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# Assessment of Aquatic Toxicity of River Keya Using *Daphnia magna*

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**Abstract** This study assessed the aquatic toxicity of the River Keya during its passage through the Hsin Chu City, Taiwan. The River Keya receives effluent from the Union Wastewater Treatment Plant of Hsin Chu Science Industrial Park (UWTP-HCSIP) primarily covering more than 500 semiconductors-related high-tech industries. Although the effluent from the UWTP-HCSIP has met the current environmental regulations of Taiwan, the aquatic environment of the River Keya was frequently subjected to unknown interferences. The aim of present study was to assess the acute toxicity of the River Keya using *Daphnia magna*. Six sampling sites were arranged where four were at downstream from the confluence of the effluent from the UWTP-HCSIP to the River Keya, and the other two were at the upstream to serve as references. *Daphnia magna* was employed to test the static 24-h acute toxicity, and the data obtained were further analyzed using the Probit and Spearman-Karber statistical models. Results showed a significant increase of the electric conductivity, $BOD_5$, and COD in those sampling sites downstream of the confluence point. The toxicity unit (TU) of the river water showed an increase-then-decrease fashion, and up to a 3-fold increase of TU was found downstream the UWTP-HCSIP effluent convergent to the River Keya. In comparison with the TU of the samples taken at the discharge point of the UWTP-HCSIP and the downstream of the convergence, the former is two times higher than the latter. These comparative results revealed that the aquatic toxicity effect is close related to the effluent from the UWTP-HCSIP, and a carry capacity of River Keya, especially for aquatic toxicity should be established.

**Keywords** River Keya, Science Industrial Park, *Daphnia magna*, Toxicity Unit

## 1. Introduction

The water quality of a river is not only determined by its natural environment, but also by the anthropogenic activities termed as point pollution sources (PPSs). The PPSs may include domestic and industrial types, and among them the centralized urban zone and high-tech industries can be the most important origins in Taiwan. In case of combination of these two origins, partially due to the mass water usages and wastewater discharge, the water environment nearby a metropolitan area will progressively become deteriorated [1-3].

The Hsin Chu Science Industrial Park, Taiwan, is located at the eastern side of Hsin Chu City, with 440,000 populations. More than 500 high-tech industrials, mainly involved in the integrated circuits, computer peripherals, telecommunication, optoelectronics, precision machinery, and biotechnology, have been established in the park at the end of December 2016[4]. The wastewaters from these high-tech industrials are firstly pipe-line collected into the Union Wastewater Treatment Plant of Hsin Chu Science Industrial Park (UWTP-HCSIP), then shield-tunneling effluent to the River Keya as receiving water. In 2016, the average daily effluent is counted at 116,000 CMD, while the average runoff of the River Keya is 215,000 CMD. It is obviously that the effluent from the UWTP-HCSIP is the main contributor (~54%) to the River Keya entering the Hsin Chu City. For years, although the effluent from the UWTP-HCSIP has met the required environmental regulations, the aquatic environment of the River Keya is frequently subjected to unknown interfering phenomena, like odorous emission, dead-fish, and floating cumulated bubbles on water surface.

For Taiwan's environmental regulations, the water quality has been mainly monitored on the physical and chemical parameters, so as the River Keya conducted in the past. However, those physical and chemical parameters are often impractical and inadequate to provide potential toxic information entering the environment, especially effluent from the high-tech industrials with unknown hazardous chemicals. It is reported for years that living organisms are capable of direct response to toxic chemical, and the use of bioassay has been an alternative and increasing important approach in monitoring the aqueous pollutants [5-7]. Therefore, the purpose of this study is to assess the aquatic toxicity using *Daphnia magna* in water samples collected in the River Keya, as it passes through the confluence of the HCSIP's UWTP effluent.

## 2. Materials and Methods

### 2.1. Study Area

The stretch of the River Keya studied is approximately 12 km in length and enters from the outlet of the Qin Cao Lake through the central part of the Hsin Chu City. Its catchment area is around 46 $km^2$. The effluent of the Union Wastewater Treatment Plant of HCSIP (UWTP-HCSIP) is shield-tunneling discharged into the River Keya, as schematically shown in Figure 1. This effluent delivers an amount of treated industrial wastewater into the River Keya represents approximately 54 % of the total amount of river water. Six sampling sites were arranged to evaluate the actual impact of the HCSIP effluent on the River Keya. Sampling sites at 1 and 2 are intended to provide background reference. They are situated 2.5 km and 1.0 km, respectively, upstream from its confluence point of the HCSIP effluent. Sites 4, 5, 6 are situated 2.5 km, 4.5 km, and 7.0 km, respectively, downstream of the convergence point, site 3. As shown in Figure 1, no other significant industrial discharge is presented between site 1 and 4, while dilution with rain water and water from small tributaries are expected.

### 2.2. Sampling

All lab wares for sample collection and storage were cleaned appropriately to minimize sample contamination. Samples at all sites were collected using two 1-liter plastic bottles from the center of the river except site 3. At site 3, the samples were drawn directly from the drop of the effluent entering the river. Samples were also collected at the discharge point of the UWTP-HCSIP before the effluent entering into the shield-tunnel. The collection bottles were flushed in order to facilitate rapid immersion. The samples were all kept in acid cleaned, sealed high density polyethylene bottles and stored in cooler for transport to the laboratory where they were analyzed right after arrival. The samples were taken at two different times of the year; the first sampling was made in May; the second was in August. All samples were collected between 10:00 and 17:00 on workdays.

### 2.3. Physical and Chemical Measurements

Water collected at all sampling sites and effluent samples from the UWTP-HCSIP were analyzed to determine the general physical and chemical parameters. Temperature, pH, dissolved oxygen (DO), and electric conductivity were measured with a portable multi-parameter probe (sensION156, Hach Instrument) as samples were collected at the sampling locations, and the measured values were referred to 20 $^{o}$C. Heavy metal ions were analyzed using inductively coupled plasma-atomic emission spectrophotometer (Perkin Elma, Optima 2100). The biochemical oxygen demand ($BOD_5$) was determined by the modified Winkler method, and the chemical oxygen demand (COD) by the dichromate method. They were all determined by following standard procedures and recommended analysis methods [8-9].

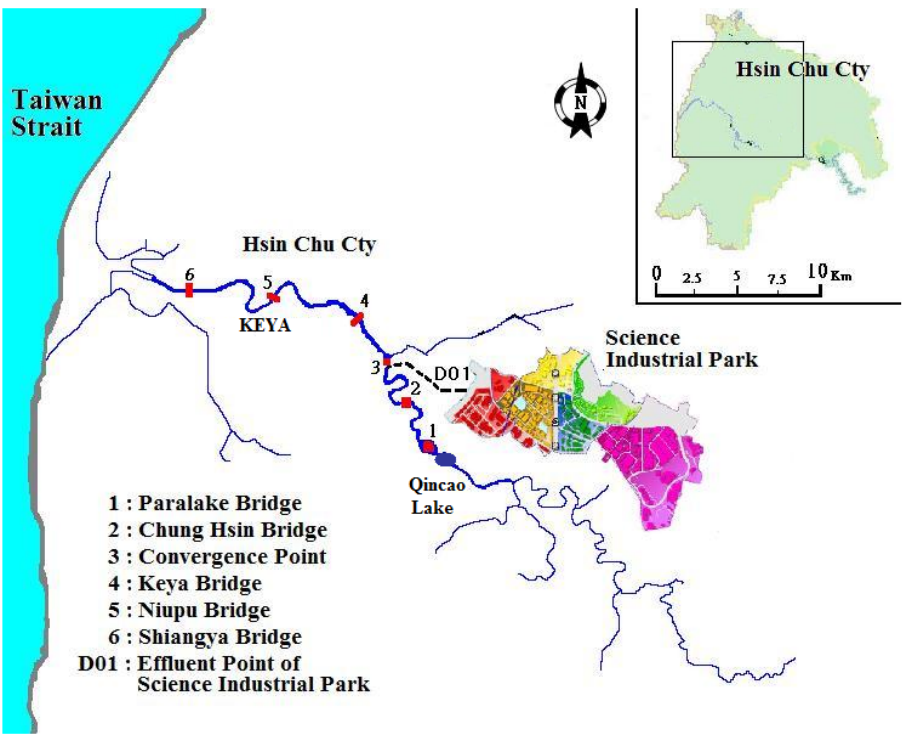


**Figure 1.** Location Map of Sampling Sites in the River Keya, Hsin Chu City, Taiwan

### 2.4. Bioassay

Static 24-h acute toxicity was conducted using *Daphnia magna*. The stock of *Daphnia magna* was originally obtained from the Freshwater Aquaculture Research Center, Chu Pei Station (Chu Pei City, Taiwan). Culture conditions were adapted from the Standard Procedure listed in the National Environmental Analysis Laboratory [9]. Culture media was synthetic fresh water (96.0 mg $NaHCO_3$, 60.0 mg $CaSO_4 \cdot 2H_2O$, 60.0 mg $MgSO_4$, 4.0 mg KCl per liter of double distilled water adjusted to pH=7.6 ± 0.2) with a hardness in the range of 80~100 mg/L as $CaCO_3$. Culture vessels were 3 L clear glass jars filled with approximately 2 liter of culture media. The neonates' daphnia (age < 24 h) were obtained from batch cultures maintained in laboratory aquarium at 25 ± 1 °C and a 16:8-h light: dark photoperiod with a 15-min transition cycle. Prior to the tests the adult daphnia were separated from the neonates produced that were used in this study. In each test 10 neonates daphnia were exposed to sampled water that was diluted using a 0.5 dilution series (100, 50, 25, 12.5, 6.25 % and control). Each test volume was set to 100 ml and was carried out in duplicate. The number of living and dead neonates was noted every 6-h after the initiation of each trial. Neonates daphnia observed as motionless and without a discernable heartbeat were considered to be dead. All $LC_{50}$ values and their 95 % confidence limits were calculated using the Probit and Spearman-Karber statistical models [8-9]. The Toxicity Unit (TU) is defined as the reciprocal of $LC_{50}$ multiplying by 100, or the reciprocal of the dilution ratio for $LC_{50}$, as shown in Eq.(1):

$$TU = \frac{1}{LC_{50}} \times 100 \quad (1)$$

# 3. Results and Discussions

### 3.1. Physical and Chemical Measurements

Physical and chemical measurements of the pH, temperature, DO, electric conductivity, COD, and $BOD_5$ at the sampling sites and the Taiwan's regulation are given in Table 1.As shown in Table 1, that pH, temperature, and DO all appear to be a relatively constant level over the all sampling periods and locations upstream or downstream. At site 3 and thereafter downstream from the confluence of the UWTP-HCSIP effluent, there were dramatically differences in COD and electric conductivity. The significant increase in COD levels indicates that effluent from the UWTP-HCSIP to the River Keya may have contributed a heavy loading of inorganic chemicals that can only be decomposed chemically. In addition, the electric conductivity was up to 6 times higher than those at reference sites. Metal ions concentrations of the sampling sites are shown in Table 2. It is clear that concentrations of W, $Cr^{+6}$, Pb, and Cu are higher after confluence of the UWTP-HCSIP effluent (site 3) than the upstream reference (sites 1 and 2), up to 66 times for Cu. It appeared that the water quality of the River Keya changed in composition after receiving effluent from the UWTP-HCSIP. The semiconductor-related industrials have been reported to apply a variety of inorganic metal chemicals that are highly soluble [10]. It can be deduced that a great amount of dissolved metal ions may have entered the River Keya that resulted in water quality with high electric conductivity. The study results revealed that the River Keya is evidently affected by the effluent of the UWTP-HCSIP.

**Table 1.** Physical and chemical parameters of sampling sites in the River Keya

| Sampling Site | Month | pH | Temperature (°C) | Dissolved Oxygen (mg/l) | Electric conductivity (μS/cm) | COD (mg/l) | $BOD_5$ (mg/l) |
|---|---|---|---|---|---|---|---|
| | | 6.0-9.0 | - | >4.5 | - | - | <4 |
| 1 | May | 6.78 | 26.5 | 4.38 | 377 | 18.7 | 6.8 |
| | August | 7.80 | 27.9 | 5.33 | 614 | 11.0 | 3.7 |
| 2 | May | 6.79 | 27.7 | 4.39 | 439 | 16.8 | 5.7 |
| | August | 7.26 | 28.5 | 4.38 | 620 | 9.8 | 1.8 |
| 3 | May | 7.05 | 26.7 | 3.92 | 2160 | 20.9 | 19.4 |
| | August | 7.28 | 27.9 | 4.12 | 1835 | 30.8 | 12.2 |
| 4 | May | 7.21 | 28.0 | 4.12 | 1487 | 21.1 | 8.9 |
| | August | 7.39 | 28.7 | 4.73 | 2670 | 20.8 | 3.6 |
| 5 | May | 7.26 | 28.8 | 4.45 | 1881 | 22.0 | 10.5 |
| | August | 7.51 | 29.4 | 4.01 | 2140 | 28.5 | 7.6 |
| 6 | May | 7.27 | 27.7 | 5.15 | 1303 | 23.3 | 7.8 |
| | August | 7.28 | 29.9 | 3.05 | 1908 | 19.1 | 7.2 |
| D01 | May | 6.96 | 26.6 | 4.51 | 2900 | 28.9 | 16.4 |
| | August | 6.97 | 27.3 | 4.73 | 3100 | 38.8 | 23.8 |

**Table 2.** Metal ions concentrations of sampling sites in the River Keya (μg/l)

| Sampling Site | Month | W | Cd | $Cr^{+6}$ | Pb | As | Cu |
|---|---|---|---|---|---|---|---|
| 1 | May | 0.89 | <DL | 0.06 | 1.70 | 0.06 | 0.50 |
| | August | 1.04 | <DL | 0.12 | 0.80 | 0.30 | 0.25 |
| 2 | May | 0.67 | 0.03 | 0.15 | 0.58 | 0.07 | 0.22 |
| | August | 0.24 | <DL | 0.23 | 0.10 | 0.04 | 0.01 |
| 3 | May | 5.24 | <DL | 6.93 | 6.93 | 0.08 | 6.80 |
| | August | 6.77 | 0.01 | 4.10 | 4.10 | 0.04 | 5.22 |
| 4 | May | 3.45 | <DL | 4.19 | 7.52 | 0.05 | 5.20 |
| | August | 6.14 | 0.03 | 2.86 | 2.58 | 0.09 | 4.75 |
| 5 | May | 4.87 | <DL | 5.42 | 5.09 | 0.02 | 5.24 |
| | August | 5.05 | 0.01 | 4.21 | 2.57 | 0.05 | 4.88 |
| 6 | May | 6.47 | <DL | 4.25 | 4.28 | 0.01 | 2.47 |
| | August | 4.45 | 0.03 | 3.57 | 3.54 | 0.09 | 3.18 |

DL: Detection Limit.

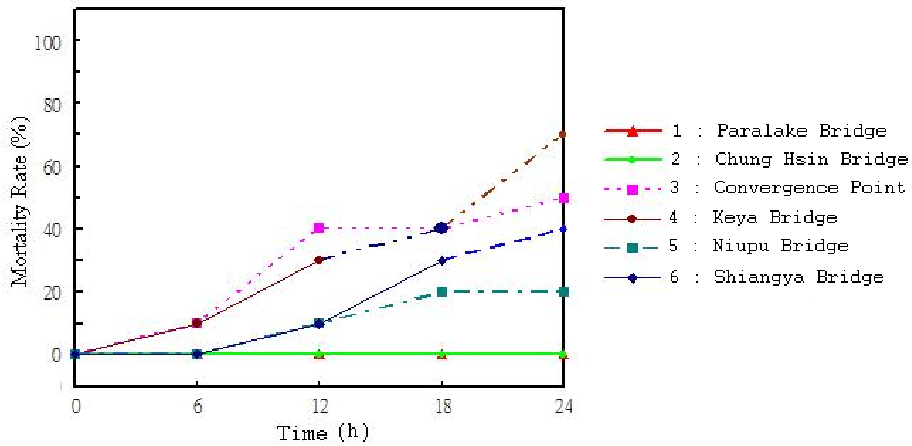


**Figure 2**. Response of *Daphnia magna* exposed to the six sampling sites

### 3.2. Acute Toxicity Tests

As a large quantity of industrial effluents entering a river, a toxicity monitoring is helpful to determine if it is with healthy water quality of a river. The mortality rate of the *Daphnia magna* within 24 hours in the collected water samples can be used to calculate the acute toxicity. From Figure 2, the *Daphnia magna* in the water samples from the sampling sites 1 and 2 of the upstream have no mortality rate. While the mortality rate becomes noticeable for *Daphnia magna* in the water samples after the convergence (site 3), and getting higher for that in samples from Keya Bridge (site 4), and lower for that samples collected at Niupu Bridge (site 5). This result indicates that as upstream of the River Keya (site 1 and site 2), the water has minor acute toxicity and still has carry capacity. However, as seen from Figure 3, the mortality rate is about 20 % higher for neonate daphnia in the water samples at the discharge point (D01) of the UWTP-HCSIP than that from the convergence (site 3). It is clear that this effluent of UWTP-HCSIP is quite a source for the aquatic toxicity.

Figure 4 shows the TU of each sampling point. The water from the upstream of the convergence showed insignificant toxicity on neonate daphnia, while that from the downstream showed a high TU value. It is highest in sample collected at the Keya Bridge (site 4), following a minor value at Niupu Bridge (site 5), and an increase of TU at Shiangya Bridge (site 6). Figure 5 shows the TU comparison of the samples taken at the discharge point (D01) of the UWTP-HCSIP and downstream of the convergence (site 3). The former is two times higher TU value than the latter. Based on this result, the source of toxicity may be partially attributed to the high concentration of metal ions chemicals originally from the high-tech industrials. It is suggested that an integral investigation be conducted downstream after the convergence of UWTP-HCSIP, including source of domestic sewage, industrial wastewater discharge points, and sediments along the River Keya.

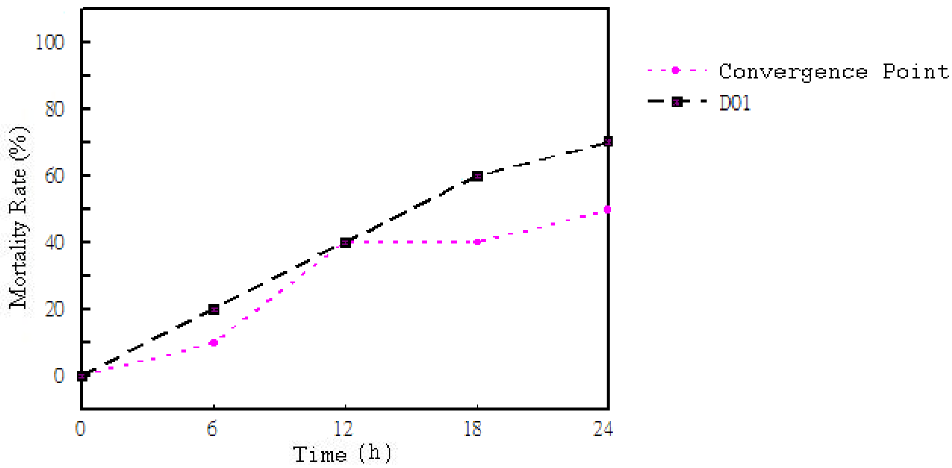


**Figure 3**. Comparative response of *Daphnia magna* at convergence point and effluent of UWTP-HCSIP (D01)

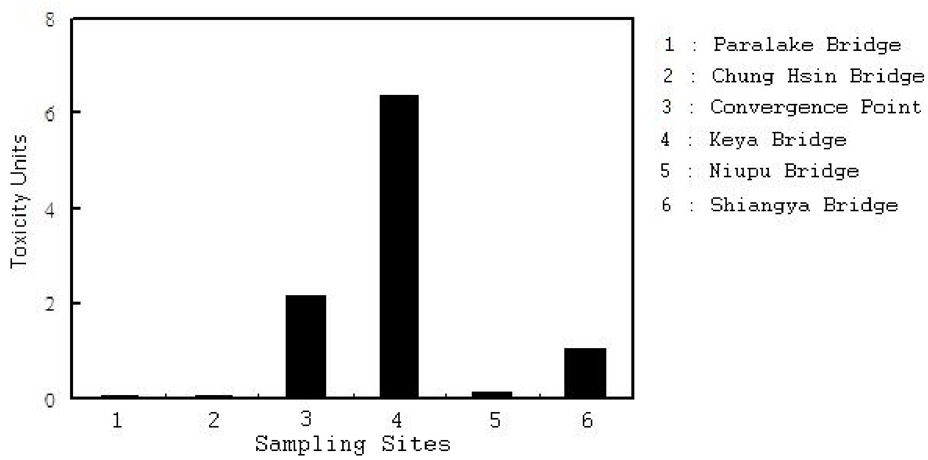


**Figure 4**. Toxicity units at six sampling sites

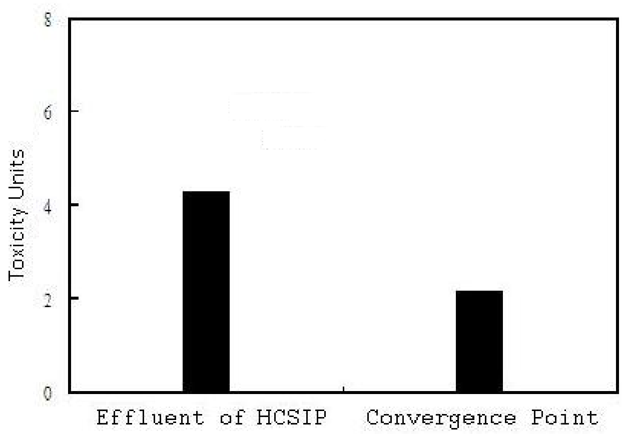


**Figure 5**. Comparatives of toxicity units at convergence point and effluent of UWTP-HCSIP (D01)

## 4. Conclusions

Aquatic toxicity is a composite index, and can be used to evaluate the water environment. This study collected water samples from the River Keya and conducted acute toxicity test on *Daphnia magna*. The results showed that $BOD_5$, electric conductivity, and COD have an increase at the convergence, indicating that the effluents from the UWTP-HCSIP have a significant effect on the water quality of River Keya. The downstream of the convergence showed obvious aquatic toxicity, and the toxicity unit (TU) at Keya Bridge was the highest. Comparing the TU of the samples taken at the discharge point (D01) of the Hsin Chu Science-based Park Sewage Treatment Plant and the downstream of the convergence, the former is two times higher than the latter. This indicates that an integral investigation be conducted downstream after the convergence of UWTP-HCSIP effluent. The aquatic toxicity monitoring and toxicity measurement on the effluents should be programmed. In addition, this study suggests that a carry capacity of River Keya, especially for aquatic toxicity should be established to well conserve the ecology and environment.

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