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Ecological risk assessment of a contaminated stream using multi-level integrated biomarker response in *Carassius auratus*☆

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ABSTRACT

The goal of this study was to evaluate the adverse effects of wastewater effluents on freshwater crucian carp, *Carassius auratus*, inhabiting Sincheon stream using the integrated biomarker response (IBR) at the genotoxic (micronucleus [MN] test), oxidative stress (activity of catalase [CAT] and glutathione *S*-transferase [GST], and level of lipid peroxidation [LPO]), histopathological (degree of tissue changes [DTC]), and physiological (condition factor [CF] and liver somatic index [LSI]) levels. The CF and LSI were significantly ($p < 0.05$) enhanced in fish from downstream sites (DS1 and DS2) as compared to that of upstream (US) fish samples. Moreover, a significant increase in morphometric indices (DTC) was observed in *C. auratus* collected from downstream sites ($p < 0.05$) and histopathological responses showed the degree of pathogenicity in the order of liver > kidney > gills. The activities of CAT, GST, and LPO in fish from the DS1 and DS2 sites were notably increased in gills, liver, and kidney compared to that of fish from the US site. Additionally, the MN test level in *C. auratus* from the DS1 and DS2 were significantly increased ($p < 0.05$) when compared with that of the US site. Considering the higher bioaccumulation of Cd, Co, Cr, Mn, Ni, and Pb in gills, liver, and kidney of *C. auratus* collected from downstream sites compared to that of the upstream site ($p < 0.05$), the observed toxicity was likely attributable to metal accumulation. The multi-level IBR index was higher at the DS1 site (15.08) than at the DS2 (1.02) and the reference US (0.00) sites. Therefore, these findings demonstrated that wastewater effluent discharge induces significant DNA damage, oxidative stress, and tissue injuries in *C. auratus* and suggested that the multi-level IBR approach should be used to quantify these effects on fish in streams and rivers.

1. Introduction

Wastewater effluents from industrial complexes represent an important point source of water pollution. Wastewater effluents consist of a highly complex mixture of chemicals, such as polycyclic aromatic hydrocarbons, solvents, heavy metals, pesticides, pharmaceuticals, volatile organic compounds, and flame retardants (Bolong et al., 2009). Therefore, environmental concerns arise regarding whether the residual concentrations of these compounds in bodies of freshwater are potential risks for humans and biota or they meet the prescribed water quality criteria for human consumption and other activities, such as the operation of fisheries, aquaculture, and tourism (Faria et al., 2010).

Although chemical analyses allow the qualitative and quantitative measurement of these compounds, quantifying all the pollutants in the effluent water is not feasible. Furthermore, chemical analyses alone are limited to assess the synergistic/antagonistic effects of mixtures of pollutants in real field conditions (Kerambrun et al., 2011). Therefore, an alternative monitoring technique involving biomarkers (physiological, biochemical, and cellular or molecular responses) has been widely accepted for assessment of environmental quality in freshwater systems (van der Oost et al., 1996). A number of studies have demonstrated the effects of wastewater effluents on the physiology (Diamond et al., 2016; Giraudo et al., 2016), immunity (Gagné et al., 2016), oxidative stress (Diamond et al., 2016; Gagné et al., 2016), histopathology, and infectious agents (Giraudo et al., 2016) of fish housed in or caught from contaminated water bodies.

No single biomarker adequately provides a complete diagnosis of the effects of effluent on organisms in field situations.

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Consequently, the use of a battery of biomarkers is a sound procedure among existing techniques to assess the overall adverse effects and to understand how an organism responds to the pollution load in the field (Kim and Jung, 2016). Thus, the integrated biomarker response (IBR) approach plays an important role in the evaluation of the effects of effluent mixtures (Beliaeff and Burgeot, 2002; Houde et al., 2014). The IBR index was also useful in laboratory studies for a number of toxicants (Kim et al., 2010, 2014), but the application of this technique in natural aquatic environments is still scarce.

This study was designed to evaluate the influence of effluent discharge, predominantly from sewage and wastewater treatment plants (STPs and WWTPs, respectively), in Sincheon stream. Our recent study demonstrated that textile dyeing effluent discharged into this stream was acutely toxic to *Daphnia magna* because of Zn contamination originating from the Fenton process in WWTP (Na et al., 2017). *Carassius auratus*, a representative of this stream with ecological relevance and economic importance, was considered the experimental model species. This species is commonly known as the crucian carp and is very common in Korean streams. Our previous study demonstrated that *C. auratus* provides a rapid early warning of pollutant exposure in field situations (Samanta et al., 2016). Although most IBR studies use molecular (mRNA expression) to biochemical biomarkers (antioxidant enzymes), the present study investigated the use of multi-level biomarker responses, including physiological, histopathological, oxidative stress, and genotoxic markers, to obtain a holistic and integrative overview of how these wastewater effluents affect fish health, and ultimately assessed their use for pollution monitoring in aquatic systems.

2. Materials and methods

2.1. Fish collection and physiological analyses

Fish sampling was conducted in August 2016 at an upstream reference site (US) and two downstream contaminated sites (DS1 and DS2) in Sincheon stream, which is located in Gyeonggi-do, Korea (Fig. 1). The stream receives wastewater effluents from the STP located in Dongducheon-si, Gyeonggi-do, Korea, which accommodated approximately 41 companies (the color processing industry was predominant) in addition to domestic sewage, and discharged approximately 79,200 m^3/day of effluent into the stream. Additionally, the stream also received approximately 19,000 m^3/day of industrial effluent from the WWTP located in Yeoncheon-gun, Gyeonggi-do, Korea from an industrial complex, which accommodated approximately 35 textile and dyeing companies. The US site (37°94′12.20″N, 127°06′07.45″E) was situated 530 m upward from the effluent discharge point of the STP. This site received little contamination and was considered the reference site. The DS1 site (37°95′92.63″N, 127°35′40.82″E) was 1 km downstream of the STP, and the DS2 site (38°00′81.47″N, 127°07′70.16″) was 800 m downward the WWTP. The distance between the two sites was approximately 7 km.

Fish (*C. auratus*) samples were collected from various habitats, including riffles, runs, and pools at sampling sites using a casting net (5 × 5-mm mesh, 15 times per site) and a skimming net (4 × 4-mm mesh, 40 min per site). After the collection of fish, 10 individual fish were selected that were >10 cm in total length from each sampling site. The fish were then anesthetized using 150 mg/L of tricaine methanesulfonate (MS-222; Sigma-Aldrich, St. Louis, MO, USA). After blotting the fish on filter paper, total body length and total weight were measured to calculate the condition factor [CF = whole fish weight (g) x 100/whole fish length $(cm)^3$] (Htun-Han, 1978) and liver somatic index [LSI = liver weight (g)/whole fish weight (g) × 100] (van der Oost et al., 2003).

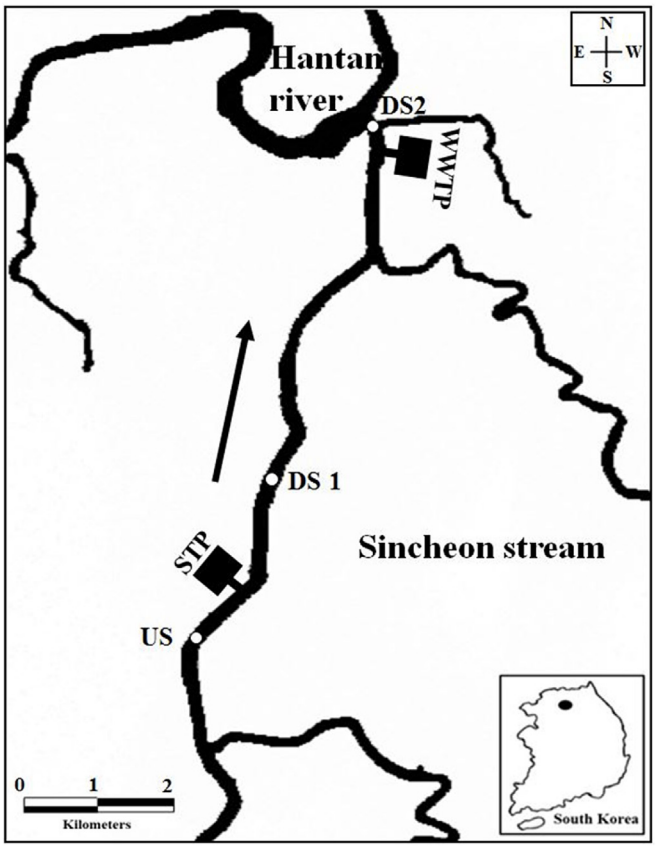

Fig. 1. Sampling sites of upstream (US) and two downstreams (DS1 and DS2) in the Sincheon stream and location of sewage and wastewater treatment plants (STP and WWTP).

For histopathological evaluation, the gills (5–15 gill filaments), liver (2–3 mm tissue), and kidney (3 mm tissue) were dissected, taking care to keep the tissue as intact as possible, and then fixed in 10% neutral formalin solution in the field. For oxidative stress analyses, tissues, namely the gills, liver, and kidney, were dissected, flash frozen with liquid nitrogen, and finally placed in an ice chest packed with dry ice at the site. These samples were then transferred to the laboratory within 2 h and stored at −80 °C until further analysis. All fish handling procedures followed the guidelines of the Institutional Animal Care and Use Committee of Korea University.

2.2. Water sampling and chemical analyses

Ambient water (US, DS1, and DS2) and effluent (EF1 and EF2 from STP and WWTP, respectively) samples were collected on February 2, April 25, and May 25, 2016, near the sites where fish sampling was conducted. The samples were transported in polyethylene containers on ice to the laboratory within 2 h, and then stored at 4 °C until further analysis. Dissolved oxygen (DO) concentration, water temperature, pH, and electrical conductivity (EC) of the samples were measured in the field using a multi-parameter water quality meter (YSI- 556, Yellow Springs Instruments, OH, USA).

Metal concentrations were determined in triplicate using an inductively coupled plasma-optical emission spectrophotometer (ICP-OES) (730 Series, Agilent Technologies, CA, USA). Water samples were treated with 10% HNO_3 (Merck, Germany) at 100:1 ratio,