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Application of toxicity identification evaluation procedure to toxic industrial effluent in South Korea

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HIGHLIGHTS

- TIE method was applied to pharmaceutical industrial effluent in South Korea.
- Toxicity of the effluent was greater than 16 toxic unit.
- Non-polar organic compounds were identified as the major contributor.
- Three compounds related to the pharmaceutical production were identified.
- Activated carbon adsorption was efficient to reduce toxicity of the effluent.

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ABSTRACT

Toxicity identification evaluation (TIE) was applied to the effluent from a pharmaceutical industrial complex, following the US EPA TIE guidelines. The whole effluent toxicity (WET) test found toxicity greater than 16 toxic units (TU) in the effluent. Dissolved non-polar organic compounds were identified as the major contributor to the observed toxicity in the TIE manipulations in phases I and II. Among the 48 organic compounds identified, three compounds (i.e., acetophenone, benzoimide, and benzothiazole) were related to the pharmaceutical production procedure; however, no contribution to toxicity was predicted in the compounds. The results of the ECOSAR model, which predicts toxicity, indicated that the alkane compounds caused significant toxicity in the effluent. The toxicity test and heavy metal analysis, which used IC and ICP/MS, identified that particulate and heavy metals, such as Cu and Zn, contributed to the remaining toxicity, except dissolved organics. The results showed the applicability of the TIE method for predicting regional effluents produced by the industrial pharmaceutical complex in this study. Although the location was assumed to be affected by discharge of pharmaceutical related compounds in the river, no correlations were observed in the study. Based on the results, advanced treatment processes, such as activated carbon adsorption, are recommended for the wastewater treatment process in this location.

1. Introduction

The whole effluent toxicity (WET) test was first developed by U.S. EPA to monitor and prevent the probable negative effects of effluent on the ecosystems of receiving streams. Subsequently, based on the U.S. EPA test guidelines, many other countries have applied WET to monitor wastewater. In 2011, the WET test method was included in South Korea's Water Quality and Ecosystem Conservation Act (WQECA). The adaptation of this law has several advantages for the monitoring of effluents in industrial wastewater, particularly the identification of unknown chemical mixtures (US EPA, 1991).

Because WET does not provide information about specific chemical species related to the toxicity of effluent, the toxicities identified by the WET method need to be further studied with the Toxicity identification evaluation (TIE) procedure for confirming major toxicant species. The TIE procedure was developed to characterize causative toxicants in effluent. The TIE method developed by US EPA includes the physicochemical manipulation of effluent and a biological toxicity test to separate and screen potential toxicant groups sequentially, as well as qualitative and quantitative chemical analyses for the toxicant candidate groups in phase

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I and II. The procedures used in TIE can be modified according to the characteristics of the effluent. The TIE method has diverse applications and is highly efficient in identifying the toxicants in effluents from different sources. Using ion exchange resin manipulation, Mount and Hockett (2000) found that hexavalent chromium resulted in acute toxicity in an industrial effluent. Using solid phase extraction manipulation, Hongxia et al. (2004) found that 2-propylbezaldehyde oxime caused acute toxicity of *Daphnia magna* in municipal wastewater.

As an emerging contaminant, pharmaceuticals have been spotlighted since the early 20th century. However, little attention has been paid to the application of the WET and TIE methods to investigate effluent produced by the pharmaceutical industry. In the present study, we applied WET and the TIE test to the effluent of a pharmaceutical industrial complex located in South Korea. Effluent toxicities were estimated in the process of biological screening with *D. magna* and toxicity characterization procedures, which was followed by conducting the TIE phase I. Compounds and ions in the effluent were identified using gas, liquid, and ion chromatography equipped with mass spectrometry (GC–MS, LC–MS, IC), induced coupled plasma atomic emission spectroscopy (ICP–OES), and ICP equipped with a mass spectrometry detector (ICP–MS), in TIE phases I and II.

2. Materials and methods

2.1. Sampling site description

Effluent samples were taken from a treatment plant located in an agro-industrial complex near the tributary of the Gum River in Chungnam Province, South Korea. The treatment plant was established in 1993. It has design capacity of 250 m^3/day and discharges 233 m^3/day of effluent after extended aeration activated sludge process (aerobic). Eleven plants operate in the agro-industrial complex, nine of which are the facilities of a pharmaceutical company that produces antibiotics and food additives. Eighty-seven percent of the wastewater discharged into the wastewater treatment plant (WWTP) originates from the nine pharmaceutical manufacturers. The remaining two industrial plants manufacture lithium batteries and damp-proof food wrapper. The lithium battery plant discharges approximately 30 m^3/day of wastewater. The damp-proof food wrapper plant rarely discharges wastewater because it uses little water during production. The target WWTP is not equipped with a chlorination tank for disinfection, but its operating condition complies with the requirements of the WQECA discharge permit.

2.2. Sample collection, treatment, and analysis

Effluent samples were collected five times using the grab sampling method in May, June, September, October, and November 2009. The first three samples were used for the bioassay (WET with *D. magna*) and the third and fourth samples were used in the TIE procedure. The last two samples (fourth and fifth samplings) were used for pollutant control experiment with activated carbon. The collections of the samples were carried out at the end of the outlet in the treatment system, which is directly connected to the secondary sedimentation tank. An amber glass bottle and an aseptic polyethylene bag (quality certified by the manufacturer) were separately utilized in collecting organic and ionic compound samples. The oxidation–reduction potential (ORP), total dissolved salt (TDS), pH, and conductivity were directly measured on the sampling site. The samples were iced during transportation and then stored in a refrigerator for subsequent use in the experiment. Basic water quality parameters, such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solid (SS), total nitrogen (T-N), total phosphorus (T-P), and hardness, were measured in the lab, according to the Standard Methods for the Examination of Water (Ministry of Environment of Korea, 2008). Ion concentrations were measured using IC and ICP–OES to quantify the amount of anion and cation in the samples. For comparison of water characteristics under operating conditions of the WWTP, samples of influent were collected and measured in the same manner as the samples of effluent were. For bioassay, iced samples were placed in the clean room to increase the sample temperature to 20 ± 1 °C.

2.3. Toxicity tests and statistics

In compliance with the WET test guidelines and phases I and II of the TIE guidelines, the toxicity of raw and manipulated effluent samples was investigated in a bioassay of *D. magna*. The WET and toxicity tests were conducted at the start of the TIE procedure and immediately after each TIE phase I manipulation (USEPA, 1991, 1993, 2002). WET tests were conducted within 36 h after sample arrival. In brief, third-brood neonates less than 24 h old were used as the test organisms in the toxicity tests. Room temperature was maintained at 20 ± 1 °C and 50% humidity under illumination conditions of 16 h light and 8 h dark. For quality control, reference toxicity tests were conducted every week using sodium dodecyl sulfate. If the results of the reference tests were beyond the accepted range (2 × standard deviation), the *Daphnia* adults used for the test were excluded from the toxicity test. Test solutions were prepared by the serial dilution of effluents with synthetic hard water at 100%, 50%, 25%, 12.5%, and 6.25% of effluent; 30 ml glass beakers containing 25 ml of the solutions were used, and five neonates were exposed to the solutions, including the control test set. Every test set was replicated four times, and the test results were expressed as LC_{50} values. The LC_{50} values were calculated using the trimmed Spearman-Karber, probit, and graphical methods, based on the mortality patterns observed. The probit and trimmed Spearman-Karber programs were downloaded from the US EPA web site. The resulting values were then converted to toxic units (TU = $100/LC_{50}$).

2.4. Toxicity identification evaluation

2.4.1. Phase I manipulation

Toxicity characterization was performed according to the US EPA TIE guidelines (USEPA, 1991, 1993). The phase I manipulations consisted of filtration, C18 solid phase extraction (SPE), sodium thiosulfate reduction, and EDTA chelation. The manipulations were conducted separately on the effluent samples. GF/C filtrations (Whatman, England) were used to screen the suspended particulates. SPE with C18 SPE cartridges (Sep-Pak® Vac 6 cc, Waters, Ireland) was conducted to remove non-polar organic compounds. Sodium thiosulfate manipulation was used to eliminate oxidative compounds (e.g., chlorine). EDTA was used to remove the heavy-metal ion effect by forming a chelate complex.

The methodologies used in each manipulation are as follows. Before the filtering procedure, GF/C filters were thoroughly rinsed with an analytical grade of methanol and nitric acid. The particulates were filtered from the effluent under low pressure by using a vacuum pump. After filtration, the suspended particulates stacked on the GF/C filter were harvested into *Daphnia* culture media by ultrasonication for one hour. In addition, the stacked particulates were extracted by treating with methylene chloride and nitric acid. The extracts were then analyzed by ICP/MS in TIE phase II. Organic pollutants were adsorbed on the C18 SPE cartridges (Sep-Pak® Vac 6 cc, Waters, Ireland), which were extracted sequentially using a serial elution. Two filtered samples were separately loaded to different C18 cartridges and eluted for extraction using

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