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Potential Risks of Effluent from Acid Mine Drainage Treatment Plants at Abandoned Coal Mines

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Abstract The lethal and sublethal toxicity of effluent from three acid mine drainage treatment plants were monitored from August 2009 to April 2010 using *Daphnia magna* (reference species) and *Moina macrocopa* (indigenous species). Acute lethal toxicity was observed in Samma effluent due to incomplete neutralization of acid mine drainages by the successive alkalinity producing system (SAPS). Additionally, there was no significant difference in toxicity values (TU) between *D. magna* and *M. macrocopa* ($p < 0.05$). Toxicity identification results of the final effluent collected in January 2010 showed that Al and Zn were key toxicants in addition to acidic pH. Unlike the Samma effluent, both Hwangji and Hamtae effluent had pH values that were near neutrality and showed either no acute toxicity or toxicity values less than 1 TU. However, the feeding rates of *D. magna* and *M. macrocopa* were significantly reduced when compared to the control ($p < 0.05$). These findings suggest that the Hamtae and Hwangji effluent likely have a sublethal effect on aquatic organisms in receiving water bodies.

Keywords Acute toxicity · Feeding rate inhibition · Heavy metals · Acid mine drainage · Toxicity identification evaluation

Acid mine drainage (AMD) is a serious environmental problem in the mining industry because it typically has a low pH and contains high concentrations of toxic metals, which may adversely affect aquatic ecosystems (Yim et al. 2006). Thus, bioassessment of AMD has been studied using different test-organisms such as microorganisms, daphnids, shrimp and fish. Cherry et al. (2001) reported significant increases in the mortality of *Corbicula fluminea* in AMD receiving waters when compared to control water. Denicola et al. (2002) also demonstrated that significant decreases in the percent survival of *hydropsychid caddisflies* (Hydropsyche sp.) occurred in a stream receiving AMD. In addition to lethal effects of AMD, sublethal effects on aquatic organisms have been observed. Macedo-Sousa et al. (2007) showed significant decreases in both feeding activity and locomotion of *Echinogammarus meridionalis* in mixed AMD samples when compared to original river water. Additionally, Gerhardt et al. (2005) reported that AMD induced the inhibition of locomotion toward *Daphnia magna* and *Gambusia holbrooki*.

However, toxicity assessment of mining effluent from active and passive treatment systems was limited in these studies. Pagnanelli et al. (2008) found that active treatment efficiently reduced the toxicity of AMD toward *Lepidium sativum*, but that 100 % immobility was still observed for *D. magna*. In addition, the toxicity of the effluent of passive treatment systems toward *L. sativum* gradually decreased when compared to untreated AMD, while residual toxicity for *D. magna* remained. Neculita et al. (2008) also demonstrated that a 10 d hydraulic retention time effluent from

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sulfate-reducing passive bioreactors was not acutely toxic toward *Oncorhynchus mykiss*, but was toxic toward *D. magna*.

Given the findings presented above, acute toxicity and feeding rate tests were applied to monitor the lethal and sublethal effects of effluent from AMD treatment plants using *D. magna* (reference species) and *M. macrocopa* (indigenous species). Additionally, toxicity identification evaluation (TIE) was used to characterize, identify and to fundamentally reduce major toxicants in the effluent.

Materials and Methods

Sampling was conducted at three AMD treatment plants located in Gangwon-Do, South Korea from August 2009 to April 2010. The Samma AMD treatment plant (Samma), which treats approximately 400 m^3/day of AMD, consists of a successive alkalinity producing system (SAPS), an oxidation pond and a constructed wetland. The Hwangji AMD treatment plant (Hwangji), which treats about 1,050 m^3/day of AMD, consists of an oxidation pond, SAPS and a constructed wetland. Samples of the raw wastewater (RW), primary effluent (PE), secondary effluent (SE) and final effluent (FE) were collected after each treatment. Only RW and FE were collected for the Hamtae AMD treatment plant (Hamtae), which treats about 8,000 m^3/day of AMD using neutralization and sedimentation processes.

For dissolved metal analyses, samples were filtered immediately using a 0.45 μm syringe filter in the field and then saved with concentrated nitric acid in sterile polyethylene bottles. For total metal analyses, concentrated nitric acid was added immediately to the sample in the field. In addition, samples for toxicity tests were collected separately without any treatment. All samples were transported to the lab on ice and then stored at 4 °C throughout the entire study period. The samples were transported to the lab on ice and then stored at 4 °C throughout the entire study period. The initial toxicity tests and water quality analyses were conducted upon arrival of the samples.

Samples were filtered using a 0.45 μm syringe filter and then analyzed for DOC (dissolved organic carbon) and anions using a Shimadzu TOC analyzer model 5000A (Kyoto, Japan) and a Dionex ion chromatography model ICS-2000 (Dionex, USA) respectively. The hardness was measured according to the Standard Methods for the Examination of Water and Wastewater (APHA 1998).

Metal concentrations were analyzed using an inductively coupled plasma-optical emission spectrophotometer (ICP-OES, Varian Vista PRO, CA, USA). For metal analysis, all vessels and experimental apparatuses were rigorously acid-cleaned prior to use. Standard solutions were freshly prepared and standard calibration curves with an $r^2 > 0.995$ were achieved daily.

Acute toxicity tests were conducted according to the Organization for Economic Co-operation and Development standard procedures (OECD 2004) using neonates of both *D. magna* and *M. macrocopa* (less than 24 h old). In each test, one control and five or more dilutions of each samples with four replicates were prepared, after which five neonates with 10 mL of test solution were added to each well. Toxicity tests were conducted at 20 ± 2 °C with a 16 h light: 8 h dark photoperiod for 48 h.

The immobilization (defined as no response to gentle agitation) of the test species was calculated using the following equation:

$$\text{Immobilization}\,(\%) = \frac{N_{\text{immobilized}}}{N_{\text{total}}} \times 100$$

where $N_{\text{immobilized}}$ is the number of immobilized animals and N_{total} is the total number of animals used in the test. The immobilization was also used to calculate the EC_{50} (50 % effective concentration) values by a graphical method, Probit analysis or the Trimmed Spearman-Karber method (USEPA 2002). For comparison, EC_{50} values were transformed into toxic units ($TU = 100/EC_{50}$).

Toxicity identification evaluation (TIE) was conducted according to the TIE procedures developed by the USEPA. In phase I of the TIE (USEPA 1991), a baseline test, pH adjustment, pH adjustment/filtration, graduated pH, and cation and anion exchanges were included to characterize the classes of the toxicants. For ion exchange experiments, columns were prepared using 50 mL syringes filled with either anion (Amberlite IRA-410, chloride form, Aldrich, USA) or cation (Amberlite IR-120, sodium form, Aldrich, USA) exchange resins. For mixed-bed ion exchange, samples were applied to both the anion and cation exchange columns. Except for graduated pH tests, the pH of the samples after each manipulation was readjusted to the initial pH with NaOH and HCl, and acute toxicity tests were then conducted to determine the change in toxicity.

In the TIE phase II test (USEPA 1993a), metals suspected as key toxic materials were measured using a Varian ICP-OES (Varian Vista PRO, CA, USA). During phase III of the TIE (USEPA 1993b), the key toxicants were confirmed using a mass balance approach. To accomplish this, a known amount of a suspect toxicant was returned to the sample after the suspect toxicant was removed. Toxicity tests were then conducted to confirm whether the toxicity was recovered due to the addition of the suspected toxicant.

Feeding rate tests were conducted according to the procedure established by Allen et al. (1995) with brief modification. In each test, five neonates less than 24-h old were exposed to 15 mL test solutions with three replicates. *Chlorella vulgaris* was then added at an initial concentration of 2×10^6 cells mL^{-1}. All feeding rate tests were conducted at 20 ± 2 °C in the dark for 24 h. In addition,

four vessels were prepared without the addition of animals and kept under the same conditions to confirm the initial concentration of *Chlorella*. After 24 h of exposure, each vessel was vigorously shaken to suspend any settled cells and the *Chlorella* cells were then counted using a hemacytometer and subsequently removed.

The mean concentration of *Chlorella* was used to calculate the feeding rate based on a simplified version of Gauld's equation (Gauld 1951):

$$F = \frac{V \times (C_0 - C_{24})}{n \times t}$$

where, F = the feeding rate (cells ind^{-1} h^{-1}), V = the volume of medium in the test vessel (mL), C_0 = the initial cell concentration (cells mL^{-1}), C_{24} = the final cell concentration (cells mL^{-1}), n = the number of animals in each test vessel, t = the duration of exposure (h).

The feeding rate inhibition (%) compared to the control was then calculated using the following equation:

$$\text{Feeding rate inhibition}\,(\%) = \frac{F_{\text{control}} - F_{\text{sample}}}{F_{\text{control}}}$$

where F_{control} and F_{sample} are the feeding rate of animals exposed to the control and the sample, respectively.

SAS 8.2 (SAS Institute Inc., Cary, NC, USA) was employed for all statistical analyses. Significant differences were determined by analysis of variance (ANOVA) followed by Dunnetts test ($p = 0.05$).

Results and Discussion

The results of acute toxicity and chemical characteristics of Samma are given in Table 1. Raw wastewater (RW) was acutely toxic toward both *D. magna* and *M. macrocopa*, and the toxicity rapidly decreased as treatment proceeded. However, the final effluent (FE) was still toxic toward both species likely due to low pH condition. The pH of samples and acute toxicity were significantly related ($p < 0.05$); however, they were not well correlated ($r^2 = 0.4314$ and $r^2 = 0.3622$ for *D. magna* and *M. macrocopa*, respectively).

Toxicity identification evaluation (TIE) was conducted for FE collected in January 2010 to identify the cause of the acute toxicity. Both *D. magna* and *M. macrocopa* showed similar results of TIE phase I (Fig. 1a). The toxicity rapidly reduced by adjustment of the pH to 11 followed by filtration and ion exchanges. These findings indicated that toxic materials may have been cationic materials, such as metals, which likely formed hydroxide precipitates at high pH.

Among metals, the Al and Zn concentrations in FE were found to exceed the EC_{50} value for both species (Table 1). Moreover, the Al and Zn concentrations were reduced in a trend similar to the toxicity by the TIE phase I procedures (Fig. 1b, c). However, the complete removal of Al and Zn by anion exchange manipulation was somewhat extraordinary (Fig. 1). Several studies have demonstrated that anionic species such as $Al(SO_4)^-$ and $Zn(SO_4)_2^{2-}$ could be

Table 1 Chemical characteristics and toxicity of Samma effluents

Sample		pH	Dissolved metals (mg L^{-1})			Acute toxicity (TU)	
			Al	Cu	Zn	*D. magna*	*M. macrocopa*
08/2009	RW	2.88	76.41	0.030	3.06	23.0	25.1
	PE	4.15	35.45	<DL	1.75	6.1	6.1
	SE	3.19	36.04	<DL	1.69	11.7	6.1
	FE	3.10	31.87	<DL	1.39	5.7	5.7
10/2009	RW	2.97	93.60	0.173	4.42	22.6	24.2
	PE	3.88	47.81	0.043	2.98	6.5	8.6
	SE	3.31	49.48	0.028	2.88	11.7	8.3
	FE	3.25	41.66	0.020	2.67	5.9	6.1
01/2010	RW	3.19	64.33	0.047	3.53	23.4	20.4
	PE	4.85	16.31	0.022	4.15	3.1	3.7
	SE	4.87	15.41	0.024	4.46	4.1	3.0
	FE	4.59	16.60	0.021	4.46	3.7	3.0
04/2010	RW	2.94	67.70	0.012	2.11	22.6	17.7
	PE	3.30	46.70	<DL	2.63	8.0	6.3
	SE	3.32	49.85	<DL	2.64	7.5	6.1
	FE	3.25	49.53	<DL	2.64	6.5	6.1
EC_{50} (mg L^{-1})	*D. magna*		11.31	0.022	1.54		
	M. macrocopa		8.06	0.011	2.84		

RW raw wastewater, *PE* primary effluent, *SE* secondary effluent, *FE* final effluent, *TU* toxic unit, *DL* detection limit

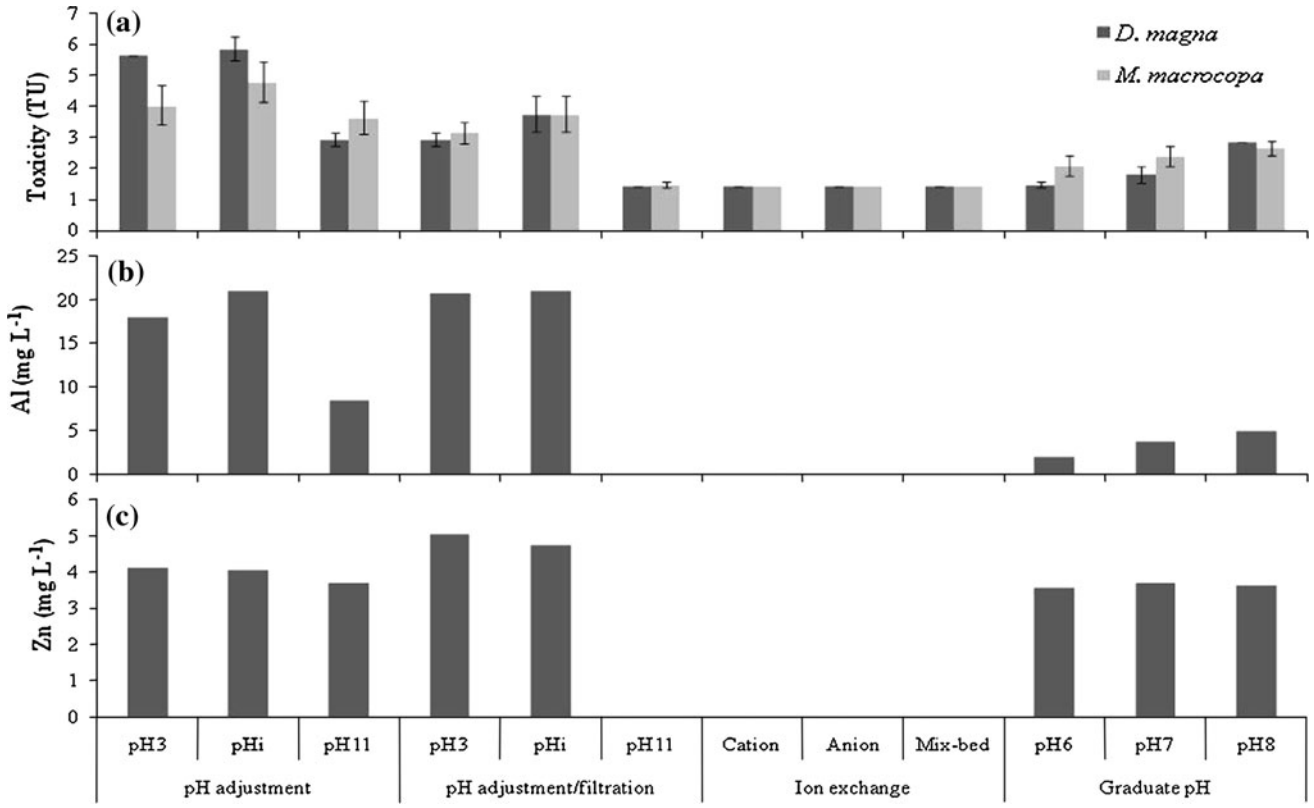

Fig. 1 Toxicity identification of the final effluent of Samma collected in January 2010: **a** TIE phase I results using *D. magna* and *M. macrocopa*, **b** Al concentrations and **c** Zn concentrations. The TU value of pH_i (initial pH) is baseline toxicity, and *error bars* represent the 95 % confidence intervals

absorbed by anion exchange resins (Matus and Kubova 2005; Karamalidis and Voudrias 2007; Yi et al. 2010). In this study, the concentration of sulfate in the FE collected in January 2010 was 1,983 mg L^{-1}. Consequently, high concentrations of sulfate appeared to result in the formation of Al and Zn sulfate complexes. These findings suggest that chemical species should be considered in the procedure of toxicity assessment and identification evaluation.

To confirm that Al and Zn were key toxic materials in FE, a mass balance test was conducted. As shown in Fig. 2a, b, toxicity toward both *D. magna* and *M. macrocopa* was recovered to the initial levels by the addition of Al (16.45 mg L^{-1}) and Zn (4.45 mg L^{-1}) after pH 11/filtration and mixed-bed ion exchange treatment, except for samples subjected to cation exchange ($p < 0.05$). Considering that FE contained cationic metals such as Cu, Fe and Mn in addition to Al and Zn, these metals might contribute to the observed toxicity of FE. Overall, the above results indicate that Al and Zn were major toxic materials in the FE sample in addition to the acidic pH.

Final effluent of Samma showed acute toxicity toward *D. magna* and *M. macrocopa* likely due to incomplete neutralization of acid mine drainage (Table 1). In the SAPS, AMD flow moves downward through a layer of limestone gravel to add alkalinity. In situations in which the AMD contains appreciable amounts of Al, accumulation of precipitates gradually decreases permeability, which may cause failure of the drain (Johnson and Hallberg 2005; Matthies et al. 2010). Indeed, Skousen (1997) reported that a SAPS receiving > 40 mg L^{-1} Al was plugged with Al precipitates despite flushing. Considering that the average concentration of Al in RW was 82 mg L^{-1}, incomplete neutralization of AMD was likely due to clogging of the limestone layer of SAPS by Al precipitates.

The acute toxicity (immobilization) of Samma effluent toward *D. magna* was largely removed as the effluent was neutralized and was further reduced by filtration (Fig. 3). The dissolved concentrations of toxic metals after pH adjustment and filtration did not exceed the EC_{50} concentration of *D. magna*, except pH 5.5 (data not shown). These findings suggest that particulates as well as dissolved metals likely contributed to the observed toxicity of Samma effluent. Several studies have demonstrated that nano- and micro-size particles of Al_2O_3, FeO, CuO and $ZnSO_4$ might induce lethal and sublethal toxicity toward aquatic organisms, and that particulate metals were more toxic than dissolved metals in some cases (Heinlaan et al. 2008; Strigul et al. 2009). These results suggest that filtration

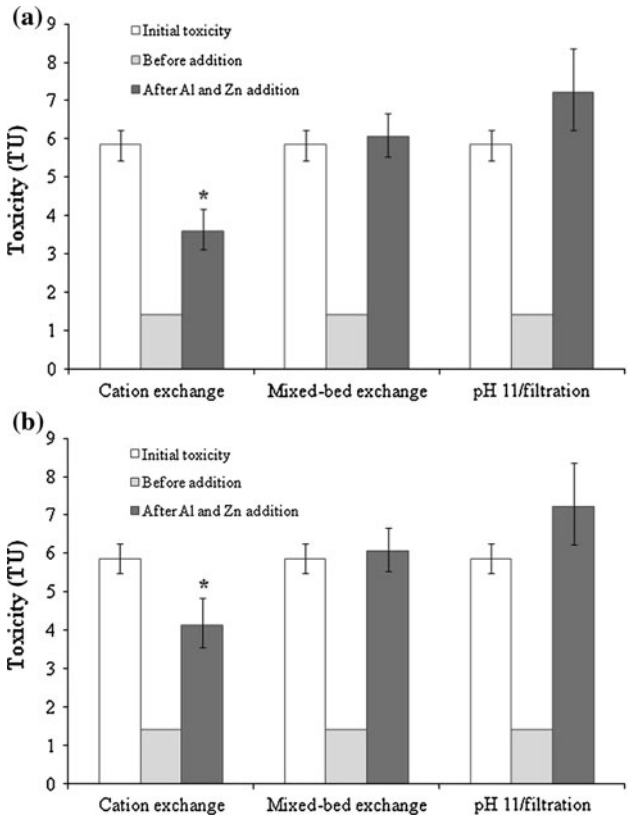

Fig. 2 Toxicity confirmation of the final effluent of Samma collected in January 2010 by the mass balance test using **a** *D. magna* and **b** *M. macrocopa*. *Error bars* represent the 95 % confidence intervals. Concentration of Al and Zn found in filtered final effluents (16.45 mg L^{-1} and 4.45 mg L^{-1}, respectively) was added. Significant differences (*asterisk*) from the control were determined by Dunnett's test at $p < 0.05$

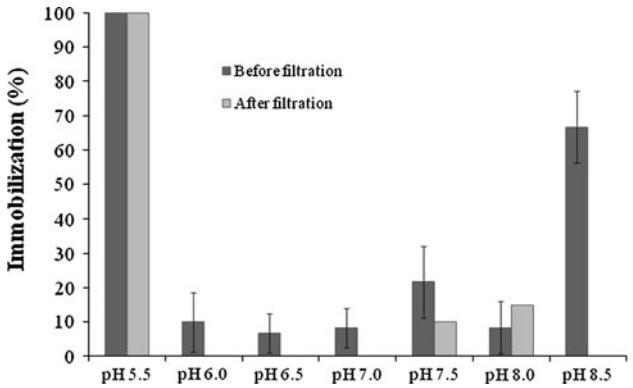

Fig. 3 Effects of pH adjustment and filtration on the immobilization of *D. magna* in the Samma final effluent collected in October 2009

processes such as sand filters should be adopted to remove particulates from the final effluent. Hamoda et al. (2004) demonstrated that solid (SS, VSS) and organics (BOD, COD) in the wastewater were removed by sand filtration.

Unlike the Samma sample, both Hwangji and Hamtae samples had near neutral pH values and showed acute toxicity less than 1 TU toward *D. magna* or *M. macrocopa* (Tables 2 and 3, respectively). Additionally, dissolved concentrations of toxic metals in FE samples of Hwangji and Hamtae did not exceed its EC_{50} values for both species (data not shown). However, feeding rates of *D. magna* and *M. macrocopa* were significantly inhibited when compared to the control ($p < 0.05$) though acute toxicity of the final effluent of Hwangji and Hamtae was not observed. Pestana et al. (2007) demonstrated that effects on the feeding behavior of individuals can be translated to effects on populations and, more importantly, to effects on vital ecosystem functions. In addition, several studies showed that feeding assays can indicate the first responses to environmental perturbations, making them at least as sensitive as other physiological measures (Maltby and Crane 1994; McLoughlin et al. 2000; Pestana et al. 2007). Furthermore, Barata et al. (2008) reported that the 24 h *D. magna* feeding assay was a cost-effective and sensitive test compared with the existing standardized bacteria bioluminescent, algal growth and fish acute tests. These findings suggest that the feeding rate test is much more sensitive than the lethal toxicity test and is a promising bioassay tool for risk assessment of industrial effluents.

The inhibition of the feeding rates of *D. magna* and *M. macrocopa* exposed to different concentrations of Hwangji FE are shown in Fig. 4a, b, respectively. Both species showed a concentration dependant feeding rate, and *D. magna* appeared to be more sensitive than *M. macrocopa*. However,

Table 2 Chemical characteristics and toxicity of Hwangji effluents

Sample		pH	Acute toxicity (TU)		Feeding rate inhibition (%)	
			D. magna	*M. macrocopa*	*D. magna*	*M. macrocopa*
08/2009	RW	6.57	<1	<1	ND	ND
	PE	6.67	<1	<1	ND	ND
	SE	7.31	NT	NT	ND	ND
	FE	7.24	NT	NT	ND	ND
10/2009	RW	6.25	NT	NT	ND	ND
	PE	6.43	NT	<1	ND	ND
	SE	6.94	NT	NT	ND	ND
	FE	6.88	NT	NT	ND	ND
01/2010	RW	6.29	<1	NT	ND	ND
	PE	6.55	<1	<1	ND	ND
	SE	6.87	NT	NT	ND	ND
	FE	7.01	NT	NT	97.2	99.7
04/2010	RW	6.37	NT	NT	ND	ND
	PE	6.74	<1	NT	ND	ND
	SE	7.13	NT	NT	ND	ND
	FE	7.25	NT	NT	36.0	34.9

RW raw wastewater, *PE* primary effluent, *SE* secondary effluent, *FE* final effluent, *TU* toxic unit, *NT* not toxic, *ND* not determined

Table 3 Chemical characteristics and toxicity of Hamtae effluents

Effluent		pH	Acute toxicity (TU)		Feeding rate inhibition (%)	
			D. magna	*M. macrocopa*	*D. magna*	*M. macrocopa*
08/2009	RW	6.69	NT	NT	ND	ND
	FE	7.66	<1	<1	ND	ND
10/2009	RW	6.53	NT	<1	ND	ND
	FE	7.27	<1	<1	ND	ND
01/2010	RW	6.65	<1	<1	ND	ND
	FE	7.83	NT	NT	87.0	84.3
04/2010	RW	6.75	<1	<1	ND	ND
	FE	8.01	NT	NT	84.3	32.7

RW raw wastewater, *FE* final effluent, *TU* toxic unit, *NT* not toxic, *ND* not determined

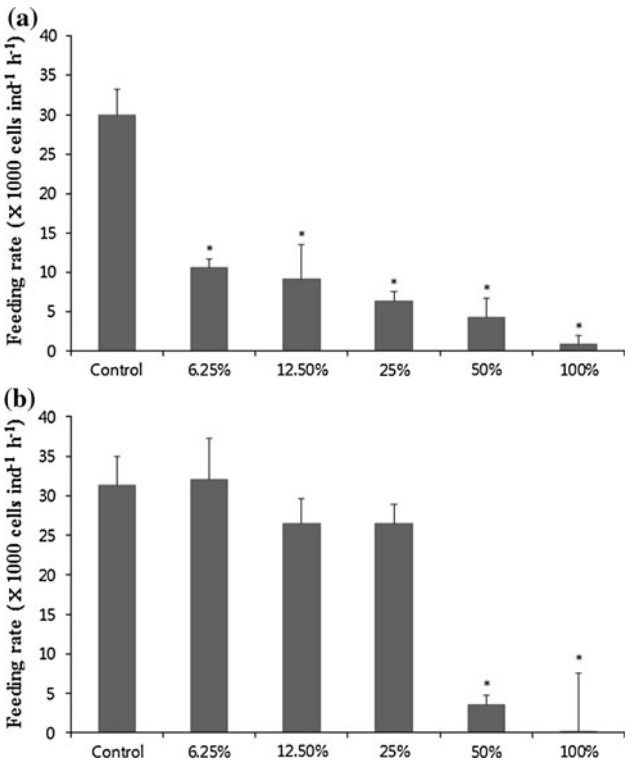

Fig. 4 Feeding rate inhibition of **a** *D. magna* and **b** *M. macrocopa* as a function of concentrations of the final effluent of Hwangji collected in January 2010. Significant differences (*asterisk*) from the control were determined by Dunnett's test at $p < 0.05$

there was no significant difference in acute toxicity values (TU) between *D. magna* and *M. macrocopa* ($p < 0.05$). Yi et al. (2010) reported that *M. macrocopa* was more sensitive than *D. magna* for acute toxicity tests, but the opposite was found in the case of the feeding rate inhibition. In addition, Ji et al. (2008) demonstrated that *M. macrocopa* showed greater sensitivity than *D. magna* in both acute and chronic toxicity tests. These results indicate that the causes of feeding rate inhibition might differ from those of acute toxicity; however, further study is necessary to confirm this.

In conclusion, effluents from acid mine drainage (AMD) treatment plants showed either lethal or sublethal toxicity toward *D. magna* and *M. macrocopa*. For Samma, malfunction of SAPS induced acute toxicity of the final effluent (FE), which was completely removed by successive neutralization and filtration. These findings suggest that an additional process (i.e. rapid sand filtration) should be adopted to remove toxic particulate metals. In this study, however, physiochemical properties of the particulates were not investigated. Thus, the lethal and sublethal toxicity of nano- and micro-size particles in AMD toward aquatic organisms should be investigated further.

The FE of Hwangji and Hamtae inhibited the feeding rates of *D. magna* and *M. macrocopa*, although acute toxicity was not observed. Moreover, feeding rate inhibition was concentration dependant, suggesting that a sublethal toxicity test (i.e. feeding rate inhibition) must be considered for regulation of AMD effluent. Additionally, given that a domestic species rather than the international standard species is more desirable, toxicity tests using *M. macrocopa* are more desirable in the toxicity-based discharge limits of Korea.

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References

Allen Y, Calow P, Baird DJ (1995) A mechanistic model of contaminant-induces feeding inhibition in *Daphnia magna*. Environ Toxicol Chem 14:1625–1630

APHA (American Public Health Association) (1998) Standard methods for the examination of water and wastewater, 20th edn. Washington, DC

Barata C, Alañon P, Gutierrez-Alonso S, Riva MC, Fernández C, Tarazona JV (2008) A *Daphnia magna* feeding bioassay as a cost effective and ecological relevant sublethal toxicity test for environmental risk assessment of toxic effluents. Sci Total Environ 405:78–86

Cherry DS, Currie RJ, Soucek DJ, Latimer HA, Trent GC (2001) An integrative assessment of a watershed impacted by abandoned mined land discharges. Environ Pollut 111:377–388

Denicola DM, Stapleton MG (2002) Impact of acid mine drainage in benthic communities in stream: the relative roles of substratum vs. aqueous effects. Environ Pollut 119:303–315

Gauld T (1951) The grazing rate of marine copepod. J Mar Biol Assoc 26:695–706

Gerhardt A, Bisthoven LJD, Soares AMVM (2005) Evidence for the stepwise stress model: *Gambusia holbrooki* and *Daphnia magna* under acid mine drainage and acidified waater stress. Environ Sci Technol 39:4150–4158

Hamoda MF, Al-Ghusain I, Al-Mutairi NZ (2004) Sand filtration of wastewater for tertiary treatment and water reuse. Desalination 164:203–211

Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A (2008) Toxicity of nanosized and bulk ZnO, CuO, and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere 71:1308–1316

Ji K, Kim Y, Oh S, Ahn B, Jo H, Choi K (2008) Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid on freshwater macroinvertebrates (*Daphnia magna* and *Moina macrocopa*) and fish (*Oryzias latipes*). Environ Toxicol Chem 27:2159–2168

Johnson DB, Hallberg KB (2005) Acid mine drainage remediation options: a review. Sci Total Environ 338:3–14

Karamalidis AK, Voudrias EA (2007) Release of Zn, Ni, Cu, SO_4^{2-} and CrO_4^{2-} as a function of pH from cement-based stabilized/solidified refinery oily sludge ans ash from incineration of oily sludge. J Hazard Mater 141:591–606

Macedo-Sousa JA, Pestana JLT, António AG, Nogueira JA, Soares AMVM (2007) Behavioural and feeding responses of *Echinogammarus meridionalis* (Crustacea, Amphipoda) to acid mine drainage. Chemosphere 67:1663–1670

Maltby L, Crane M (1994) Responses of *Gammarus pulex* (amphipoda, crustacean) to metalliferous effluents: identification of toxic conponents and the importance of interpopulation variation. Environ Pollut 84:45–52

Matthies R, Aplin AC, Jarvis AP (2010) Performance of a passive treatment system for net-acidic coal mine drainage over five years of operation. Sci Total Environ 408:4877–4885

Matus P, Kubova J (2005) Complexation of labile aluminium species by chelating resins Iontosorb-a new method for Al environmental risk assessment. J Inorg Biochem 99:1769–1778

McLoughlin N, Yin D, Maltby L, Wood RM, Yu H (2000) Evaluation of sensitivity and specificity of two crustacean biochemical biomarkers. Environ Toxicol Chem 19:2085–2092

Neculita C, Vigneault B, Zagury GJ (2008) Toxicity and metal speciation in acid mine drainage treated by passive bioreactors. Environ Toxicol Chem 27:1659–1667

OECD (2004) *Daphnia sp*. Acute immobilization test. OECD guidelines for testing of chemicals no. 202, Paris, France

Pagnanelli F, Michelis ID, Muzio SD, Ferella F, Vegliò F (2008) Bioassessment of a combined chemical-biological treatment for synthetic acid mine drainage. J Hazard Mater 159:567–573

Pestana JLT, Re A, Nogueira AA, Soares AMVM (2007) Effects of cadmium and zinc on the feeding behaviour of two freshwater crustaceans: *Atyaephyra desmarestii* (Decapoda) and *Echinogammarus meridionalis* (AMphipoda). Chemosphere 68:1556–1562

Skousen J (1997) Overview of passive systems for treating acid mine drainage. Green Lands 27:34–43

Strigul N, Vaccari L, Galdun C, Wanze M, Liu X, Christodoulatos C, Jasinkiewicz K (2009) Acute toxicity of boron, titanium dioxide, and aluminum nanoparticles to *Daphnia magna* and *Vibrio Fischeri*. Desalination 248:771–782

USEPA (1991) Methods for aquatic toxicity identification evaluations: phase I. Toxicity characterization procedures. EPA 600/6-91/003, Washington DC

USEPA (1993a) Methods for aquatic toxicity identifications evaluations: phase II. Toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/080, Washington DC

USEPA (1993b) Methods for aquatic toxicity identifications evaluations: phase III. Toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/081, Washington DC

USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA/821/R-02/012, Washington DC

Yi X, Kang SW, Jung J (2010) Long-term evaluation of lethal and sublethal toxicity of industrial effluents using *Daphnia magna* and *Moina macrocopa*. J Hazard Mater 178:982–987

Yim JH, Kim KW, Kim SD (2006) Effect of hardness on acute toxicity of metal mixtures using *Daphnia magna*: Prediction of acid mine drainage toxicity. J Hazard Mater B138:16–21