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Comparative Whole Effluent Toxicity Assessment of Wastewater Treatment Plant Effluents using *Daphnia magna*

Jin Sung Ra · Byoung Cheun Lee · Nam Ik Chang · Sang Don Kim

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Abstract An approach to compare the toxicities employing the whole effluent toxicity (WET) test, using *Daphnia magna* and chemical analysis with GC/MS and ICP/MS, was conducted to the nine South Korean wastewater treatment plants (WWTPs). From the chemical analysis and bioassay experiments, heavy metals (i.e., Cu and Zn) were found to be the major compounds causing toxic effects toward *D. magna*. In the whole effluent toxicity (WET) tests using *D. magna*, toxicities were observed in 34% of the effluent samples. However, the biological toxic unit (TU) value showed a non-toxic response (i.e., 0 TU) in many samples despite the response indicated by the chemical TU values. This may be due to the species sensitivity, environmental parameters, mixture effects, and limitation of the chemical analyses.

Keywords Whole effluent toxicity (WET) · *Daphnia magna* · Chemical analysis

The biologically based toxicity test, also known as the whole effluent toxicity (WET) test, has become a powerful tool in the monitoring of effluents (US EPA 1991). Nevertheless, the chemical monitoring method that assesses the individual chemical concentration also plays an important role in the identification of toxicants as well as the regulation of their discharge limits. Traditionally, the quality of wastewater treatment plant (WWTPs) effluents discharged into streams is mostly regulated by the concentration of their individual toxicities. These chemical-based toxicity assessments enable scientists to link individual toxicities to the damage caused to the environment. This approach is dependent on the available information of the chemicals present in effluents and their potential toxicities (Sarakinos et al. 2000). However, there are two major limitations to this approach. First, the chemical-based methods are unable to detect all chemicals when present at low concentrations, as there are potentially hundreds or thousands of chemicals in real aquatic environments. Second, this method has trouble in predicting the toxicity of combinations of chemicals in effluents. Due to these limitations, biological WET testing methods have been introduced as alternatives. WET testing is an integrative tool that measures the toxicity of effluents and accounts for the uncharacterized sources of toxicity as well as their toxic interactions, but cannot explain the origin or identity of chemicals affecting toxicity (Aguayo et al. 2004). Therefore, these chemical identification and biological toxicity test methods should be used together for regulatory purposes. Many studies have recently been conducted on the toxicity of mixtures of toxicants from sewage effluents (Ra et al. 2006; Sarakinos et al. 2000), which showed increased or decreased toxic effects on aquatic organisms according to the chemical characteristics when in combination. The aim of this study was to compare the observed biological toxicity in various WWTP effluents toward *D. magna* with that calculated based on chemical analyses using GC/MS and ICP/MS and; thereby, evaluate the application of the whole effluent toxicity (WET) test to the monitoring of WWTP effluents.

J. S. Ra · B. C. Lee · S. D. Kim (✉)
Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology, 1 Oryong-dong, Buk-gu, Gwangju 500-712, South Korea
e-mail: sdkim@gist.ac.kr

N. I. Chang
Youngsan-river Environmental Research Laboratory, 1110-8 Oryong-dong, Buk-gu, Gwangju 500-480, South Korea

Materials and Methods

Effluent samples were collected from nine WWTPs, including five domestic and four agro-industrial wastewaters, located in the Youngsan River basins (total length, 115 km; watershed area, 2.8×10^3 km^2) in the South Jeolla province, Korea. Effluent samples were collected for about 3 years, between October 2002 and May 2005. The general characteristics of the WWTPs are given in Table 1. The samples were collected as subsurface grabs with 2 L amber glass bottles, which were filled as to allow no space. Liquid–liquid extraction was adopted for the analysis of organic chemicals in the nine WWTP effluents. One liter of effluent sample was shaken for 10 min after the addition of 50 mL of dichloromethane and 15 g of NaCl and extracted to 1 mL for gas chromatography/mass spectrometry (GC/MS, Varian 3400 CX-Saturn 2000, CA, USA) analysis. Metal samples collected from the treatment plants were analyzed using inductively coupled plasma mass spectrometry (ICP/MS, Perkin Elmer-Elan 6000, MA, USA).

Daphnia magna were cultured and handled according to the procedures outlined in the US EPA manual (1993). Organisms were fed every other day with a suspension of yeast, CEROPHYLL® and trout chow (YCT) supplemented with *Selenastrum capricornutum*. The synthetic freshwater had a pH of 8.0 ± 0.2, hardness of 170 ± 10 mg/L and alkalinity of 110 ± 10 mg/L, as $CaCO_3$. Reference toxicities of pollutants were acquired from bioassay experiments, performed in accordance with the US EPA test guideline for *D. magna* (US EPA 1993), and the ECOTOX database of the US EPA (2006). The metal toxicity from the ECOTOX database was plotted as a function of the water hardness. A toxic unit (TU) approach was introduced to calculate the mixed toxicity of all toxicants in an effluent, which was compared to the overall effluent toxicity of the same effluents to derive a relationship. Forty eight-hour acute WET tests were conducted according to the US EPA guideline using *D. magna* (US EPA 1993). The toxic unit was calculated; $TU = 100/LC_{50}$. If the mortality in a 100% effluent concentration was between 10% and 49%, the TUs were derived as follows; $TU = 0.02 \times \%$ mortality. A toxic unit of zero was allocated to mortalities between 0% and 10% in 100% effluent exposure (State of Michigan 2002).

Results and Discussion

The compounds analyzed by GC/MS and ICP/MS in the nine-wastewater treatment plant effluents are summarized by frequency in Table 2. The toxic compounds are categorized into four major chemical groups: phenols, phthalates, pesticides, and heavy metals. The most frequently detected toxicants were heavy metals (i.e., Cu, Zn, and Cr). Of organic compounds, phthalates were frequently detected in sewage treatment plant effluents as a result of their use in PVCs and other resins, as well as plastics for building materials and home furnishings, food packaging, and insect repellents (Aguayo et al. 2004). Most pesticides were found during the summer farming season, as this is the period they are used and sprayed in Korea. The total chemical TU of the treatment plants at each sampling time was calculated based on the results of chemical analyses and bioassay experiments. The toxicant concentrations obtained from the GC/MS and ICP/MS analyses were divided by each reference toxicity (LC_{50}) to give individual TU values. Table 2 shows the cumulative TUs (summation of TU of target chemicals in 99 samples) and the detected frequency of compounds showing TU greater than 0.01. Notably, although pesticides do not show high detection frequencies, they contributed greatly to the cumulative TU due to their strong toxicity toward *D. magna*. For example, EPN (ethyl paranitrophenyl), an organophosphorous insecticide, was detected only once during the 11 sampling periods at the nine-wastewater treatment plant, but showed a high TU of 5.41. Copper and zinc also showed high cumulative TU values as well as frequent detection. According to these results, most of the toxicity of the WWTP effluents tested in this study was attributed to heavy metals and pesticides. Therefore, the selection of

Table 1 Characteristics of the nine South Korean wastewater treatment plants

WWTPs	Treatment	Flow (m^3/day)	Origin of waste water
A	Activated sludge	600,000	Factory/domestic waste
B	Activated sludge	120,000	Domestic waste
C	Activated sludge	22,500	Manure, domestic waste
D	RBC	11,000	Manure, domestic waste
E	RBC	11,000	Manure, domestic waste
F	Extended aeration	600	Fruit processing waste
G	Activated sludge	1,300	Dye, domestic waste
H	Activated sludge	1,200	Food/fruit processing waste
I	Activated sludge	750	Beverage/lens waste

Table 2 Detection frequency of toxic compounds with a toxic unit value >0.01 and their cumulative toxic unit values

Compounds	Frequency	Percentile	Compounds	Cumulative TU	Percentile
Cu	32	18.18	Cu	9.9	32.32
Zn	32	18.18	Dichlofenthion	5.53	18.05
Cr	31	17.61	EPN	5.41	17.66
Ag	22	12.50	Zn	3.79	12.37
Ni	13	7.39	Diazinon	1.67	5.45
Cd	9	5.11	Ag	1.41	4.60
Di-*n*-butyl phthalate	8	4.55	Fenobucarb	0.83	2.71
Fenobucarb	6	3.41	Isofenphos	0.6	1.96
Diazinon	4	2.27	Cr	0.42	1.37
Pb	4	2.27	Ni	0.27	0.88
p-Octylphenol	3	1.70	Cd	0.21	0.69
Dichlofenthion	3	1.70	Di-*n*-butyl phthalate	0.2	0.65
Isofenphos	2	1.14	Pb	0.13	0.42
bis-Phthalate	2	1.14	Al	0.12	0.39
Al	2	1.14	*p*-Octylphenol	0.09	0.29
EPN	1	0.57	bis-Phthalate	0.02	0.07
Pendimethalin	1	0.57	Chlornitrofen	0.02	0.07
Chlornitrofen	1	0.57	Pendimethalin	0.01	0.03

chemicals for monitored in WWTP effluents should be based on their detection frequency and cumulative toxicity. Figure 1 shows that the levels of phenols ranged from 0.12 to 10.82 μg/L for 4-methyl-2,6-di-tert-butyl phenol and 2,3,4,6-tetrachlorophenol, respectively. The concentration of octylphenol, which shows high toxicity to *D. magna*, was lower than 0.87 μg/L in all effluents. The levels of phthalates showed similar values to those of the phenolic compounds, ranging from 0.12 to 13.2 μg/L for diethyl phthalate and diisobutyl phthalate, respectively. The concentration of phthalates was high at the agro-industrial wastewater treatment plants. Pesticides in the effluents were detected from 0.1 to 42.65 μg/L for probenazole and chloroneb, respectively. The measured concentrations of heavy metals varied widely between the treatment plants, indicating the mean concentration of 35.3 μg/L for Al, 0.11 μg/L for Cd, 11.1 μg/L for Cu, respectively.

The reference toxicity results were obtained from bioassay tests using *D. magna* and the US EPA ECOTOX database with regard to 92 chemicals via GC/MS and ICP/MS analyses. Due to the low detection level, but high LC50 concentration of phthalates, they would not be expected to reveal high toxic effects toward aquatic organisms. Of the organic compounds, pesticides presented the highest toxicity, with observed LC_{50} values of as low as several μg/L. Some pesticides, classified as herbicides or fungicides, did not cause high toxicity toward *D. magna*. For example, simazine, herbicide used for the control broad-leaved and grassy weeds on a variety of deep-rooted crops, has a toxicity of 10 mg/L. Other herbicides, such as thiobencarb and chloroneb, also exhibit low toxicities toward *D. magna*. Conversely, ethyl-*O*-(*p*-nitrophenyl) phenylphosphonothionate (EPN), an organophosphorous insecticide, showed the highest toxicity of the pesticides detected. Organophosphorous pesticides are known to inhibit type "B" esterase by binding to the active site and phosphorylating the enzyme (Hsieh et al. 2004; Barata et al. 2004). Due to their modes of action, the range of toxicities of the pesticides varied widely, from ng to mg/L. The difference in the toxicity patterns between phenols and phthalates was negligible, but the mean reference toxicity was slightly high for compounds with a phenolic group (Fig. 1). This is because their mode of action affecting toxicity is narcotic. The LC_{50} values of low

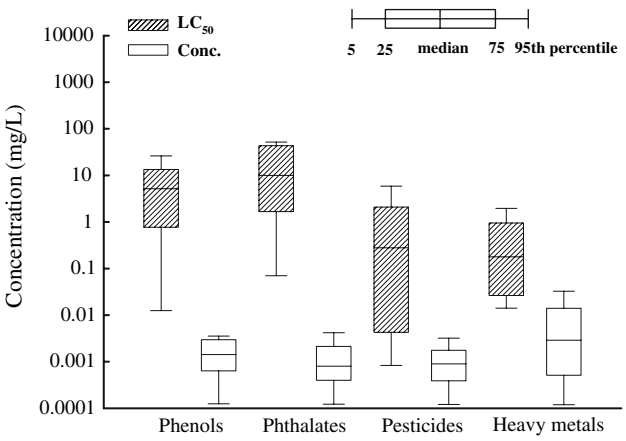

Fig. 1 The results of median lethal concentrations (LC_{50}) using *D. magna* and the measured concentrations of three chemical groups found in effluents. LC_{50} values of heavy metals were calculated based on the hardness of water

solubility chemicals (i.e., esprocarb and probenazole) are expressed with the highest exposure concentration, and were not included in the toxic unit calculation because their median lethal concentration (LC_{50}) was above their solubilities. Metal toxicities collected from the ECOTOX database (http://www.epa.gov/ecotox/) are expressed as a function of the water hardness in aquatic environments, as increased hardness will reduce the toxicity of metals to aquatic organisms due to competition between the hardness and the metals (Paquin et al. 2000). The toxicities of the heavy metals increased in the following order: Ag > Cd > Cu > Cr(VI) > Zn > Pb > Ni > Al, based on the hardness concentration of 100 mg/L as $CaCO_3$. The data used in this study were consistent with the results reported by Hsieh et al. (2004).

The results of WET tests are summarized in Table 3. The maximum violation ($TU_{acute} < 0.3$) frequencies were observed at treatment plants D and E. Seven of the 11 samples resulted in acute toxicity towards *D. magna*. The highest TU obtained with *D. magna* was 5.32 at treatment plant F, which treats agro-industrial wastewater. However, the other agro-industrial wastewater treatment plants, G, H and I, exhibited very low average TU, ranging from 0.027 to 0.190, which also showed very few violation frequencies. In terms of the mean discharge TU, five wastewater treatment plants violated the discharge limit. Treatment plant A, which has the largest discharging volume, exhibited a low TU value, 0.098. This plant sustains more steady flow rate than the other small discharging volume plants. Therefore, plant A can be achieved with stable treatment efficiency on toxicity. From the effluent classification scale in TU proposed by Persoone et al. (1993), two WWTPs (D and F) showed toxic characteristic (i.e., TU > 1) and seven were classified as slightly toxic (i.e., 0 < TU < 1.0). However, the results revealed a wide variation among the TU values. For example, treatment plant F, with a mean calculated TU of 1.65, showed a large change, from 0.31 to 8.40. Furthermore, the calculated and WET TU values did not match, showing a low regression coefficient ($r^2 = 0.023$) when all 99 TU values were plotted (Fig. 2). The extremely low correlation indicates a limitation in the application of the calculated TU values in WWTPs toxicity monitoring. This may be due to the low threshold value of *D. magna* to effluents. In many samples, the biological TU showed a non-toxic response (i.e., 0 TU), despite the chemical TU values indicating a response.

This markedly reduced the correlation between the plotted data. In addition, effluent toxicity obtained from the chemical analyses might encounter a number of limitations due to the complicated water characteristics. The aquatic parameters, such as dissolved organic matter (DOM), synthetic organic ligands and suspended solids (SS), primarily influenced the toxicities of metals, but also those of organic compounds (Plette et al. 1999; Ma et al. 2002). In this study, metals constituted a major portion of the toxicity, as shown in Table 2, with Cu and Zn accounting for more than 45% of the combined toxicity. Therefore, considering the change in the metal toxicity of waters is important when evaluating the toxicity of WWTP effluents. Moreover, the combined toxic effect of chemicals may affect the biological toxicity either antagonistically or synergistically. These complicated environmental factors in aquatic systems make the toxicity prediction of chemicals in effluents difficult. The weakness of chemical analyses for the detection of thousands of chemicals, potentially resulting in many unidentified chemicals, could be one of the reasons for the uncertainty in the measurement accuracy. To solve this problem, a simultaneous determination technique is necessary for the detection of

Table 3 Mean toxic units of the wastewater treatment plant effluents obtained from chemical analyses and WET tests

Treatment plant	Mean chemical TU[a]	Mean biological TU[b]
A	0.67 (0.35–1.63)	0.098 (0.00–0.60)
B	0.53 (0.29–1.03)	0.326 (0.00–2.27)
C	1.35 (0.37–4.34)	0.695 (0.00–3.33)
D	0.78 (0.15–2.00)	1.533 (0.00–2.95)
E	0.75 (0.16–2.11)	0.797 (0.00–2.67)
F	1.65 (0.31–8.40)	1.757 (0.00–5.32)
G	0.47 (0.18–1.34)	0.124 (0.00–1.36)
H	0.26 (0.06–0.99)	0.190 (0.00–1.39)
I	0.54 (0.09–2.97)	0.027 (0.00–0.30)

[a] TUs are calculated from the concentrations of individual compounds in effluents

[b] 100% of effluents are divided by the LC_{50} value from WET tests

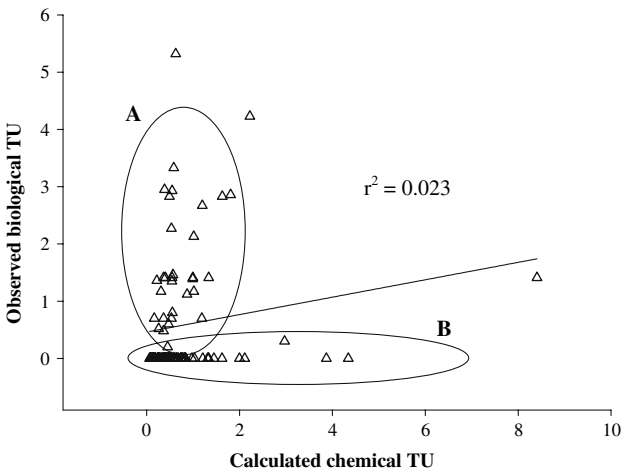

Fig. 2 The correlation between calculated and observed toxic units of the effluents (n = 99). Circles indicate the skewed results, mainly due to the presence of unidentified chemicals (**A**), and the insensitivity of *D. magna* to effluents (**B**), respectively. TU means toxic unit

major chemicals frequently present in WWTP effluents, which also show high toxicities. Kadokami et al. (1995) reported a technique for the simultaneous determination of 266 chemicals in water using GC/MS, an advanced chemical measurement, with more confined categories, should be suggested based on the frequency of detection and the degree of toxicity. Consequently, the biological method (i.e., WET testing), which measures the potential and real toxicity of mixed chemicals, should be used for monitoring the toxicity of WWTP effluents containing various hazardous chemicals.

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