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Toxicity characteristics of sewage treatment effluents and potential contribution of micropollutant residuals

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Abstract

Background: A typical sewage treatment plant is designed for organic and nutrient removal from municipal sewage water and not targeted to eliminate micropollutants such as pesticides, pharmaceuticals, and nano-sized metals which become a big concern for sustainable human and ecological system and are mainly discharged from sewage treatment plant. Therefore, despite contaminant removal by wastewater treatment processes, there are still remaining environmental risks by untreated pollutants in STP (sewage treatment plant) effluents. This study performed aquatic toxicity tests of raw wastewater and treated effluents in two sewage treatment plants to evaluate toxicity reduction by wastewater treatment process and analyze concentration of contaminants to reveal potential toxic factors in STP effluents.

Methods: Water samples were collected from each treatment steps of two STPs, and acute and chronic toxicity tests were conducted following USEPA (United States Environmental Protection Agency) and OECD (Organization for Economic Cooperation and Development) guidelines. Endpoints were immobility for mortality and reproduction effect for estrogenicity.

Results: Acute EC_{50}s (median effective concentration) of influents for Seungki (SK) and Jungnang (JN) STPs are 54.13 ± 32.64% and 30.38 ± 24.96%, respectively, and reduced to 96.49 ± 7.84% and 100%. Acute toxicity reduction was clearly correlated with SS (suspended solids) concentration because of filter feeding characteristics of test organisms. Chronic toxicity tests revealed that lethal effect was reduced and low concentration of influents showed higher number of neonates. However, toxicity reduction was not related to nutrient removal. Fecundity effect positively increased in treated wastewater compared to that in raw wastewater, and no significant differences were observed compared to the control group in JN final effluent implying potential effects of estrogenic compounds in the STP effluents.

Conclusions: Conventional wastewater treatment process reduced some organics and nutritional compounds from wastewater, and it results in toxicity reduction in lethal effect and positive reproductive effect but not showing correlation. Unknown estrogenic compounds could be a reason causing the increase of brood size. This study suggests that pharmaceutical residues and nanoparticles in STP effluents are one of the major micropollutants and underline as one of estrogenic effect factors.

Keywords: Toxicity reduction, Wastewater treatment, Micropollutants, Nanoparticle, Pharmaceuticals

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Background

A typical sewage treatment plant (STP) is limited for organic and nutrient removal from municipal sewage water. Suspended solids (SS) are also one of the main targets to remove from wastewater. STP processes generally consist of primary settlement process for SS removal and biological treatment process for nutrient such as nitrogen and phosphorus removal. Biological treatment, which is called as the secondary treatment process, includes aerobic, anaerobic, and anoxic processes. Thus, it is expected that other micropollutants such as pharmaceuticals, personal health care products, and detergents are retained at the effluent stream which are subsequently released to the aquatic environment (Fent et al. 2006).

Environmental levels of pharmaceuticals varied from ppbs to ppts according to its media and discharge patterns (Vieno et al. 2005; Loraine and Pettigrove 2006) and their usage pattern (Balakrishna et al. 2017). Carbamazepine, an antidepressant drug, is one of the most detectable pharmaceuticals because of its persistency in the environment even though metformin showed the highest concentration of 8100 ng/L (Kot-Wasik et al. 2016). Pharmaceutical residues in the aquatic environment have been a big concern in terms of its consistent discharge from sewage but it is also considered nontoxic because of relatively low environmental levels and nonsevere acute toxicity resulting in a low risk to humans and aquatic animals.

Nanoparticles are also one of the major micropollutants which should be issued for human health and water environment. Nanomaterials are defined as less than 100 nm of their size and are well known as they show different physicochemical characteristics according to size. Drastically increased usage in the industry resulted in more detection in the environment. Health effects on human and the ecosystem have been studied but some toxicologists doubt its ecological effect because of its size distribution in the environment. Titanium oxides and zinc oxide are one of high-ranked compounds among industrial nanomaterials in terms of annual usage in Korea (Kim et al. 2014), and relevant detections have been reported as they are used for mainly consumer products which finally flows into water environment though STP effluent (Gottschalk et al. 2013). Like pharmaceuticals, ENM (engineered nanomaterials) were measured in the level of ppbs and ppts.

The main objective of this study is to investigate toxicity reduction by performing WET (whole effluent toxicity) tests on raw wastewater, treatment process effluents, and final effluents of sewage treatment plants. In addition, factors which could influence toxic effect in the effluent were studied by contaminant and nutrient removal analysis and by literature reviews.

Methods

Sample collection

Grab samples were collected in 2-L Mediland® disposable sampling bags once a week from SK STP (Seungki Sewage Treatment Plant) in Incheon and JN STP (Jungnang Water Reclamation Center) in Seoul, South Korea, at each wastewater treatment process stage (Fig. 1) from July 15 to November 24, 2009, and transported to the laboratory and stored at 4 °C (7 days at most). SK and JN STPs are one of the major STPs in Incheon and Seoul, which hire biological treatment process; one is MLE (Modified Ludzack-Ettinger) and the other A^2O (Aerobic-Anaerobic-Oxidation). The daily loads for SK and JN STPs and the daily volumetric capacities are 320,000 m^3/day and 460,000 m^3/day (This is the capacity for A^2O process line used in this study. The total capacity of JN STP is 1,590,000 m^3/day.), respectively.

Test organisms and culturing conditions

Daphnia magna cultures were held in 3-L beakers containing moderately hard water (80–100 mg/L as $CaCO_3$) prepared following the US EPA Guideline (USEPA 2002). Cultures were maintained in a growth chamber at 21 ± 1 °C and 16-h light: 8-h dark photoperiod was employed. The media were renewed every 2 days, and the daphnids were fed daily with 5 ml YCT (Yeast:Ceropyll®:Tetramin®) and 3 ml living *Chlorella* (1×10^7 cells/ml). Water qualities of the culture media were maintained as follows: pH, 7.4~7.8; hardness, 80~100 mg/L as $CaCO_3$; and alkalinity, 57~64 mg/L as $CaCO_3$.

Acute and chronic toxicity tests

Acute toxicity tests (48 h) were conducted using the grab samples with concentration range of 6.25, 12.5, 25, 50, and 100 vol% in accordance with the recommended procedure outlined in US EPA WET test (USEPA 2002). The immobility of test organisms was measured for endpoint. Standard reference tests were performed twice a month using sodium chloride as the test chemical to compare sensitivity of the test organisms over time.

Chronic toxicity tests (21 days) were conducted using the same range of concentration as mentioned under OECD TG (test guideline) 211 (OECD 2008). Ten neonates for each concentration were randomly selected and placed individually in 40-ml test solution of ten replicates. The test solutions were renewed every other day, and daphnids were fed with 150 μl YCT and 100 μl live *Chlorella* algae daily. The endpoints observed include molting, mortality, brood size, and number of brood and sex ratio of offspring. All experiments were carried out in the growth chamber at 21 ± 1 °C and a 16-h light:8-h dark photoperiod.

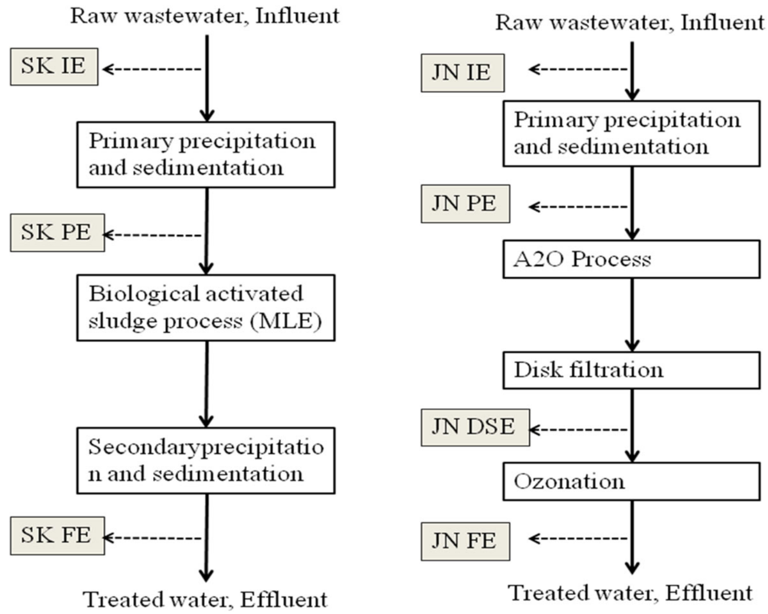

Fig. 1 Wastewater treatment processes and sampling processes points for Seungki (SK) and Jungnang (JN) STPs. IE, influent, PE, primary treatment effluent, DSE, disk filter effluent, FE, final effluent

Analytical methods and data analyses

Measurement of the water quality parameters were done as follows: suspended solids (SS)—gravimetric methods, ammonia (NH_4-N) and phosphate (PO_4-P)—Branne + Luebbe Automatic Analyzer 3 with digital colorimeter, and NO_3-N and NO_2-N—Ion chromatography (Dionex ICS-100).

In statistical analysis, median effective concentration(EC_{50}) for acute toxicity results and 25% inhibitory concentration(IC_{25}) for chronic reproductive results were calculated using probit analysis, Spearman-Karber and trimmed Spearman-Karber methods with ToxStat (Ver 3.5, West Inc., Cheyenne, WY, USA). Statistically significant differences in toxicity were determined using ANOVA test with SPSS (Ver. 10.0). Nominal concentrations were used throughout the course of the study.

Results and discussion

Characteristics of wastewater

The summary of water quality parameters and analysis results are presented in Table 1. Alkalinity and hardness levels for SK STP influent, primary treatment, and final effluents are higher than those of the moderately hard water (test media). However, the values of all the samples of JN STPs were within the range of the moderately hard water. Suspended solids (SS) contents of overall samples from SK STP were higher than those of JN STP except for influent samples. In JN STP, a significant reduction of suspended solids from biologically treated wastewater was the result of the secondary treatment employed using disk filtration. There are similarities in ammonia, nitrate, and nitrite levels in both STPs' raw wastewater,

Table 1 Water quality parameters obtained from the grab samples collected from Seungki and Jungnang STPs

Water quality parameter	Seungki Sewage Treatment Plant			Jungnang Water Reclamation Center			
	SK RW	SK PE	SK FE	JN RW	JN PE	JN DSE	JN FE
pH	8.1 ± 0.1	8.1 ± 0.2	7.9 ± 0.7	7.9 ± 0.3	7.9 ± 0.2	7.8 ± 0.2	7.8 ± 0.1
DO (mg/L)	6.1 ± 2.2	6.2 ± 2.6	8.2 ± 1.0	7.2 ± 1.0	7.5 ± 1.6	8.5 ± 0.1	8.7 ± 0.1
SS (mg/L)	68.3 ± 3.5	54.7 ± 6.4	29.3 ± 4.2	161.7 ± 26.2	41.3 ± 4.7	2.8 ± 0.4	1.9 ± 0.4
Alkalinity (mg/L $CaCO_3$)	154.1 ± 27.2	178.6 ± 70.4	99.4 ± 16.3	118.1 ± 1.8	126.4 ± 3.0	57.5 ± 9.4	59.1 ± 6.2
Hardness (mg/L $CaCO_3$)	201.7 ± 20.2	226.7 ± 25.2	170 ± 20	116.7 ± 28.9	126.7 ± 15.3	106.7 ± 5.8	105 ± 5
NH_4-N (mg/L)	26.0 ± 1.4	21.7 ± 0.2	9.2 ± 0.2	21.7 ± 0.3	21.0 ± 0.2	1.7 ± 0.0	1.2 ± 0.8
NO_3-N (mg/L)	16.1 ± 2.1	12.7 ± 1.1	8.6 ± 1.8	14.1 ± 1.0	13.8 ± 1.7	9.4 ± 1.4	6.4 ± 1.6
NO_2-N (mg/L)	2.5 ± 0.0	1.9 ± 0.8	1.8 ± 0.6	3.2 ± 0.4	2.7 ± 0.7	1.7 ± 0.1	1.1 ± 0.1
PO_4-P (mg/L)	3.9 ± 0.0	2.8 ± 0.1	0.9 ± 0.0	3.3 ± 0.0	1.9 ± 0.0	1.4 ± 0.1	0.60 ± 0.0

primary treatment effluents, and final effluents. Phosphate concentration of raw wastewater is approximately doubly higher in SK STP than in JN STP but reduced to a similar level in final effluents both.

Acute toxicity test results

Results of the 48-h acute toxicity tests using *Daphnia magna* for the raw wastewater, treatment process effluents, and final effluents collected from SK and JN STPs generally indicate that the effective concentrations (EC_{50}s) increase with respect to the sequence of treatment (Fig. 2). There were no significant differences ($p > 0.05$) between the raw wastewater samples and primary treatment effluents for both SK and JN STPs. However, EC_{50}s of the samples after the secondary treatment were significantly increased to > 100% indicating total acute lethal toxicity reduction of sewage wastewaters.

The data also revealed that the A^2O process employed in JN STP could reduce daphnia acute toxicity with consistent results as indicated by narrower range of EC_{50} values. EC_{50}s of raw water samples varied regarding sampling time and toxicity reduction by the process is also varied (Fig. 3). For SK STP, the overall toxicity of the effluents was low during the summer and high during the winter implying decreased removal efficiency at low temperature. Specifically, the EC_{50}s of the raw wastewater and primary effluent varied dramatically with the seasons. This may be also brought about by the amount of rainfall received by the sewage intake system during the summer season, since heavy rains occur during summer, but fewer in winter (Ra et al. 2008).

It is generally assumed that the dissolved fraction of a toxic substance in surface water is mainly responsible for the toxicity to aquatic organisms. However, toxic compounds are often adsorbed or chemically bound to suspended particles in the water column, depending on the physico-chemical conditions. *Daphnia magna* are filter-feeding organisms, and contaminated particles might end up in the gastrointestinal tract and exert toxic effects (Weltens et al. 2000). In this study, an average 19.6% suspended solids (SS) reduction was observed in SK PE while a more effective decline was measured in JN PE with 74.44% average SS reduction. Although a secondary precipitation and sedimentation tank was employed after biological treatment in SK STP, final effluent SS were still moderately high with an average of 29.33 mg/L. In contrast, disk filtration treatment employed in JN STP proved to be effective in decreasing the SS of the wastewater to 2.83 ± 0.35 mg/L.

Chronic toxicity tests

Raw wastewater and primary treatment effluents of JN STP exhibited mortality at low effective concentrations (EC_{50}) of 8.94 ± 0.50% and 18.19 ± 0.77%, respectively. On the other hand, SK STP raw wastewater and primary treatment effluent EC_{50}s were identical to each other with values at 18.75%. From the acute toxicity results, it was surmised that suspended solids in the water column may have highly contributed to the mortality of the *Daphnia magna*. Chronic exposure to suspended solids alone has been reported to induce detrimental and stimulate sublethal effects to daphnids as in their fecundity, growth, and juvenile survival at concentrations of 50 and 100 μg/L (Kirk 1992; Kirk and Gilbert 1990; Arruda et al. 1983; McCabe and O'Brien 1983). The result supports the previous study with the significant difference ($p < 0.05$) of the EC_{50}s of the SK and JN STPs' raw wastewater at SS values of 68.3 ± 3.5 mg/L and

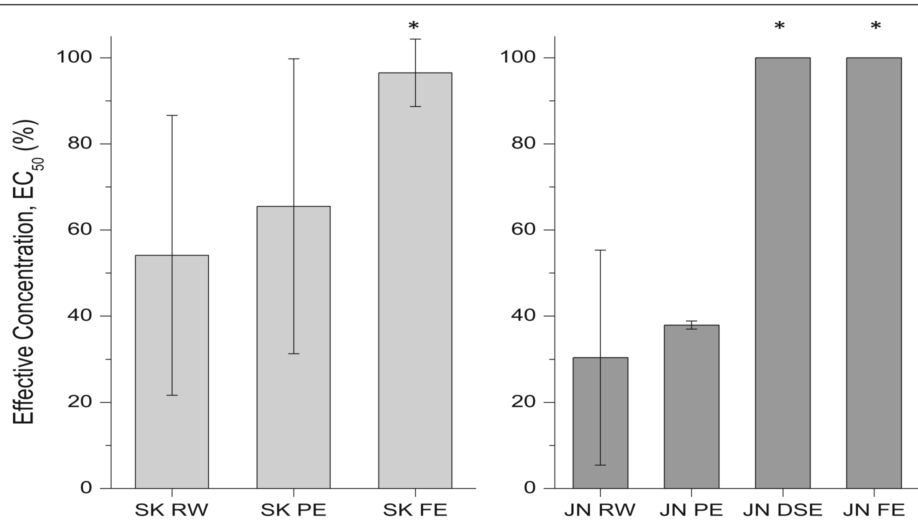

Fig. 2 Acute 48 h effective concentrations (EC_{50}s) of wastewater effluents from SK and JN STPs. Asterisks indicate significant difference with respect to raw wastewater samples

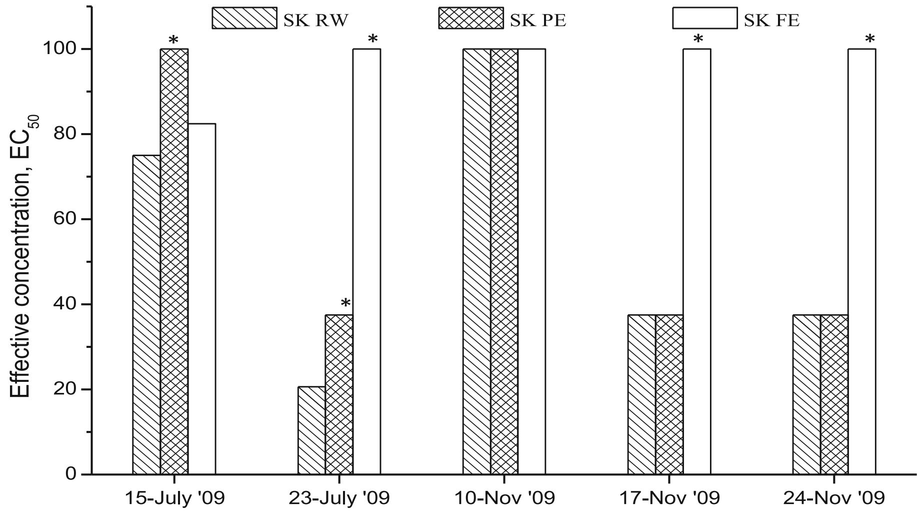

Fig. 3 Seasonal variations of the EC_{50}s of SK STP effluents. Asterisks indicate significant difference with respect to raw wastewater samples

161.7 ± 26.2 mg/L, respectively. Significant reduction of the toxicity was noted with JN STP effluent after the primary treatment with EC_{50} improvement from 8.94 ± 0.50% to 18.19 ± 0.77%. Additionally, disk filtration effluent of JN STP did not exert mortality to the daphnids (data not shown). In contrast, no improvement on the EC_{50} was observed in SK STP effluent because suspended solids content in the effluent remained relatively high at 54.7 ± 6.4 mg/L.

Besides mortality, average lifespan reflects the overall health and fitness of the test organisms during the 21-day chronic exposure. Effluents from JN STP exhibited substantially truncated average daphnid lifespan, particularly those tested in raw wastewater at 8.34 days and primary treatment effluent at 11.14 days. Conversely, disk filtration and ozonation effluents influenced protracted average lifespan on the test organisms with values 20.80 and 20.78 days, respectively.

An extension of at least 1 day to the total days to gravidity of *Daphnia magna* prompts delay in the first day reproduction and may serve as an indication of the ensuing effect of exposure to suspended particles on their population (Fig. 4). SK STP effluents exhibited the described phenomenon strongly with first day of brooding at 9.0, 8.20, and 8.60 for raw wastewater, primary treatment effluent, and final effluent. JN STP effluents reflected the opposite trend showing that time of the first brood recorded were 7.0, 7.50, 7.30, and 7.90 days. Previous studies reported that the duration of exposure to suspended solids played a more important role than suspended solids concentration in the delay of the number of days to gravidity of *Daphnia magna* (Robinson et al., 2009). However, data in this study support that concentration of SS could be more influential. Also significant correlation of nutrient removal by treatment process and toxicity reduction was not observed.

Figure 5 illustrates the fecundities of the effluent samples from SK STP and JN STP. The production of offspring per female for SK STP follows an inverted U-shaped trend and SK PE showed more clearly. On the other hand, JN STP did not follow th pattern implying less impact of SS and nutrient concentration on fecundity. In the water column of contaminated water, many pollutants adsorb to the suspended solids such as sediments, clay particles, and natural organic materials (Rodgers et al. 1987). Although many physicochemical parameters are involved, the adsorption behavior for organics, for example, endocrine disruptor chemicals (EDCs), pharmaceuticals and personal care products (PPCPs), pesticides, and heavy metals, can be predicted roughly from the compound's log K_{ow} or oil/water partitioning coefficient (Admiraal et al. 1998). Allison and Allison (2005) summarized the partitioning coefficients of a myriad of heavy metals found in suspended matter. Works by Kim et al. (2007) enlisted the partitioning coefficients of some pharmaceuticals and disclosed the potential risks they impose to the receiving body of waters and its aquatic ecology. Several studies have shown removal of pesticides and pharmaceuticals from sewage effluent (Ternes et al. 2003; Ikehata et al. 2006; Ikehata et al. 2008; Okuda et al. 2008; Svenson et al. 2003; Andersen et al. 2003; Braga et al. 2005) but organic micropollutants pass through the STPs and enter the aquatic environment (Fent et al. 2006; Kummerer 2009). Especially, estrogenic compounds hardly removed by wastewater treatment processes (Andersen et al. 2003) and STP effluent denoted estrogenic effect (Nakada et al. 2004).

As the scope of this study did not include toxicity identification evaluation, we can only attribute the sublethal effects of the sample effluents on the fecundity of the *Daphnia magna* on suspended materials and particle-bound organic chemicals such as EDCs, PPCPs, and pesticides. One possible effect of contaminated particles on particle-feeding

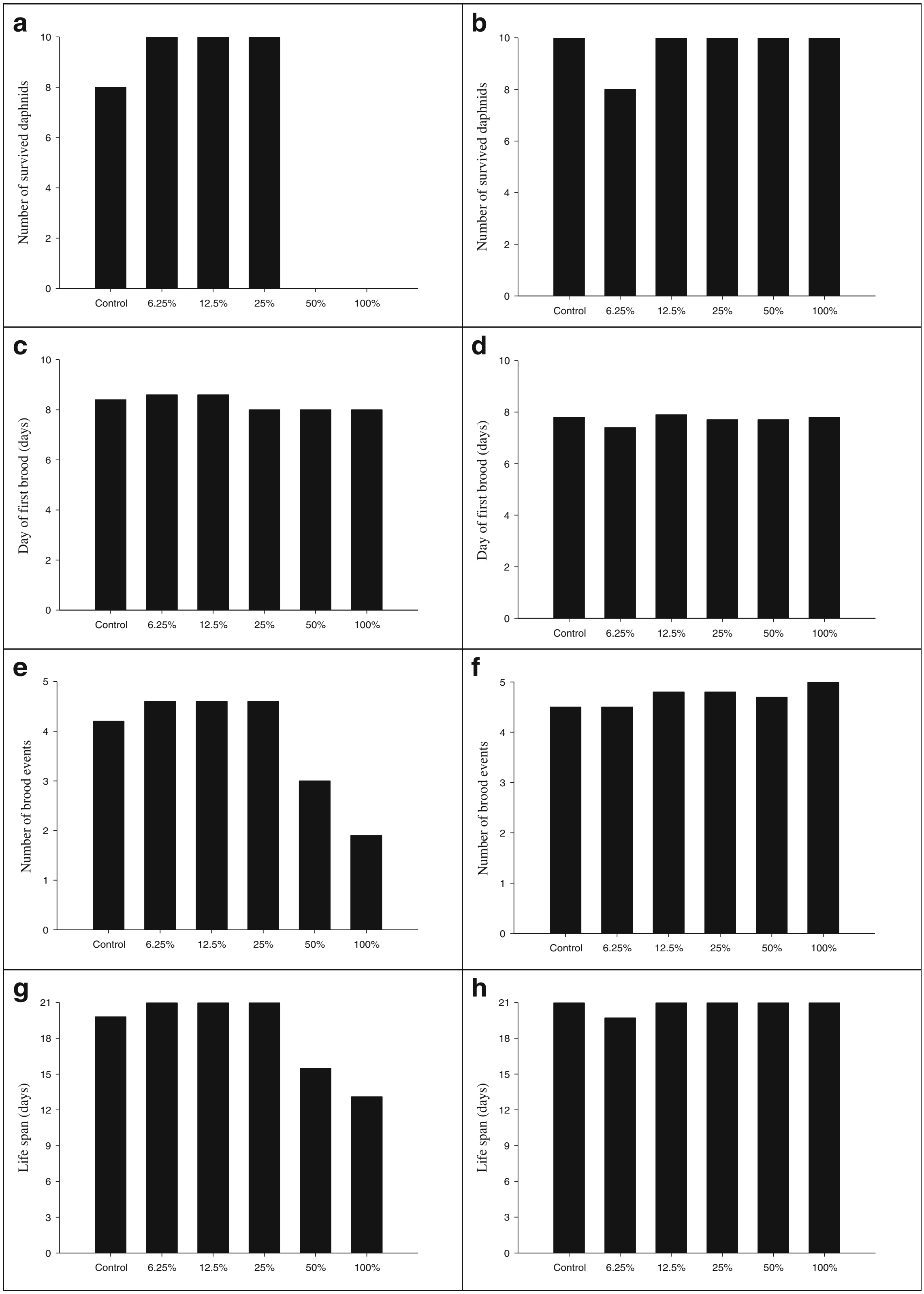

Fig. 4 Comparison of chronic toxicity test endpoints for SK and JN STPs final effluent. **a**, **c**, **e**, **g** for SK STP efluent and **b**, **d**, **f**, **h** for JN STP effluent

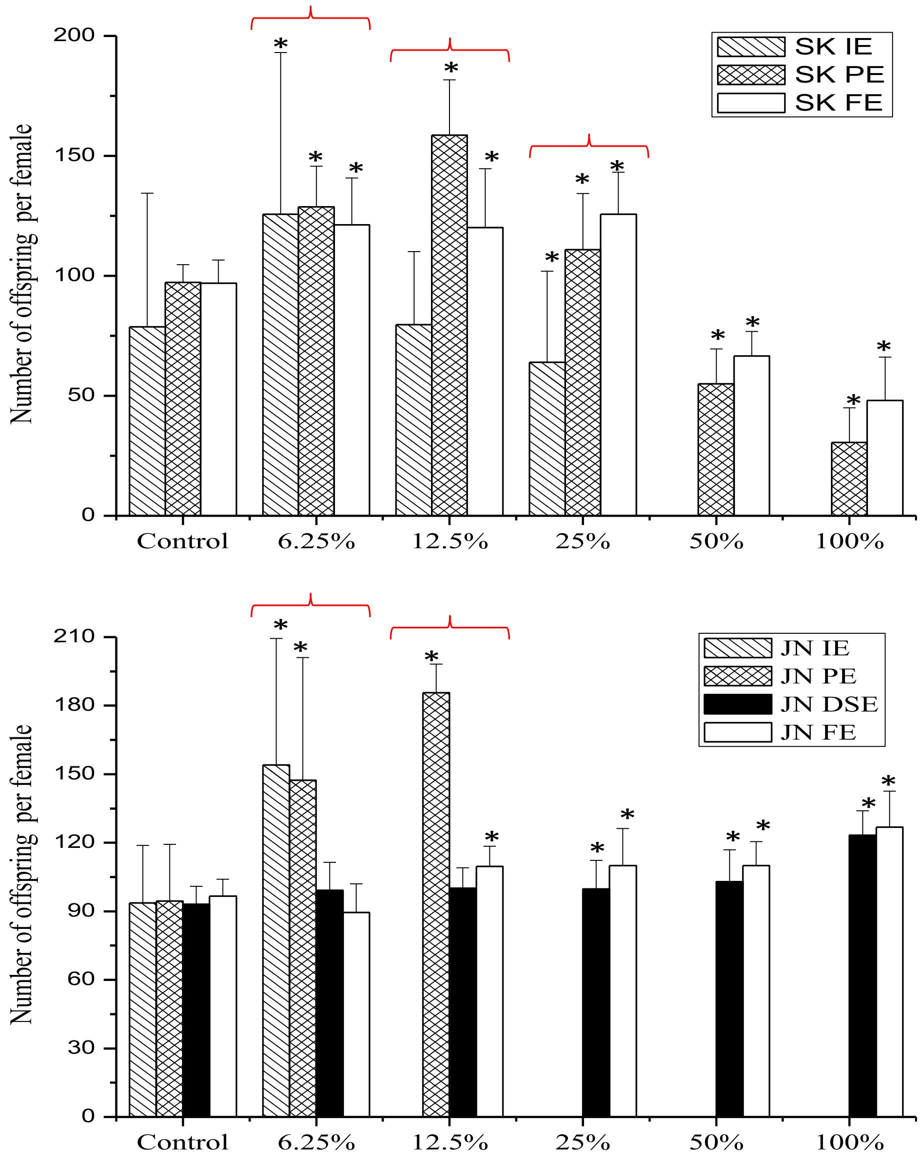

Fig. 5 Fecundities of *Daphnia magna* tested for SK and JN STP effluent samples. Asterisks indicate significant difference from control

organism like daphnids is a drastic reduction in food intake causing serious chronic effects on the population level. It has been shown many times that toxic products reduce food uptake in daphnids. Moreover, contaminated food particles affect food uptake even more than dissolve contaminants (Allen et al. 1995). The consequences to population growth of mortality during acute tests are commonly acknowledged, even though the relationship between survival and population growth is not a simple function (Caswell, 1989). Both field observations and laboratory studies have demonstrated that suspended materials can have deleterious effects on planktonic species including cladocerans (Bridges et al. 1996). For SK FE, JN DSE and JN FE where none or positive effects on daphnid fecundity were observed can be accredited to be result of ingestion of fine-grained sediment particles with nutritive values as shown in previous study (Dillon 1993; Ankley et al. 1994).

Sun et al. (2008) evaluated the estrogenic activities of the wastewater effluent samples and reported that since some estrogenic substances such as estradiol, bisphenol, and nonylphenol were discharged in conjugated forms, the microbial activity could deconjugate and reactivate them during the early steps of treatment. Hence, there are higher population densities for raw wastewater and primary treatment for both SK and JN STPs. Secondary treatment has been considered as one of the most effective treatment of processes to reduce the estrogenic activity in wastewater (Kirk et al. 2000; Leush et al. 2006). However, still some estrogenic compounds such as estrone showed only 6.3% of removal efficiency and implied high ecological risk (Belhaj et

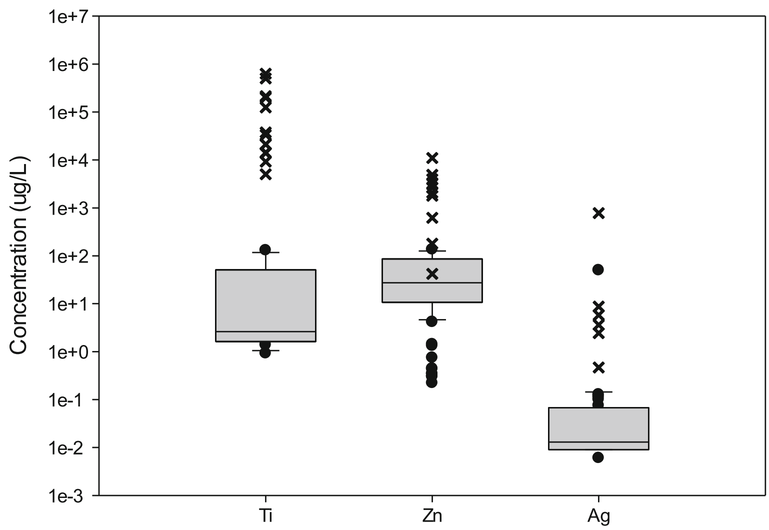

Fig. 6 Environmental level (●, other countries, **X**, South Korea) of nanoparticles and its toxicity level (▢) on aquatic organisms Kim et al. (2010); Kim et al. (2014); Yang et al. (2013); Kiser et al. (2009); Westerhoff et al. (2011); Mitrano et al. (2012); Johnson et al. (2011a); Gottschalk et al. (2009); Johnson et al. (2011b); Khosravi et al. (2012); Hendren et al. (2013)

al. 2016). In addition, estrone in effluent showed higher level than in raw wastewater (Mailler et al. 2015; Belhaj et al. 2015), and pharmaceutical residues showed large variation of its removal efficiencies (Yang et al. 2017).

Another micropollutants, nanoparticles are also easily removed in STPs up to 95% even though some are not totally removed (Tiede et al. 2011) and environmental concentration of nanoparticles are higher than toxicity levels so that it is hardly said nanoparticles are responsible for effluent toxicity (Fig. 6). However, many studies reveal a negative effect of nanoparticles on animals, especially on reproductive organs and reported that females are more vulnerable according to nanoparticle characteristics, exposure routes, and test animals (Brohi et al. 2017). It implicates that the potential role of nanoparticles on reproductive effect should not be neglected and further toxicity evaluation at low concentration is needed.

Conclusions

In this study, acute and chronic toxicity tests were successfully employed for the evaluation of the reduction of toxicity of sewage water effluents for two sewage treatment plants in SK STP, Incheon and JN STP, Seoul, Korea. Acute and chronic lethal effect decreased depending on SS removal by wastewater treatment process. As estrogenid effect, fecundity of daphnids was significantly increased in the influent and primary effluents implying presence/attribution of endocrine disrupting chemicals which controls reproduction and embryogenesis in mysid crustaceans. Pharmaceutical residues and nanoparticles in the effluents could be suggested as possible sources for estrogenic effect referring to previous study. However to identify and clarify the potential role of micropollutants on reproduction, further toxicity evaluation is needed.

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DMF conducted toxicity tests and statistical analysis. YK designed the study, drafted and revised manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate
Not Applicable.

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Not Applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

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