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Daphnia magna as a Test Species for Toxicity Evaluation of Municipal Wastewater Treatment Plant Effluents on Freshwater Cladoceran in Turkey

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Abstract

Aquatic toxicity of municipal wastewater was evaluated in an acute toxicity test using water flea, *Daphnia magna* as an freshwater aquatic experimental animal model. Toxicity test were performed on samples of both untreated (raw) and treated wastewaters were collected Manisa municipal effluents. Undiluted untread and treated effluents were very toxic to *D. magna*and cause to death of all exposed daphnids. Dilution of wastewaters was observed to decrease percentage of influence of biological toxicity based on dilutional rate. Acute toxic effect of untreated wastewater on *D. magna* was more than that of treated wastewater. In addition, the longer the period of exposure to *D. magna*, the more significantly toxic effect increased.

Keywords: Water flea, acute toxicity, municipal wastewater.

Türkiye Tatlısu Kladoseranları Üzerine Belediye Atıksu Arıtma Tesisisi Atıklarının Toksisite Değerlendirmesinde Bir Test Türü Olarak *Daphnia magna*

Özet

Evsel atıksuların sucul toksisitesi, bir tatlısu sucul deneysel hayvan modeli olan su piresi Daphnia magna akut toksisite kullanılarak değerlendirildi. Toksisite testleri hem arıtılmamış (ham) ve hem de arıtılmış Manisa evsel atıksuları ile gerçekleştirildi. Hiç seyretilmeyen arıtılmamış ve arıtılmış atıksular *D. magna* üzerine çok toksik olduğu ve maruz kalan tüm dafnidlerin ölümüne neden oldu. Atıksularda seyrelme yapıldığında seyrelme oranına bağlı olarak biyolojik toksik etkinin azaldığı görüldü. Yine arıtılmamış atıksuların *D. magna* üzerine akut toksik etkisi arıtılmış atıksulardan daha fazla bulundu. İlave olarak *D. magna'* nın atıksulara maruz kalma süresi arttıkça toksik atkinin de belirgin bir şekilde artığı bulundu.

Anahtar Kelimeler: Su piresi, akut toksisite, evsel atıksu.

Introduction

Urban activities are a major source of pollution in aquatic ecosystems. Many freshwater ecosystems are increasingly degraded due to the input of wastewater-borne pollutants (Fent, 1996; Wang *et al*., 2003; Nakada *et al*., 2006; Lindqvist *et al*., 2005). Excessive discharge of some constituents, such as suspended solid, nutrients, microorganisms and toxic compounds from municipal wastewater into aquatic ecosystems cause water quality is not suitable for survival or growth of aquatic organisms. Municipal wastewater contains a broad spectrum of contaminants (Rowsell *et al*., 2010). They can contain: 1) suspended solids; 2) disease-causing pathogens (e.g., bacteria and viruses); 3) decaying organic wastes; 4) nutrients; and 5) about two hundred different identified chemicals (Environment Canada, 2001).

Most wastewater treatment plants not treat all types of contaminants and a high part of emerging compounds especially organic chemicals and their metabolites may escape elimination in treatment plants and enter the aquatic environments. A number of studies have demonstrated the occurrence of organic contaminants in municipal wastewater treatment plant effluents. These studies mostly concered with occurance of pharmaceuticals, personal care products, diagnostic imaging contrast agents, estrogens and pesticides in wastewaters (Paxéus, 1996; Ternes, 1998; Daughton and Ternes, 2000; Ternes *et al*., 1999; Boyd *et al*., 2003; Lindstrom *et al*., 2002; Lishman *et al*., 2006; Odjadjare and Okoh, 2010; Rowsell *et al*., 2010, Zein *et al*.,2015).

Hazard assessment of municipal wastewater in Turkey is based on physicochemical parameters e.g. total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD) and pH. Bioassays for evaluating treated municipal wastewaters are not used. However, the complexity of wastewaters limits analyses of all chemicals and these give rise to the suspicion of the safety of wastewater treatment plants effluents. At the same time volume of effluents is too much. For this reason, toxicity tests have been carried out to assess the potential effects of wastewater discharges on aquatic life. One of the most internationally used bioassays for monitoring of effluents is the acute toxicity test with daphnid cladocerans and in particular that performed with *Daphnia magna*. Standard methods have been developed for this assay that were gradually endorsed by national and international organisations dealing with toxicity testing procedures, in view of its application within a regulatory framework (Persoone *et al*., 2009).

A number of species of Daphnidae family are used for toxicity test very common. Besides they are well recommended for testing (USEPA, 2002), the organisms are generally available throughout the year and easily cultured in the laboratory. In addition to this, daphnids are sensitive to a variety of pollutants and have been widely used as the biotests organisms for evaluating different toxic substances (ISO, 1996; Sarma and Nandini, 2006; Sánchez- Meza *et al*., 2007; Oral *et al*., 2007; Tatarazako and Oda, 2007).

In this study, a common species such as the freshwater flea *D. magna* (Cladocera, Crustacea) was used to assess the acute toxicity of untreated and treated wastewaters from the municipal wastewater treatment plant in Manisa, Turkey. The objective of this study was to investigate the safety for discharging municipal wastewaters effluents into fresh water organisms to protect aquatic biota and achieve water quality standards.

Materials and Methods

Manisa Municipal Wastewater Treatment Plant (MMWTP) provided treatment to wastewater from approximately 332.346 residents and discharges to Gediz River. In 2007, the average daily flow of MMWTP was 7000 m^3/day. Samples of raw influent and mechanically and biologically treated effluent from MMWTP were collected in June 2007. The samples were transferred to the laboratory in cooled box and stored in darkness in refrigerator (+4^oC) prior to chemical analyses and performance of bioassays. The bioassays were performed within a week.

All chemical analysis of MMWTP influents and efluents were performed in accordance with Standard Methods (APHA, 1998).

In this research, *D. magna* were cultured and handled according to the procedures outlined in the U.S. Environmental Protection Agency (USEPA) manual (USEPA, 2002). The acute and whole effluent toxicity tests performed followed the U.S.EPA guideline (USEPA, 2002). Four replicates of five neonates (less than 24 h old) were used for each treatment and control without feeding. The test concentrations ranging from 0.1% to 100% were set by dilution of untreated (raw) and treated wastewater effluents. In each treatment schedule 20 daphnids were scored for their frequencies of immobilized daphnids.

$$TU = 100/LC_{50} \qquad (1)$$

The toxicity data were classified according to the hazard classification system for wastewaters discharged into the aquatic environment as shown in Table 1 (Persoone *et al*., 2003).

The LC_{50} values were determined at 24, 48, 72 and 96 hours by probit analysis. The calculations were done using Probit Programme Version 1.5, which is available in USEPA (USEPA, 2002). Following to this, toxicity values (LC_{50}) were transformed into Toxic Units (TU) according to the equation 1.

Results

Wastewater Characterization

A large amount of municipal wastewaters are discharged directly or indirectly into the water bodies in Turkey. 63.3% and 36.4% of wastewaters are discharged into the recipient environment treated and untreated, respectively. Almost half of municipal wastewaters (49.6%) is dumped into freshwaters such as rivers, lakes, reservoirs and dams (TurkStat, 2013), with great amount various contaminants in municipal wastewaters becoming a major threat to freshwater organisms in particular. Water fleas, as *D. magna*, are small inverte brate crustaceans and a key source of food for many fish in fresh water ecosystems. 41.24% of all known species of fish are found in fresh water (Marzan *et al*., 2014), which indicates that the subject

Table 1. Hazard classification system for waste waters discharged into the aquatic environment (Persoone *et al*., 2003)

TU	Class	Toxicity	Symbol
< 0.4	Class I	No acute toxicity	☺
< 0.4 < TU < 1	Class II	Slight acute toxicity	☹
1 < TU < 10	Class III	Acute toxicity	☠
10 < TU < 100	Class IV	High acute toxicity	☠☠
TU > 100	Class V	Very high acute toxicity	☠☠☠

is of increasing importance.

In order to characterize the water quality parameters of MMWTP influents and efluents, such as pH, COD, BOD_5 and TSS were measured. Results of chemical analyses of wastewaters are presented in Table 2. One of the important parameters in municipal wastewater is pH, whose measurements show that there were no significant differences between treated and untreated wastewaters in pH value with optimum intervals. According to results of COD and BOD examination, their values in wastewaters of MMWTP decrease after treatment, which proves that the system works well. Following the treatment process, both COD and BOD values were found to be within the discharge limits in Turkey (Turkish Official Newspaper, 2004). Measurement of TSS values indicated that they were within the standard limits of wastewater discharge in Turkey both in treated and untreated wastewaters with a significant reduction in TSS value only after the process. On the other hand, chemical characteristics of MMWTP waste effluents tested in the study are consistent with the regulation of effluent discharging criteria of European Union (EU) (1991) (Table 2). Values of treated MMWTP effluents were in agreement with EU discharge limits.

The mean untreated wastewawater COD, BOD_5 and TSS were found to be 288.0 mgO_2/L, 96.0 mgO_2/L and 57.3 mg/L respectively. After treatment all were observed as 44.2 mgO_2/L, 16.0 mgO_2/L and 26.5 mg/L respectively. The findings revealed that the mean percentage removal in COD, BOD_5 and TSS after treatment procedure were found to be 84.7%, 83.3% and 53.8% respectively.

Whole Effluent Toxicity Test

Results of physicochemical parameters measured in wastewaters showed a significant decrease in the values while those of toxicity tests on *D. magna* did not indicate such a reduction (Table 3). None of *D. magna* individuals allowed to live in 100% untreated and treated wastewaters managed to survive even in the shortest time of testing.

However, the value of toxicity in immobilized *D. magna* was defined as LC_{50} and TU varying with testing time (Table 4).

Although all the MMWTPs wastewaters were discharged under the regulations for the conventional water quality standards, revealed toxicities in the whole effluent toxicity tests with *D. magna.* In addition, biological toxic effect increased based on the duration of the test. LC_{50} value of the tests performed with untreated wastewater was 24.9 after 24 hours while it dropped to 15.8, 12.7 and 11.3 following 48, 72 and 96 hours, respectively. Accordingly, TU values were measured as 4.0, 6.3, 7.9 and 8.8 after 24, 48, 72 and 96 hours, respectively. Toxic effect of treated waters on *D. magna* immolization was less than that of untreated waters on it. However, toxic effect of treated wastewaters on *D. magna* increased in longer periods, which was similar to that of untreated wastewaters on it. Although LC_{50} value was 32.9 following 24 hours, it decreased to 29.4, 25.1 and 23.8 after 48, 72 and 96 hours, respectively. Consequently TU values were found as 3.0, 3.4, 4.0 and 4.2 after 24, 48, 72 and 96 hours, respectively (Table 4).

Discussion

Consequences of the study on acute toxicity of treated municipal wastewaters on *D. magna* in Korea

Table 2. Chemical characteristics of untreated and treated wastewaters collected from MMWTP

	COD mgO_2/L	BOD_5 mgO_2/L	TSS mg/L	pH
Untreated WW	288.0	96.0	57.3	7.3
Treated WW	44.2	16.0	26.5	7.5
Discharge limits in Turkey (Turkish Official Newspaper, 2004)	180.0	50.0	70.0	6-9
Discharge limits EU (91/271/EEC)	125.0	25.0	35.0	-

Table 3. Mortality of *D. magna* exposed to different concentrations of both untreated and treated wastewaters collected from MMWTP

Wastewater Concentrations %	Mortality %							
	24 h		48h		72 h		96 h	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
3	5	0	15	0	15	0	15	0
10	20	5	20	15	30	25	35	25
30	40	15	70	35	80	40	85	45
100	100	100	100	100	100	100	100	100

Table 4: Toxicity evaluation of untreated and treated wastewaters collected from MMWTP using *D. magna*

Hour	LC_{50}	95% Confidence Limits	TU	Class	Toxicity
Untreated					
24	24.9	17.2 - 37.2	4.0	III	Acute toxicity
48	15.8	10.7 - 23.4	6.3	III	Acute toxicity
72	12.7	8.7 - 18.5	7.9	III	Acute toxicity
96	11.3	7.8 - 16.4	8.8	III	Acute toxicity
Treated					
24	32.9	24.7 - 44.6	3.0	III	Acute toxicity
48	29.4	21.2 - 41.4	3.4	III	Acute toxicity
72	25.1	17.7 - 36.2	4.0	III	Acute toxicity
96	23.8	16.9 - 34.1	4.2	III	Acute toxicity

indicated that of TU values in five different municipal wastewaters after 48 hours, only one wastewater TU value exceeded 1 (1.31) while the other four TU values remained below 1 (0.10-0.80) (Ra *et al*., 2007), as compared to which treated wastewaters of MMWTP were found to have significantly more toxic effect on *D. magna*. Because municipal wastewaters are complex mixtures, it is natural for them to show different physicochemical properties based on time and location, from which it could be concluded that toxic effect remains variable as well.

The present study showed that municipal wastewaters could be harmful to biota even if they evidently met standards for wastewater discharge limits, since measurement of sufficient amount of physicochemical parameters requires determination of standards of discharge limits. However, as we previously cited, municipal wastewaters could include complex toxic, carcinogenic and mutagenic organic and inorganic substances which are all difficult to determine (Ricking *et al*., 2003; Servos *et al*., 2005; Cristale and Lacorte, 2015).

Zein *et al*. (2015) determined similar values of toxicity removal for *D. magna* after primary and secondary treatment. The potential impact of complex chemical mixtures (e.g., treated wastewater) can enhance the toxicity of exposure to the insecticide. Complex chemical mixtures exposure in aqueous systems is an input of contaminants in the environment (Zein *et al*, 2015).

No significant reduction was observed for whole effluent acute toxicity by luminescent bacteria assay and cladoceran assay (Sun *et al*., 2015).

Feeding rate inhibition and oxidative stress of effluent from a liquid crystal display (LCD) wastewater treatment plant (WWTP) to *D. magna* (reference species) and *Moina macrocopa* were monitored and raw wastewater was acutely toxic to both *D. magna* and *M. macrocopa*, but the toxicity reached less than 1 TU in the final effluent (FE) as treatment proceeded (Kim *et al*., 2012).

It has been reported that *D. magna* can be a useful analytical tool for early warning system to monitor of WWTP. Results of ecotoxicity tests presented *Daphnia* mobility ranges from 0 to 100% at the untreated and from 15 to 100% after treated (Mendonça *et al*., 2013).

In conclusion, we claim and propose that measurement of toxicity and monitoring of physicochemical parameters be performed simultaneously in large-scale treatment plants and that discharge limits be standardized in view of protection of biota in fresh waters. Furthermore, it should be considered whether there are other possible discharges into the recieving environment and if there are what types and how much they are so as to protect biota.

Based on the results of the study and on other experiences, we reported that ecotoxicological tests should be used for assessment municipal whole effluents in Turkey since they help predetermine whether municipal wastewaters are toxic in the shortest time possible. Data of the present study on water flea show that wastewater discharges into receiving environment could enable us to understand their toxic potential.

Municipal effluents which are treated and compared with standards of permissible discharge limits in Turkey have toxic potential to living *D. magna*. Therefore, whole effluent toxicity tests are needed to combine measurement of physicochemical parameters with assessment to better protect the quality of the fresh water environment.

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References

APHA (American Public Health Association), 1998. Standart methods for the examination of water and wastewater/prepared and published jointly by American Public Health Association, American Water Works Association, Water Environment Federation., 20 Edition (Joint editorial board, Lenore S. Clesceri, Arnold E. Greenberg, Andrew D. Eaton ; Managing editor, Mary Ann H. Franson). American Public Health Association, Washington DC.

http://trove.nla.gov.au/version/38126605.

Boyd, G.R, Reemtsma, H., Grimm, D.A and Mitra, S. 2003. Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, US and Ontario, Canada. Science of the Total Environment, 311(1-3): 135–49.

Cristale, J. and Lacorte, S. 2015. PBDEs versus NBFR in wastewater treatment plants: occurrence and partitioning in water and sludge. AIMS Environmental Science, 2(3): 533-546. doi: 10.3934/environsci.2015.3.533

Daughton, C.G. and Ternes, T.A. 2000. Pharmaceuticals and personal care products in the environment: Agents of subtle change?. Environmental Health Perspectives, 107 (6): 907-38.

Environment Canada, 2001. State of Municipal Wastewater Effluents in Canada (State of the Environment Report). Prepared by Indicators and Assessment Office, Ecosystem Science Directorate, Environmental Conservation Service, Environment Canada. ISBN 0-662-29972-8. Cat. No. En1-11/96E, Ottawa, Ontario, 74 pp.

E.U. (European Union) Council Directive 91/271/EEC of 21 May 1991. Concerning urban waste-water treatment (91/271/EEC), 1991, Official Journal of the European Communities, No. L 135, 30 May 1991. 40-52.

Fent, K. 1996. Organotin compounds in municipal wastewater and sewage sludge: contamination, fate in treatment process and ecotoxicological consequences. Science of The Total Environment, 185 (1–3): 151–159. doi: 10.1016/0048-9697(95) 05048-5.

ISO (International Organisation for Standardisation). 1996. Water Quality: Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) – Acute Toxicity Test. ISO 6341, Geneva, Switzerland.

Kim, S-B., Kim, W-K., Chounlamany, V., Seo, J., Yoo, J., Jo, H-J and Junga, J. 2012. Identification of multi-level toxicity of liquid crystal display wastewater toward *Daphnia magna* and *Moina macrocopa*. Journal of Hazardous Materials, 227–228: 327–333. doi:10.1016/j.jhazmat.2012.05.059.

Lindstrom, A., Buerge, I.J., Poiger, T., Bergqvist, P.A., Muller, M.D. and Buser, H.R. 2002. Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater. Environmental Science and Technology, 36: 2322–2329.

Lindqvist, N., Tuhkanen, T. and Kronberg, L. 2005. Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters. Water Research, 39 (11): 2219–2228.

Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M. and Seto, P. 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Science of the Total Environment, 367 (2-3): 544-558.

Marzan, L.W., Barua, P., Akter, Y., Mannan, A., Hossain, A. and Ali, Y. 2014. Molecular investigation on clinopathological, genetic and biochemical changes in *Channa punctata* infected with internal parasites and subjected to metals pollution in Chittagong, Bangladesh. Journal of Biomolecular Research and Therapeutics, 3: 113. doi: 10.4172/2167-7956.1000113.

Mendonça, E., Picado, A., Paixão, S.M., Silva, L., Barbosa, M. and Cunha, M.A. 2013. Ecotoxicological evaluation of wastewater in a municipal WWTP in Lisbon area (Portugal). Desalination and Water Treatment, 51: 4162–4170. doi: 10.1080/19443994.2013.768021

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K. and Takada, H. 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Research, 40 (17): 3297–3303. doi: 10.1016/j.watres.2006.06.039

Odjadjare, E.E. and Okoh, A.I. 2010. Physicochemical quality of an urban municipal wastewater effluent and its impact on the receiving environment. Environmental Monitoring and Assessment, 170: 383–394. doi: 10.1007/s10661-009-1240-y.

Oral, R., Meric, S., De Nicola, E., Petruzelli, D., Della Rocca, C. and Pagano, G., 2007. Multi-species toxicity evaluation of a cromium-based leather tannery wastewater. Desalination, 211: 48-57.

Paxéus N. 1996. Organic pollutants in the effluents of large wastewater treatment plants in Sweden. Water Research, 30: 1115–1122.

Persoone, G., Marsalek, B., Blinova,I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L. and Kolar, B. 2003. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environmental Toxicology, 18 (6): 395–402. doi: 10.1002/tox.10141.

Persoone, G., Baudo, R., Cotman, M., Blaise, C., Thompson, K. C., Moreira Santos, M., Vollat, B., Törökne, A. and Han, T. 2009. Review on the acute *Daphnia magna* toxicity test - Evaluation of the sensitivity and the precision of assays performed with organisms from laboratory cultures or hatched from dormant eggs. Knowledge and Management of Aquatic Ecosystems, 393: 1-29.

Ra, J.S., Kim, H.K., Chang, N.I. and Kim, S.D. 2007. Whole effluent toxicity (WET) tests on wastewater treatment plants with *Daphnia*

magna and *Selenastrum capricornutum*. Environmental Monitoring and Assessment, 129 (1-3): 107-113.

Ricking, M., Schwarzbauer, J., Hellou, J., Svenson, A. and Zitko, V. 2003. Polycyclic aromatic musk compounds in sewage treatment plant effluents of Canada and Sweden-first results. Marine Pollution Bulletin, 46 (4): 410-7.

Rowsell, V.F., Tangney, P., Hunt, C. and Voulvoulis, N. 2010. Estimating levels of micropollutants in municipal wastewater. Water Air Soil Pollution, 206: 357–368. doi: 0.1007/s11270-009-0112-y

Sarma S.S.S. and Nandini, S. 2006. Review of recent ecotoxicological studies on cladocerans. Journal of Environmental Science and Health, Part B, 41: 1417-1430.

Sánchez-Meza, J.C., Pacheco-Salazar, V.F., Pavón-Silva., T.B., Guiérrez-García, V.G., Avila-González Cde, J. and Guerrero-García, P. 2007. Toxicity assessment of a complex industrial wastewater using aquatic and terrestrial bioassays *Daphnia pulex* and *Lactuca sativa*. Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering, 42 (10): 1425-31.

Servos, M.R., Bennie, D.T., Burnison, B.K., Jurkovic, A., McInnis, R., Neheli, T., Schnell, A., Seto, P., Smyth, S.A. and Ternes, T.A. 2005. Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. Science of the Total Environment, 336: 155–170.

Sun, J., Quan, Y., Wang, W., Zheng , S. and Liu, X. 2015. Potential contribution of inorganic ions to whole effluent acute toxicity and genotoxicity during sewage tertiary treatment. Journal of Hazardous Materials, 295: 22–28. doi: 10.1016/j.jhazmat.2015.04.012.

Tatarazako, N. and Oda, S. 2007. The water flea *Daphnia magna* (Crustacea, Cladocera) as a test species for screening and evaluation of chemicals with endocrine disrupting effects on crustaceans. Ecotoxicology, 16: 197-203.

Ternes, T.A. 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research, 32 (11): 3245-3260.

Ternes, T.A., Stumpf, M., Kreckel, P., Mueller, J., Haberer, K., Wilken, R.D. and Servos, M.R. 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants. I. Investigations in Germany, Canada and Brazil. Science of the Total Environment, 225 (1-2): 81–90.

Turkish Official Newspaper. 2004. Su kirliliği kontrolü yönetmeliği (Water pollution control regulations Date: 31.12.2004 Number: 25687) (in Turkish).

TurkStat, 2013. Turkey's Statistical Year Book, 2013, Turkish Statistical Institute Printing Division, Ankara, Türkiye.

USEPA (United States Environmental Protection Agency). 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organism, Fifth edition, EPA-821-R-02- 012, Office of Water (4303T), Washington, DC.

Wang, C., Wang, Y., Kiefer, F., Yediler, A., Wang, Z. and Kettrup, A. 2003. Ecotoxicological and chemical characterization of selected treatment process effluents of municipal sewage treatment plant. Ecotoxicology and Environmental Safety, 56 (2): 211-217. doi: 10.1016/S0147-6513(02)00121-5.

Zein, M.A., McElmurry, S.P., Kashian, D.R., Savolainen, P.T. and Pitts, D.K. 2015. Toxic effects of combined stressors on *Daphnıa pulex*: interactions between diazinon, 4-nonylphenol, and wastewater effluent. Environmental Toxicology and Chemistry, 34 (5): 1145–1153. doi: 10.1002/etc.2908.