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Fish Exposure and In Situ Field Pilot Tests in the Abandoned Mine Drainage for a Stream Restoration

Bae, Dae-Yeul, Ju-Yong Kim[1], Kyoung-Woong Kim[1] and Kwang-Guk An*

(School of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Korea
[1]Environment Science and Engineering, Gwangju Institute of Science & Technology,
Gwangju 500-712, Korea)

The objectives of this study were to analyze ecological effects on effluents from the Sagok Stream (Chonnam province) as an abandoned mine drainage through necropsy-based health assessments and fish exposure tests, and to conduct In situ field pilot tests for restoration of stream water. Also, we analyzed water quality including general parameters and heavy metals. The tests were performed three times on April 2005, April 2006, and April 2007. Also, we constructed a reactor facility in the outflowing point of the abandoned mine for the remediation of AMD wastewater. In lab test, death rates in all three treatments were ≥50% in the experiments. Necropsy-based fish tissue assessments using the Health Assessment Index (HAI), indicated that the most frequently damaged tissue was liver (average: 20.8). Values of Health Assessment Index were lower in the control than any other treatments of T1, T2, and T3 and three treatments showed a distinct toxicity impacts by the AMD. In situ lethal test, concentration of Fe, Al and Zn decreased particularly by 85%, 99% and 94%, respectively through the disposal facility. Values of pH, ranged from 3.1 to 7.0, increased by 2.3 fold (mean=5.1) along with the reduction of metal contents. All fishes in P1 cage died 100% on 3 days later after the experimental setting, while all fishes in the P6 died 100% on 9 days later. Overall, these results evidently provide a key methodology for pilot test using the disposal facility and also clarify the toxicity of AMD once again, so this approach used in the pilot facilities here may reduce the acidic and toxic effects in the abandoned mining drainage.

Key words : restoration, HAI, mine drainage, Health Assessment Index, toxic test

INTRODUCTION

Recent studies pointed out that abandoned mines cause critical water quality problems by various metals and acidic waters in the upstream watersheds and this source frequently resulted in contaminations of water and soils naturally or by accidental spills from abandoned mines (Gray, 1998), producing in ecological and human health problems. Even after the mining activity has ceased, effluents from the acid mine drainage (AMD) caused high dissolution of heavy metals from sulphide ores in contact with oxygen (Kelly, 1991), resulting in greater toxicity in the water and on the aquatic biota. These AMD activities can consequently contaminate farmland in the downstream watersheds and drinking water resources around the abandoned mines, and do adversely affect crop growth and human health (Kim *et al.*, 2002). Nevertheless, some of soils and waters near mines have been supplied to the living of people and the agricultural activity without any risk assessment of environment and hu-

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* Corresponding author: Tel: +82-42-821-6408, Fax: +82-42-822-9690, E-mail: kgan@cnu.ac.kr

man health.

Currently, developed countries in the environments provided guidelines for the protection of regional watersheds where abandoned mines are located. In case, the new Canadian Metal Mining Effluent Regulations demand biological monitoring using several metrics of aquatic invertebrates, and then whole set of biomonitoring measurements using fish bioindicators as a net step, for the fish fauna. The main purpose of such regulations is accomplish to ultimate goals of ecosystem conservations and public health (MAC, 2002). The use of endemic species for the evaluation of contamination effects of the stream water on the local fauna is crucial for the application in water quality monitoring due to the ecological relevance.

In Korean watersheds, there are about 2500 mines, including 900 metal mines, 380 coal mines, and 1200 nonmetallic mines (KME, 2005). More than 80% of these mines are now abandoned and they have been a long-term sources of environmental pollutants such heavy metals and toxic chemicals. Aquatic ecosystem impacted by abandoned mine has been mainly evaluated only with heavy metals including other chemicals and a little simplified biological data. limnology was hate you

The best indicators for wastewater of acid mine drainage having been reported up to the present are (1) presence of algae indicators, (2) high densities of chironomids, (3) presence of iron hydroxide and (4) pH recordings (Kelly, 1991). In fact, numerous studies (Judy *et al.*, 1984 Karr and Chu, 2000) have pointed out that simple chemical monitoring may not detect an integrative health condition of water environments due to degradations of physical habitat and flow modifications. New approach using biological or ecological integrity, based on aquatic biota assemblage rather than chemical approaches, has been applied to aquatic health assessment studies in North America and Europe (Huguey *et al.*, 1996). For this reason, new biological approaches for assessing fish health such as necropsy-based health assessment index, biochemical assay, and pathological analysis are widely applied for the analysis of ecosystem health (Weber *et al.*, 2003; Jhingan *et al.*, 2003; Tierney and Farrell, 2004).

The objectives of this study were 1) to evaluate water quality in Sagok Stream, influenced by the abandoned mine drainage (AMD), 2) to determine the risk of the stream ecosystem, based on lethal tests and Health Assessment Index (HAI) tests using a fish indicator species, *Phynchocypris oxycephalus*, and 3) to evaluate efficiency of water treatments in the inlet and outlet compartments using the in situ pilot facilities.

MATERIALS AND METHODS

1. Study area and stream conditions

Abandoned mine stream is located (N 34° 56′ 12″, E 127° 37′44″) in Sagok-ri, Gwangyang-eup, Gangyang-si, Chonnam province, which is located in southern part of South Korea (Fig. 1). The mine is known as Jeom-dong gold and silver mine. Mineral deposits of this study sites are classified as hydrothermal Au-Ag bearing quartz veins and the main geology is composed of precambrian gneiss and cretaceous diorite (Jung *et al.*, 2003). It was an active mine during 1936-1989, and total gold and silver produced were 208 kg and 1,606 kg, respectively. The mining activity ceased in 1989, and after that, toxic mining water, approximately 100 tons day^{-1}, has been

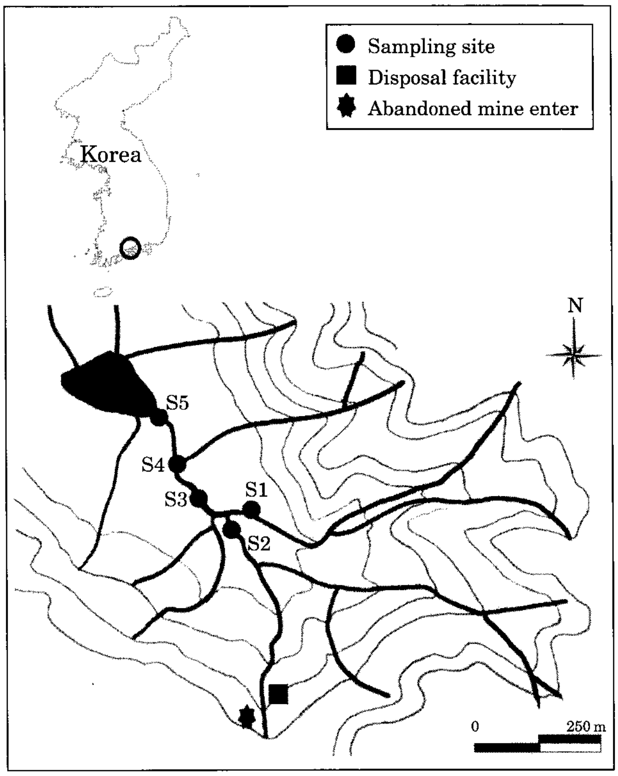

Fig. 1. The map showing the study sites in the Sagok Stream and Jeomdong abandoned mine.

Table 1. Comparisons of water temperature, dissolved oxygen (DO), turbidity, conductivity, salinity and pH in the water sampling site.

Parameters	April 2005			April 2006			April 2007		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
Temperature (°C)	14.1	14.3	15.8	13.7	12.8	15.1	16.1	15.0	16.4
DO (mg L^{-1})	7.2	7.9	7.9	8.5	8.8	8.4	10.9	11.1	10.1
Turbidity (NTU)	3.5	2.6	6.0	5.0	3.6	8.7	2.2	1.5	7.4
Conductivity (μs cm^{-1})	110	276	144	133	264	157	166	259	178
Salinity (ppt)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
pH	6.6	4.3	5.5	6.4	5.3	6.3	6.3	5.9	6.1

flow out from the acid mine drainage (AMD) without any wastewater treatment facility.

In the stream, sulfide mineral is a main composition of the sediments and are made of high chalcopyrite (Fe, S, Cu), pyrite (Fe, S), galena (Pb, S) and sphalerite (Zn, S). The contaminated sediments and high contents of metals in the AMD flow into Sagok Stream and then stored agricultural reservoirs. One of the problems in the AMD water was very acidic and toxic. And, the unmanaged AMD have been flowed into the stream and frequently resulted in massive fish kills in the downstream and down-reservoir in the watershed (residental information in the village). Especially, during periods of heavy monsoon rainfall, massive yellow sediments mainly consisted in goethite were flashed out from the AMD, expecting an environmental impact on the aquatic ecosystem (Jung *et al.*, 2003).

2. Experimental lab designs for necropsy-based health tests

In this experiments, the necropsy-based approach using the Health Assessment Index (Adams *et al.*, 1993; Blazer, 2000), was employed for the assessments of aquatic ecosystem health influenced by the AMD. The sampling collections were conducted during April 2005-May 2007 in three sampling sites. We choose 3 sampling sites for collecting the water to examine. S1 is the control site, that have no source of water pollution, indicating the uncontaminated tributary. S2 is a headwater site directly influenced by mining wastewater and colored yellow with sediments. S3 is the site merged with Site 1 (the control) and Site 2 (Fig. 1). We designed four cages in the laboratory and set up one control and three treatments of surface water from S1 (control, C), surface water from S3 (Treatment 1, T1), surface water from S2 (Treatment 2, T2), and surface water plus bottom sediments (i.e., yellow fine matter) from S2 (Treatment 3, T3; Table 1). We collected water samples using 10 L cubitainers from the control and treatments, and then stored in the cooler while the transporting in the laboratory. We poured the water tanks of the control (C), three treatments (T1, T2, and T3) and then put 20 individual numbers of fish, respectively, in the control and three treatments.

Fish species tested was *Phynchocypris oxycephalus*, which is known as a sensitive species and primary dominant species in the non-polluted control sites of the AMD. Fish size tested ranged between 10 cm and 15 cm in the total length, which was considered as adult stage. We acclimated the fish in the laboratory during 7 days before the start of the experiments. Each cages contained 10 fishes and were monitored physicochemical parameters such as temperature (°C), pH, dissolved oxygen (mg L^{-1}), and conductivity (at 25°C) along with observations of fish death. We also choose 3 individuals (F1, F2 and F3) randomly per each cage and calculated HAI to analyze the effect of AMD. For the impact analysis from the AMD, we developed 6 metric necropsy-based model including M_1 fins, M_2 spleen, M_3 kidney, M_4 liver, M_5 eyes, and M_6 gills (Adams *et al.*, 1993). Ratings of 0, 10, 20 and 30 were assigned to each metrics based on 6 metric model. The sum of those ratings (0-30) was given as values of HAI and four integrity classes including excellent (0), good (10-20), fair (30-60) and poor conditions (70-180) were adapted in the experiments.

3. In situ pilot test for the remediation of AMD wastewater

We constructed a reactor facility in the outflowing point of the abandoned mine for the remedia-

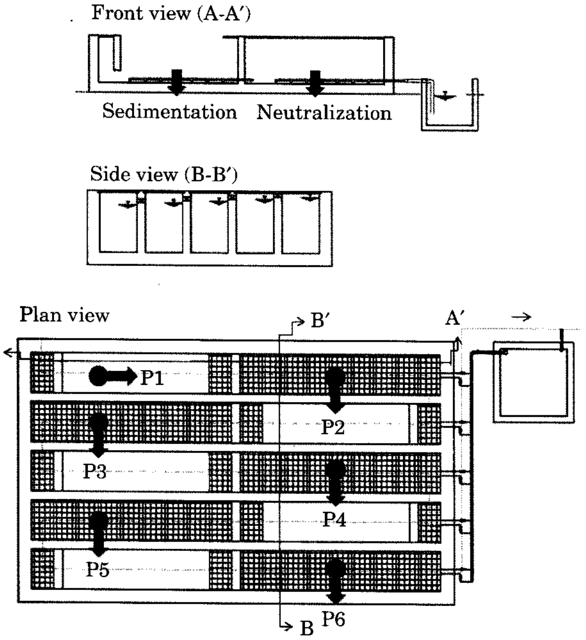

Fig. 2. The experimental design for disposal pilot facility.

tion of AMD wastewater. For the wastewater disposal facilities, dimension of the reactor was 9.8 m × 1.8 m × 5.3 m as L × H × W (Fig. 2). The pilot plant made of concrete structure, which is filled with oyster shell in the Jeomdong mine entrance and the wasterwater from the AMD passed through the facilities (Fig. 2). The species of shell was Crassostrea gigas and these shells were waste shells abudanded from the aquacultural industry in the sea. By the previous research of Kim *et al.* (1999), Oyster shell consisted in 38% of Ca including sodium and magnesium and can be adopted as neutralizer between metalic cation and AMD. The treatment capacity of the wastewater using the waste shells was designed as 30 tons per day. Reactor facility can be divided with neutralization zone and sedimentation zone (P2, P3, P4, P5 and P6) and AMD can discharged from P1 to P6 direction in order of precedence (Fig. 2).

For the pilot tests, we constructed 9 fish cages for the in situ lethal tests using sentinel fish species of Phynchocypris oxycephalus. The dimension of each fish cage is 40 × 25 × 20 cm as L × H × W and 3 cages for the control (a pristine site, no mining water; C), P1 (entrance site of untreated wastewater), and P6 (the site for treated water after pass through the oyster shell) were set up in each site (Fig. 2). In the cage, there was water channel to flow through but net on the top to prevent the escape of fish. Cage at the control site was set up in the ground of stream after digging up to soak and at the P1 and P6, was also set up by rope to located in the middle of sedimetation tank. In situ field pilot tests were performed during nine days from 4 April to 13 April 2005, and we measured pH, total dissolved solids (TDS), sulfate (SO_4 and heavy metal concentration such as Fe, Al, Zn, Mn, Cu, and Pb. We measured pH, total dissolved solids (TDS), sulfate (SO_4) and heavy metal concentration such as Ca, Fe, Al, Zn, Mn, Cu, and Pb. The TDS of the water were measured using a TDS meter Model 124 in the field. Water samples collected for cation determination were filtered through a 0.45 mm cellulose nitrate membrane filter using a hand pump, and were immediately acidified in the field to pH <2.0 by adding HNO_3. All the water samples were stored in a refrigerator at 4°C Analyses for dissolved cations (Ca, Fe, Al, Zn, Mn, Cu, and Pb) were performed using an Atomic Absorption Spectrophotometery (AAS).

The sentinel species tested was chinese minnow, *Phynchocypris oxycephalus*, was chosen, based on field survey of fish, which is locally abundant in the study area and inhabit generally in pristine mountainous up-streams in Korea. During the test period, no fish feed was supplied in the cages and also measured dissolved oxygen (mg L^{-1}), pH, water temperature (°C), turbidity (NTU), salinity, and specific conductivity (at 25 °C) in the in situ pilot tests.

RESULTS AND DISCUSSION

1. Chemical water quality in the AMD and control site

We measured chemical water quality in the water sampling site (S1, S2 and S3) for microscale lab tests. Turbidity measured on each site was augmented along the gradient from S1 (mean =3.6, range: 2.2-5.0) and S2 (mean=2.6, range: 1.5-3.6) to S3 (mean=7.4, range: 6.0-8.7) by increased water volume (Table 1). Conductivity were averaged 136, 266 $\mu s\ cm^{-1}$ in the S1 and S2 respectively, and showed 160 $\mu s\ cm^{-1}$ in S3 of the conflux area (Table 1). Values of pH was clearly increased, in company with that of conductivity, as much as 0.8 from S2 (mean=5.2, range: 4.3-

5.9) to S3 (mean=6.0, range: 5.5-6.3) and this increase seems to be caused by the result of dilution effect on S1 (mean=6.4, range: 6.3-6.6). Besides, there were no significant differences among sites on water temperature (mean=14.8°C, range =12.8-16.4°C), dissolved oxygen (mean=8.98 mg L^{-1}, range=7.2-11.1 mg L^{-1}) and salinity (all value is 0.1 ppt).

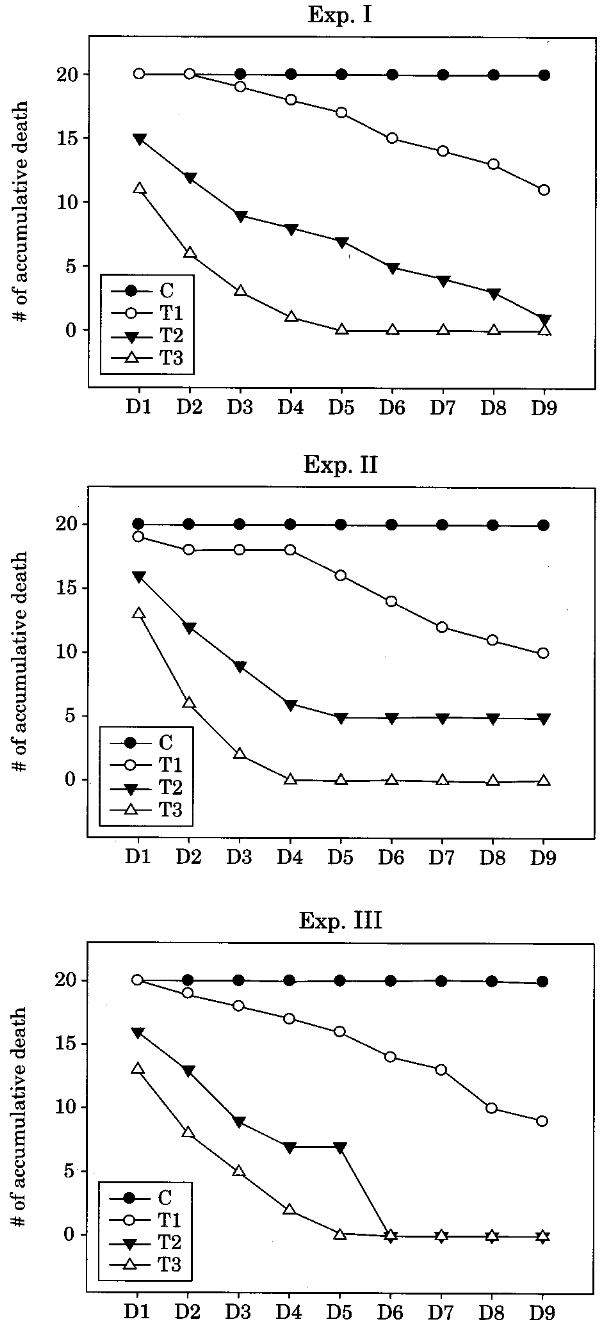

Fig. 3. Lethal effects of fish during 9 days in the pilot tests. Experimental conditions were designed as the control (pristine condition), and the two treatments (T1, T2). In the experiments, 1st, 2nd, and 3rd experiments (Exp. I, II, and III) were conducted in the April 2005, April 2006, April 2007, respectively.

2. Microscale lab tests

Microscale laboratory experiments conducted were for the toxicity impacts on the fish exposed using the stream water of the AMD. Overall experiments indicated that fish exposed, *Phynchocypris oxycephalus*, had no any death or immobility in the control during the experimental periods and the fish showed large impacts in the three treatments including surface water from S3 (Treatment 1, T1), surface water from S2 (Treatment 2, T2), and surface water plus bottom sediments from S2 (Treatment 3, T3). During the 9 day experiments, 20 fishes were exposed to the control tank (C), three treatment tanks (T1, T2, and T3), respectively. In the experiments, the death rate of fish exposed, was determined by the equation of the number of died fish/20 multiplied by 100. In the control (C), the death rate was 0%, which means that no 20 fishes exposed died (Fig. 3). In contrast, death rates in all three treatments were $\geq 50\%$ in the experiments and the numbers of fish died ranged 10-20 individuals among 20 fishes in each tank (Fig. 3).

In the treatment 1 (T1), overall death rate was 50% over the 9 day exposure, while death rates in the T2 and T3 was 70% and 100%, respectively (Fig. 3). Daily Mortality rate of fish exposed as fish number died per day (# d^{-1}), in the experiments was 0 d^{-1} in the control (C), 0.5 d^{-1} in the treatment of T1, 3 d^{-1} in the treatment of T2 and 5.2 d^{-1} in the treatment of T3 (Fig. 3). Thus, the response of fish in the T3 treatment was greater than any other treatments, indicating that lethal effect of fish on the surface water plus bottom sediments (i.e., yellow fine matter) was greater than only on the water samples and the fine yellow sediments cause high toxic effect on fish. The exposure experiments evidently suggest that the stream effluents from the AMD resulted in toxic effects to the fish exposed. Also, we observed highly paled yellow water and fine sticky sediments in the stream during the period of heavy monsoon rain, resulting in high turbidity of >100 NTU. The mine sediments was originated definitely

Table 2. Lab tests for fish exposure and necropsy-based health assessment index (HAI) in the controls (C) and treatments (T1-T3).

Seasons	Model metric	Experimental design											
		C			T1			T2			T3		
		F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3	F_1	F_2	F_3
APR, 2005	M_1	0	0	0	10	10	20	10	20	20	20	20	20
	M_2	0	0	0	0	0	10	0	20	10	0	10	10
	M_3	0	0	0	30	30	30	30	30	30	20	30	20
	M_4	0	0	0	30	30	30	30	20	30	30	30	30
	M_5	0	0	0	10	30	10	30	20	30	10	20	30
	M_6	0	0	0	30	30	30	20	20	10	30	30	30
Sum		0	0	0	110	130	130	120	130	130	110	140	140
APR, 2006	M_1	10	0	0	10	10	20	10	20	20	20	20	10
	M_2	0	0	0	0	0	0	10	20	20	0	10	10
	M_3	0	0	0	30	30	20	20	20	30	30	20	20
	M_4	0	0	0	30	30	30	30	30	30	30	30	20
	M_5	0	0	0	30	30	20	30	30	20	20	30	30
	M_6	0	0	0	20	30	30	20	10	10	30	30	30
Sum		10	0	0	120	130	120	120	130	130	130	140	120
APR, 2007	M_1	0	0	0	0	0	10	20	20	20	0	20	10
	M_2	0	0	0	0	10	10	10	10	10	0	0	10
	M_3	0	0	0	10	20	30	30	20	20	30	30	20
	M_4	0	0	0	20	20	30	30	20	20	30	30	30
	M_5	0	0	0	10	0	0	30	0	10	10	20	20
	M_6	0	0	0	30	30	30	20	10	20	30	30	30
Sum		0	0	0	70	80	110	140	80	100	100	130	120
Average		3	0	0	100	113	120	127	113	120	113	137	127
Criteria		G	Ex	Ex	P	P	P	P	P	P	P	P	P

M_1=Fins, M_2=Spleen, M_3=Kidney, M_4=Liver, M_5=Eyes, M_6=Gills

from the entrance of the AMD and passed through the stream and then deposited in bottom of down-reservoirs.

Necropsy-based fish tissue assessments using the Health Assessment Index (HAI, Adams *et al.*, 1993; Blazer, 2000), employed showed an toxic impacts on the effluents of the AMD depending on the fish tissues used in the experiments (Table 2). By the result of evaluation by methods of Adams *et al.* (1993), T1 (mean: 111), T2 (mean: 120) and T3 (mean: 126) were "poor" conditions except C3 (mean: 3), indicating signficant toxic effects by AMD. Most frequently damaged tissue was liver (average: 20.8) and spleen was less effected than other tissues. Gill was also effected with high value 18.6 and especially all individuals were the worst value, 30 in T3 by sediments in T3, attached at the gill in fish respiration. Kim *et al.* (2007) and Hellawell (1986) reported that high turbidity can cause the physical abrasion and capacity reduction of gill. Values of Health Assessment Index (HAI) were lower in the control than any other treatments of T1, T2, and T3 and three treatments showed a distinct toxicity impacts by the AMD (Table 2).

3. Chemical remediation of the mining water by pilot facilities

Concentrations of Fe, Al and Zn measured in the disposal pilot facility were reduced rapidly as the untreated water passed through the compartments from P1 to P6. This phenomenon was a results of oxidation-deoxidation reaction in the disposal pilot facility. Initial concentration of Fe, Al and Zn in the AMD prior to the treatments by the oyster shell was 6.89 mg L^{-1}, 10.43 mg L^{-1} and 0.13 mg L^{-1}, respectively (Fig. 4). However, the concentration of Fe, Al and Zn decreased particularly by 85%, 99% and 94%, respectively thro-

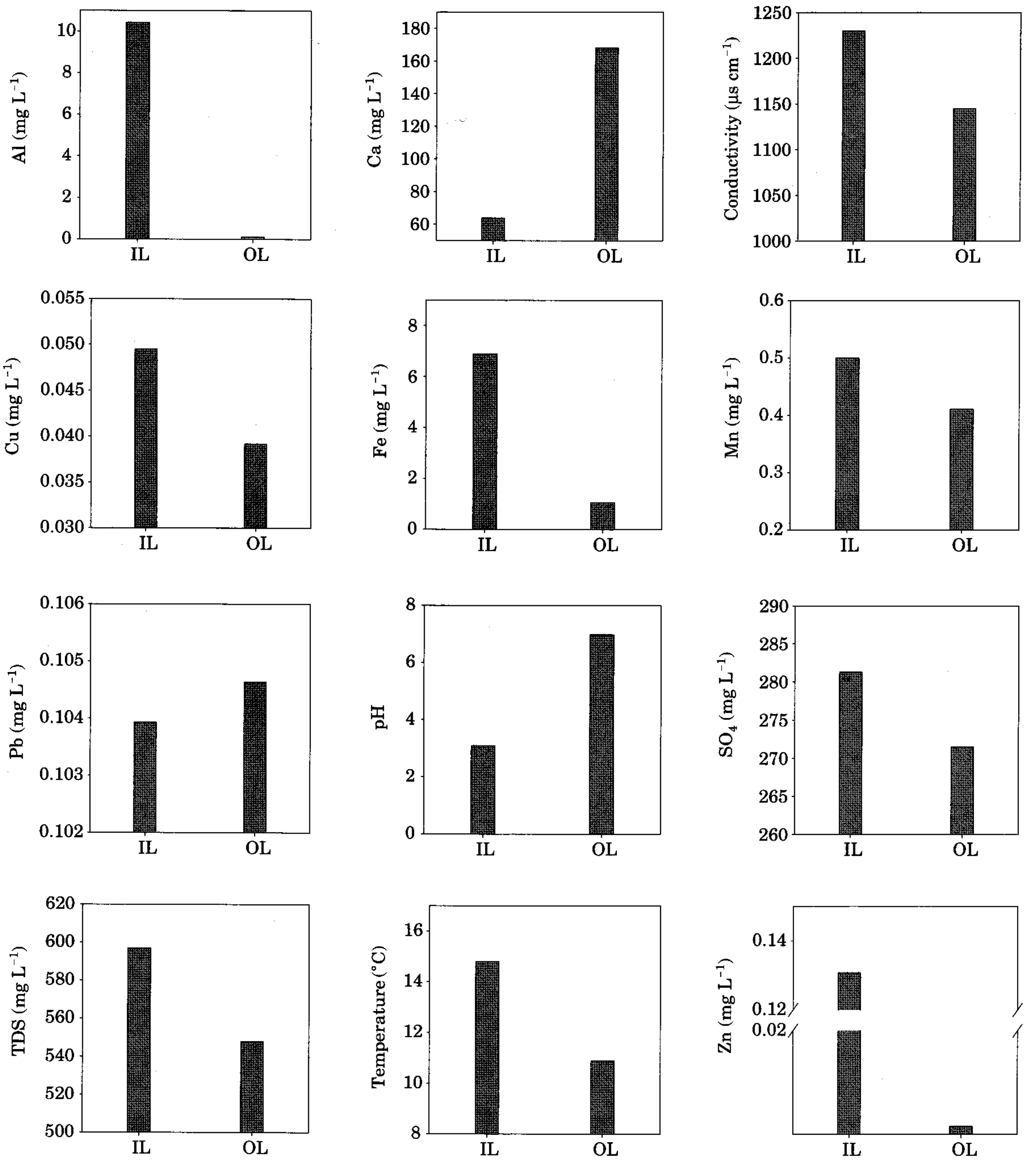

Fig. 4. Comparisons of water temperature, conductivity, total dissolved solids (TDS), pH, and various metal contents in the inlet and outlet of the disposal reactor (IL=Inlet, OL=Outlet).

ugh the disposal facility. In the mean time, concentration of Ca was increased by 2.6 fold, which seems to be caused by shell dissolved effect (Fig. 4).

One of the most important process in the pilot facilities was massive increases of pH after the water from the AMD passed through the oyster shell. Values of pH, ranged from 3.1 to 7.0, increased by 2.3 fold (mean 5.1) along with the reduction of metal contents. Values of pH increased up to more than twice in the compartments from P1 to P6. The increased high pH was caused by increases of dissolved calcium carbonate of shell. In the mean time, conductivity and total dissolv-

Table 3. Lethal effects of fish exposed on the various stream water (S1, control; P1, inlet; P6, outlet). The effect was expressed as an accumulative number of died fish. The water in the P1 is an inflowing mine water untreated, which is located in the inlet of the stream. The water in the P6 is an effluent water with non-acidic and low heavy metal treated by the oyster shell.

Experimental Compartments		D-3	D-6	D-9	Survival #
S1 (Control)	C1	0	0	1	9
	C2	0	0	4	6
	C3	0	0	5	5
P1 (Inlet)	I1	10	–	–	0
	I2	10	–	–	0
	I3	10	–	–	0
P6 (Outlet)	O1	0	1	9	0
	O2	0	0	10	0
	O3	0	0	10	0

ed solids (TDS) in the pilot facility have reduced just 6.9% and 8.2 respectively, showing not too many changes between the initial water and final water so that means the shell had little removal effects on the parameters. Also, there were not too much removal efficiencies in the metals such as of Mn, Cu, Pb and SO_4 (Fig. 4).

4. In situ fish exposure tests in the field pilot facilities

Fig. 2 shows two dimensional structures of the pilot plant made of concrete structure, which is filled with oyster shells in the Jeomdong mine entrance and 3 fish tanks for the control (S1, a pristine site, no mining water; C), P1 (entrance site of untreated wastewater), and P6 (the site for treated water after pass through the oyster shell). In situ field tests on fish lethal effects showed that there were large differences in the lethal effect of fish exposed among the control (C), P1, and P6. All fishes in P1 cage died 100% on 3 days later after the experimental setting, while all fishes in the P6 died 100% on 9 days later (Table 3). In the S1, which is located in the pristine location and have no wastewater impact, 10 of 30 fish individuals died, indicating 33% in the lethal ratio. Even if the lethal effect on fish occurred in the S1, the fish death might have been partially associated with limited food resource. Once the death in the S1 is primary factor of food limitation, we assume that death rate in the P1 and P6 may be 66% (i.e., 100-33%) on the 3rd day and 9th day, respectively (Table 3).

Overall, these results evidently provide a key methodology for pilot test using the disposal facility and also clarify the toxicity of AMD once again. Currently, there are so many abandoned acid mines in Korean watersheds (Lee *et al.*, 2007), and these watersheds have discharged the acidic water and waters with high heavy metal contents (Kim *et al.*, 2002; Lee *et al.*, 2006). resulting in massive toxic impacts on aquatic biotas of fish, macroinvertebrate, and attached algae (i.e., periphyton). In fact, residential people in the Jeomdong Mine, which is located in current our study region, have frequently observed massive fish kills such as gold fish, carps, and minnows especially, during heavy monsoon seasons. Such contaminated water is often stored in the down-reservoirs for agricultural irrigations and drained into rice paddy in the field, so this approach used in the pilot facilities here may reduce the acidic and toxic effects in the abandoned mining drainage.

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