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A Comparative Study on Toxicity Identification of Industrial Effluents Using *Daphnia magna*

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Abstract In this study, acute toxicity monitoring and toxicity identification evaluation procedures were applied to identify causative toxicants in industrial effluents. Effluents from a metal plating factory and a rubber products factory were acutely toxic toward *Daphnia magna* and the toxicity varied over different sampling events (2.9–5.9 and 1.7–7.6 TU, respectively). For the rubber products effluent, it was confirmed that zinc (5.65–13.18 mg L^{-1}) was found to be a major cause of toxicity, which is likely originated from zinc 2-mercaptobenzothiazole and zinc diethyldithiocarbamate used as vulcanization accelerators. For the metal plating effluent, it appeared that the presence of high concentrations of Cl^- and SO_4^{2-} (8,539–11,400 and 3,588–4,850 mg L^{-1}, respectively) caused the observed toxicity. These toxicants likely originated from sodium bisulfate ($NaHSO_3$) and sodium hypochlorite (NaOCl) used as reducing and oxidizing agents. Though copper was found to be present in levels much higher than the EC_{50} (50% effective concentration) value, this was not attributable to the toxicity of metal plating effluent likely due to complexation with dissolved organic matter.

Keywords Acute toxicity · *Daphnia magna* · Metal plating factory · Rubber products factory

Discharging industrial effluent can have adverse effects on receiving water bodies, even if concentrations of toxic chemicals in the effluents pass water quality criteria (Kim et al. 2008). In addition, it is difficult to assess ecological effects of toxic chemicals in wastewater based on physicochemical analysis alone (Wolska et al. 2007). Accordingly, in 2007, the Korean Ministry of Environment (MOE) announced that a new standard protocol and legislation using *Daphnia magna* acute toxicity tests to regulate wastewater effluent would be gradually implemented from 2011 (Korean MOE 2007).

Wastewater effluents often contain a large number of chemicals, however not all of these compounds are responsible for the observed toxicity. Thus, this brings a necessity for using toxicity identification evaluation (TIE) methods to characterize and identify toxicity-causing substances and ultimately to reduce effluent toxicity. The TIE has been developed by the USEPA (1991, 1993a, b) and widely used to identify and reduce major toxicants in industrial effluents (Jo et al. 2008; Yi et al. 2009; Yim et al. 2006). However, there is little information on the causes of toxicity of industrial effluents in Korea. Moreover, it has been debated whether salinity should be considered as a toxicant under all situations when applying the new discharge regulations based on *D. magna* acute toxicity tests. Thus, the objectives of this study were (1) to examine the levels of toxicity in different types of industrial wastewater effluents; and (2) to identify major toxic substances in wastewater effluents using TIE procedures.

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Materials and Methods

Five sampling events were conducted at a metal plating factory located in Banwol Industrial Complex from March 2007 to September 2008. The factory treats about 550 m^3/d of wastewater using reduction, neutralization, coagulation/flocculation, oxidation, flotation, filtration, anoxic/oxic processes. In addition, three grab samples of effluent from a rubber products factory located in Shihwa Industrial Complex were collected from October 2007 to January 2008. The factory treats about 23,000 m^3/d of wastewater using screening, neutralization, coagulation/flocculation, flotation and activated sludge processes. The samples were transported in a polyethylene container and immediately stored at 4°C. Initial toxicity tests and water quality analysis were conducted upon the arrival of the samples.

Samples were filtered through a 0.45 μm syringe filter and then analyzed for anions and dissolved organic carbon (DOC) using ion chromatography (IC, Dionex-500, CA, USA) and a Shimadzu TOC analyzer (model 5000A, Kyoto, Japan), respectively. In addition, metals were analyzed using a Varian inductively coupled plasma–optical emission spectrophotometer (ICP-OES, Varian Vista PRO, CA, USA). For metal analysis, all vessels and experimental apparatuses were rigorously acid-washed before use. Calibration curves were established daily using freshly prepared standard solutions and the r^2 values of all curves were greater than 0.995.

Acute toxicity tests were conducted according to the Organization for Economic Co-operation and Development (OECD) standard procedure (OECD 2004). Each toxicity test consisted of five dilutions and one control with four replicates per treatment and each test vessel contained 10 mL of test solution and five individuals (neonates less than 24 h). Toxicity tests were conducted at 20 ± 2°C with a 16 h light:8 h dark photoperiod for 48 h. Immobilization data were employed to calculate the EC_{50} (50% effective concentration) values using probit analysis, the Trimmed Spearman–Karber method or a graphical method (USEPA 2002). EC_{50} values were transformed into toxic units ($TU = 100/EC_{50}$) in the interest of comparison.

Toxicity identification evaluation was conducted according to the TIE procedures developed by the USEPA with some modifications. In the TIE phase I test (USEPA 1991), baseline test, pH adjustment, pH adjustment/aeration, pH adjustment/filtration, pH adjustment/C_{18} or Carb solid phase extraction (SPE), graduated pH, ethylenediaminetetraacetic acid (EDTA) addition and sodium thiosulfate (STS) addition were included to characterize classes of toxicants. Ion exchange manipulations were conducted to further characterize the toxicants according to method described by Jo et al. (2008). Except for the graduated pH tests, the pH of the samples following each manipulation was readjusted to the initial pH with NaOH and HCl before the toxicity test.

In the TIE phase II test (USEPA 1993a), metals suspected as key toxic materials were measured using the Varian ICP-OES. For metal analyses, aliquots were saved and preserved with concentrated nitric acid. Based on the results of identification, suspect toxicants were confirmed by mass balance and spiking approaches of the TIE phase III test (USEPA 1993b). In the mass balance approach, the same concentration of a suspect toxicant that was found in the filtered sample was added to samples after manipulations, which showed a toxicity reduction (e.g., ion exchange or pH adjustment to 11). Toxicity tests were then conducted before and after the addition of the suspect toxicant. For the spiking approach, the same concentration of the suspect toxicant was added to the filtered sample. Next, toxicity tests were conducted to determine if the toxicity increased linearly with the addition of the suspect toxicant.

Analyses employing mock effluent (ME) were conducted to determine if the toxicity of the effluent was primarily due to salinity (USEPA 1996). ME was produced to match the major ion concentrations of the effluent by adding reagent-grade salts to deionized water. Toxicity tests were then conducted by mixing the effluent and ME with different ratios.

Results and Discussion

The results of the initial toxicity test and chemical analyses of effluent of rubber products factory are shown in Table 1. The initial TU of the JAN effluent was much higher than that of the OCT and NOV effluent. The anion concentrations were all below the levels that were toxic to *D. magna*. However, among the toxic metals, the Zn concentrations far exceeded its EC_{50} concentration to *D. magna* and appeared to be related to the initial toxicity. The toxicity characterization (TIE phase I) results of the JAN effluent of

Table 1 Chemical characteristics and toxicity of effluents from a rubber products factory

	OCT 2007	NOV 2007	JAN 2008	EC_{50} (mg L^{-1})
TU	1.7	1.9	7.6	–[a]
pH	5.8	5.7	5.6	–
DOC (mg L^{-1})	–	8.7	17.7	–
Zn (mg L^{-1})	7.25	5.65	13.18	1.54
Cl^- (mg L^{-1})	529	790	912	3,140
SO_4^{2-} (mg L^{-1})	919	963	1,102	3,381

[a] Not determined

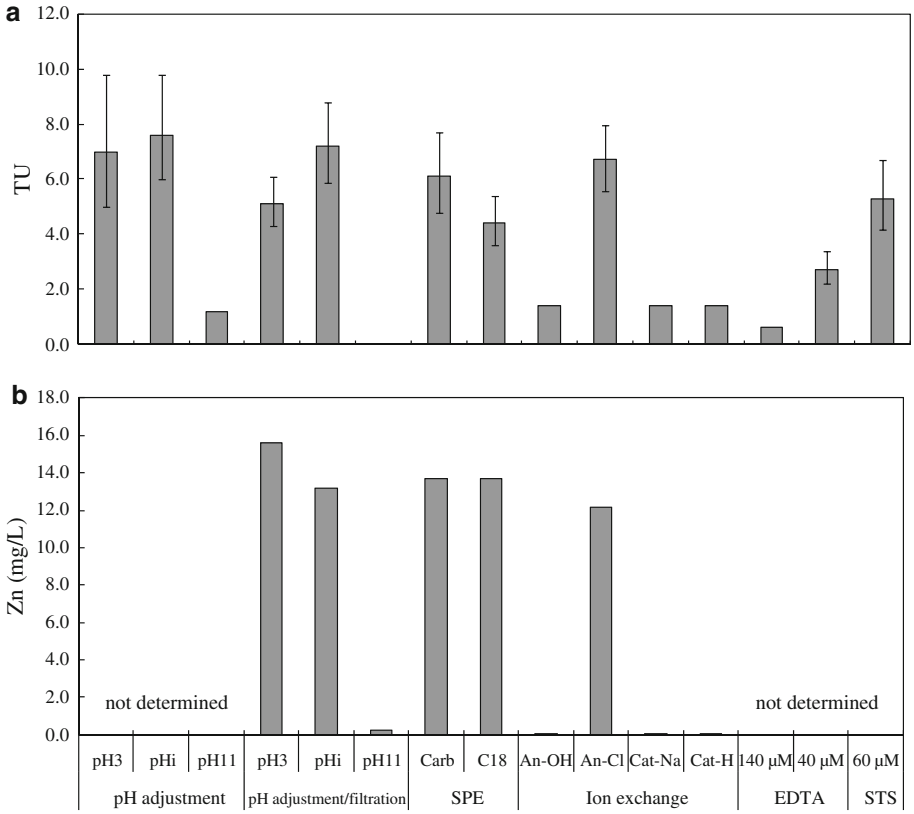

Fig. 1 Toxicity identification of JAN effluent from a rubber products factory. (**a**) TIE phase I results, and (**b**) Zn concentrations. *Error bars* represent the 95% confidence level. Zn concentrations were not measured after manipulations of pH adjustment, EDTA addition and STS addition. The TU value of pH_i (initial pH) is baseline toxicity

the rubber products factory are illustrated in Fig. 1a. The acute toxicity was completely removed by adjustment of the pH to 11 followed by filtration. Additionally, several manipulations such as adjustment of the pH to 11, cation exchange using sodium and hydrogen resins (Cat-Na and Cat-H, respectively) and addition of EDTA drastically reduced the acute toxicity.

Anion exchange using a hydroxide resin (An-OH) effectively reduced the toxicity, while there was only a slight change observed when the chloride resin (An-Cl) was used. This was likely due to precipitation in response to the drastic pH change that occurred following the An-OH manipulation. The above results suggest that the toxicants were likely cationic metals that could be chelated by EDTA and form precipitates at higher pH. Thus, the metal concentrations were analyzed to identify the possible toxicants. As shown in Fig. 1b, Zn was reduced to a very low concentration after adjustment of the pH to 11 followed by filtration and cation exchange manipulation, which drastically reduced the toxicity. Given the above results, Zn was likely a toxicant in the effluent of the rubber products factory.

Spiking and mass balance studies (TIE phase III) were conducted to determine if Zn was a key toxicant in the effluent (Fig. 2). For the spiking test, Zn was added to the filtered effluent at a concentration of 13.18 mg L^{-1}, which resulted in the toxicity approximately doubling (16.6 TU) when compared to that of the filtered sample (7.5 TU). For the mass balance, the same concentration of Zn was added to the samples after cation exchange (H and Na forms), anion exchange (OH form) and pH 11/filtration manipulations, which resulted in the toxicity of 8.8, 8.0, 8.1 and 7.2 TU, respectively. These findings indicate that the toxicity was recovered to the baseline toxicity (7.5 TU) by the concentration of Zn added. As a result, the overall TIE results showed that Zn was a key toxicant in the effluent of the rubber products factory. However, organic compounds

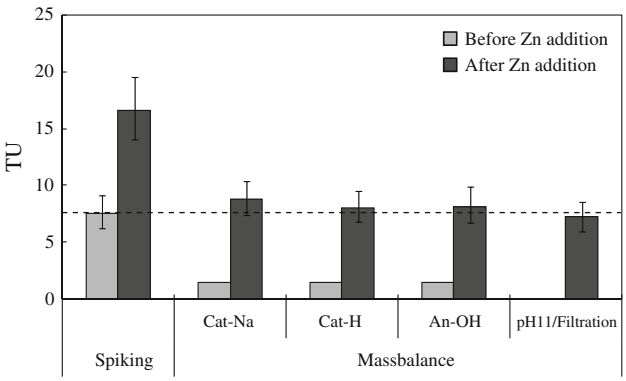

Fig. 2 Spiking and mass balance tests of JAN effluent from a rubber products factory. *Error bars* represent the 95% confidence level. The levels of Zn (13.18 mg L^{-1}) found in filtered effluent was added. The *dotted line* is the baseline toxicity of filtered effluent

Table 2 Chemical characteristics and toxicity of effluents from a metal plating factory

	MAR 2007	MAY 2007	JUN 2007	MAR 2008	SEP 2008	ME[a]	EC_{50} (mg L^{-1})
TU	2.9	3.7	5.5	5.9	3.6	–[b]	–
pH	7.34	7.70	7.30	7.39	7.42	–	–
DOC (mg L^{-1})	163	56	43	60.9	65.8	–	–
Cu (mg L^{-1})	0.098	0.410	0.482	0.581	0.215	–	0.010
Cl^- (mg L^{-1})	–	8,539	10,973	11,400	10,978	10,640	3,140
SO_4^{2-} (mg L^{-1})	–	3,641	4,745	4,850	3,588	3,600	3,381

[a] Mock effluent (ME) was produced by adding $CaCl_2$ (0.0275 M), Na_2SO_4 (0.0375 M) and NaCl (0.245 M)

[b] Not determined

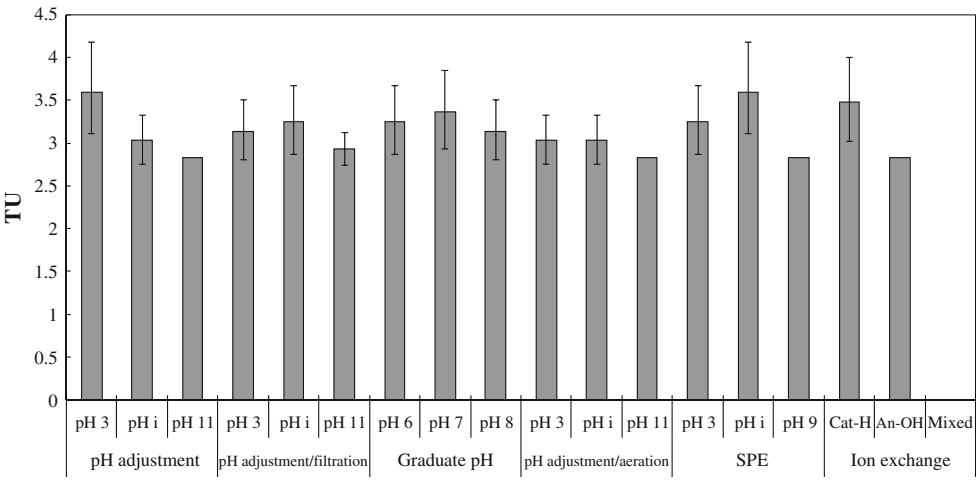

Fig. 3 TIE phase I results of SEP effluent from a metal plating factory. *Error bars* represent the 95% confidence level. The TU value of pH_i (initial pH) is baseline toxicity

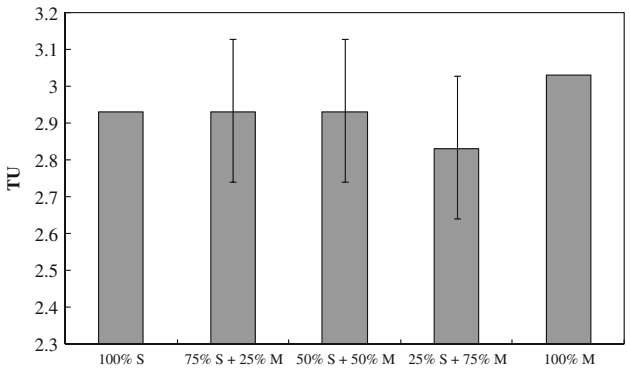

Fig. 4 Mock test results of SEP effluent (S) with increasing ratios of mock effluent (M). *Error bars* represent the 95% confidence level

likely contributed to the observed toxicity to some degree because toxicity was partially reduced after C_{18} SPE manipulation (Fig. 1a). These toxicants likely originated from zinc 2-mercaptobenzothiazole (ZMBT) and zinc diethyldithiocarbamate (ZDEC) used as vulcanization accelerators in the factory (Park et al. 2008) and this should be further investigated.

The results of an initial toxicity test and chemical analyses of effluent of metal plating factory are shown in Table 2. The toxicity of the effluent varied over different sampling events. As demonstrated by Kim et al. (2008), a single sampling occasion to regulate toxicity of wastewater effluents may not be sufficient due to toxicity variability. In addition, the concentrations of Cu, Cl^- and SO_4^{2-} exceeded their EC_{50} concentrations to *D. magna* and appeared to be toxicity causing chemicals.

The toxicity characterization (TIE phase I) results for the SEP 2008 effluent are shown in Fig. 3. The toxicity of the SEP was completely reduced after mixed-bed ion exchange (both Cat-H and An-OH), suggesting the toxic effects of total dissolved solid (TDS) (USEPA 1991). Though the Cu concentrations were found to be much higher than its EC_{50} value, there was no change in toxicity after cation exchange, pH adjustment followed by filtration or EDTA addition. Many studies have demonstrated that the toxicity of Cu was dependent on water quality parameters such as pH, organic components, hardness and some other major ions (Pernet-coudrier et al. 2008; Park et al. 2009; De Schamphelaere and Janssen 2004). In this

effluent, complexation of Cu with DOC and competition between Cu and major ions appeared to eliminate the toxic effects of Cu on *D. magna*. These findings indicate that Cu was not likely a toxicant in the effluent of the metal plating factory.

Additionally, mock tests were performed using solutions with increasing ratios of ME to SEP effluent to confirm the toxic effect of salinity (Table 2). As shown in Fig. 4, the acute toxicity remained constant over all ratios, indicating that salinity is primarily responsible for the toxicity. Martinez-Jeronimo and Martinez-Jeronimo (2007) also demonstrated that salinity conditions reduced significantly reproduction and survival in *D. magna*. As shown in Table 2, all effluents from the metal plating factory showed that the concentrations of Cl^- and SO_4^{2-} were above the EC_{50} values and that the sum of their concentrations was strongly correlated with the initial toxicity ($r^2 = 0.816$). Given the above results, Cl^- and SO_4^{2-} were major toxicants in the effluent of the metal plating factory. These toxicants likely originated from sodium bisulfate ($NaHSO_3$) and sodium hypochlorite (NaOCl) used as reducing and oxidizing agents, respectively, in the wastewater treatment plant of the factory.

In conclusion, TIE procedures successfully identified Zn and salinity as key toxicants in the effluent of a rubber products factory and a metal plating factory, respectively, which is useful information to help industries comply with the new bioassay based regulations. Considering a complexity of industrial wastewater, more toxicity identification evaluations for a larger variety of industries should be conducted to characterize the pattern of toxicity causing chemicals. Additionally, the effluent toxicity was inconsistent over different sampling events, suggesting that long-term toxicity monitoring studies are needed. It has been debated whether salinity should be considered as a toxicant under all situations (Goodfellow et al. 2000). This might depend on the types of receiving water bodies (e.g. surface water or seawater) because marine species are believed to be more tolerant of salinity than freshwater species. Thus, when applying the new discharge regulations based on *D. magna* acute toxicity tests, one should consider methods of regulating industries that discharge their effluents containing immoderate concentrations of salinity.

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