentrations (C) showing distribution tendency between delta and lake sediments.

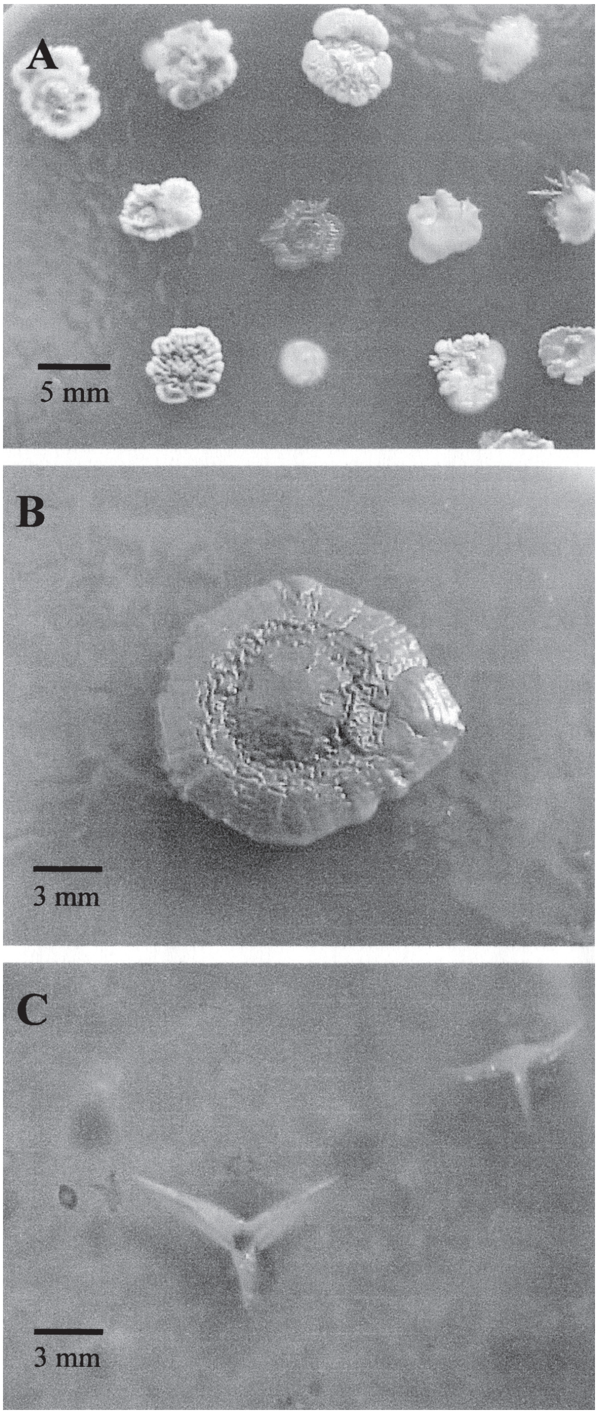

Fig. 4. Optical photographs of colonies of Fe-oxidizing microorganisms, isolated from bottom sediments of Delta Selenga River. A, mixture of microorganisms cultivated on selective medium ; B, C, colonies of isolated strains with different capacity to growth.

water and sediments, such as low mineral content and low concentration of nutrients, microorganisms in natural environments have grown with high enzymatic activities (Parfenova, 1985). Microorganisms, such as Si-utilized bacteria, utilized silica from diatoms cell walls during precipitation from the upper layer of water to bottom sediments. Fig. 2 (D, E) shows this process on the cell walls of *Cymbella* sp., found in the sediments from Lake Baikal. On epifluorescence micrographs (Fig. 2 E), large amount of bacterial cells were found. The process of accumulation and precipitation of Si is more dependent on diatoms (Fig. 2 H, G). According to chemical analysis of sediments (Fig. 3 A, B, C), weight percent concentrations of Si tend to curve similar to Al and Sr. Diatoms might be responsible for Al and Sr accumulation in river sediments.

The attempt to isolate pure strains of microorganisms associated with metal accumulation was carried out. Diversity of Fe-oxidizing bacteria, isolated from sediments, shows variety of existing strains : ones have metal color of the colony (Fig. 4 B), while others have grown and change the medium color to neutral (Fig. 4 C). These strains have different mechanisms of metal accumulation deals with either the capacity to adsorption, accumulation or transportation of metal on/in the cells (Ledin, 2000). The tendency weight percent concentration was found on Mn, Mg, and Ti similar to curve of Fe (Fig. 3 A, B, C). These metals could be involved in the processes of biomineralization corresponding with microorganisms.

5. Conclusion

According to chemical analysis of bottom sediments, optical microscopical observation and isolation of Fe-oxidizing bacteria, we can suggest that Si, Al and Sr biomineralization process is corresponding with diatoms which metal accumulation of Fe, Mn, Mg and Ti is deals with bacteria. In further perspectives, pure strains of microorganisms with metal accumulation abilities would be cultivated, and mechanisms of biomineralization process would be studied in laboratory experiments.

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