Effectiveness of ozonation and chlorination on municipal wastewater treatment evaluated by a battery of bioassays and biomarkers
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ABSTRACT
A battery of bioassays, including biological toxicity as well as in vitro mouse spleen lymphoproliferative responses and cytokine production, was conducted to compare the effectiveness of tertiary treatment methods such as coagulation (Coag) and absorption on granular activated carbon (GAC) and disinfection processes such as chlorination and ozonation in removing toxic or stress inducing agents from reclaimed wastewater. Whole effluent toxicity (WET) testing of secondary treated (ST) wastewater using as test species Vibrio fischeri, Daphnia magna and Tetrahymena thermophila as well as phytotoxicity revealed moderate toxicity effects that depend on the organism used. All bioassays exhibited decrease of the ecotoxicological responses after tertiary treatment. However, mitogenic responses were proved to be more sensitive. Endotoxin present in ST samples may be responsible for the increased strong lymphoproliferative activity as well as interleukin-1 (IL-1) production by mouse splenocytes. Tertiary treatment of ST with coagulation and/or adsorption on granular activated carbon (GAC) in combination with ozonation reduced WET to control levels. Ozonation alone or in combination with any other treatment removed endotoxin more efficiently than chlorination and thus reduced spleen lymphoproliferative responses and IL-1 production.

Key words | bioassays, biomarkers, chlorination, disinfection, lymphoproliferation, ozonation

INTRODUCTION
The shortage of water resources combined with the increased demand of water consumption became one of the major social and economic problems globally. Reclaimed water, tertiary treated municipal wastewater, recently gained particular importance in the sustainable management of water resources. Up-to-date treatment processes for the reclamation of wastewater achieve efficient removal of biological and chemical parameters; however, efficient pathogen removal is not always feasible (Harwood et al. 2004; Wéry et al. 2008). Reclaimed wastewater is widely used for irrigation, cooling water, industrial process water and enhancement of the groundwater resources (Levine & Asano 2004). The treatment efficiency and thus the respective treatment technology that should be applied for the reclamation of wastewater depends on its potential use and the regional regulatory permits. Tertiary treatment usually includes one or a combination of processes such as coagulation, disinfection and adsorption on activated carbon, prior to reuse (Samaras et al. 1995).

The final step of typical wastewater treatment is disinfection, aiming to the reduction of microbiological load and thus the minimisation of transmission of possible waterborne infectious diseases. Chemical oxidants, such as ozone, chlorine, chlorine dioxide and chloramines, are the most widely used disinfectants due to their capacity of efficient elimination of microorganisms and oxidation of micropollutants (Gottschalk et al. 2000; Bruchet & Duguet 2004). However, under certain circumstances, oxidants can...
induce formation of potentially harmful by-products or transformation products due to their reactivity with organic/inorganic materials or micropollutants (Plewa et al. 2004; Krasner et al. 2006). The kind of disinfectant to be used in each case depends on its bactericidal capacity and the method of administration to the wastewater as well as their potential ability to produce toxic by-products (Zanetti et al. 1996).

Effluents deriving from wastewater treatment plants constitute point sources for the disposal of emerging contaminants (organic trace pollutants and disinfection by-products) to the aquatic environment (Clara et al. 2005). Emerging contaminants are defined as previously unknown or unrecognised pollutants (Rodriguez-Mozaz et al. 2007) and could be comprised of compounds such as pharmaceuticals, sulphophenyl carboxylates, bacteria derived free molecules, steroids, antibiotics, pesticides, polychlorinated biphenyls, algal toxins and thousands of others. Despite the fact that most of them are present in the environment for a long time and are able to cause adverse health effects, they are generally not included in the legislation. On the other hand, the conventional methods for the monitoring of effluents are based mainly on the quantification of physicochemical parameters, while the presence of toxic emerging pollutants is usually not monitored.

Furthermore, the routine monitoring of reclaimed wastewater includes the analysis of microbiological load by the use of grab samples in order to detect standard indicator bacteria such as total or faecal coliforms (Harwood et al. 2004) but the presence of microorganism derived free molecules such as endotoxins is rarely considered. Endotoxins are components of lipopolysaccharide (LPS) complexes that constitute the outer layer of the cell wall of most Gram negative bacteria and some cyanobacteria, which are highly toxic inflammatory agents able to activate numerous cellular and humoral mediated systems (Morisson et al. 1994). Wichmann et al. 2004 indicated that the high endotoxin levels present in river-water samples isolated from the downstream of a wastewater treatment plant were responsible for in vitro proinflammatory cytokine production by human peripheral blood mononuclear cells.

Consequently traditional chemical analysis is not sufficient in order to depict the possible effects of reclaimed wastewater on the environment, while biological or ecotoxicological toxicity testing provides an overall direct estimation of the environmental hazard of effluents. These methods are based on the exposure of selected test species to samples and determination of certain end points such as lethal effect and growth ability (Ma et al. 2005). Whole effluent toxicity (WET) was incorporated in the US-EPA guidelines for effluent assessment. According to these guidelines effluent mixtures as a whole should be tested for their potential toxic effects as well as toxic interactions prior to and their discharge to the environment (US EPA 2002).

Therefore, there is a need to determine bioassays and sensitive biomarkers, based on in vitro bioassays that will be able to assess the environmental impact of wastewater effluents and thus the effectiveness of the tertiary wastewater treatment. In previous data we have already shown that mouse spleen cells, mitogenic responses as well as cytokine production could serve as sensitive bioassays and biomarkers for the evaluation of the quality of tertiary treated effluents (Kontana et al. 2008).

In the present work the efficiency of various tertiary treatment methods was investigated in order to define the optimum method or combination of methods that are able to eliminate toxic effects of the effluents. The aims of the work included (i) the examination of coagulation and activated carbon efficiency combined with ozonation on the physicochemical and microbiological characteristics of secondary treated effluents, (ii) the study of effluent ecotoxicity by using Vibrio fischeri, Daphnia magna, protozoa and three plant species as test organisms and immunological responses by using in vitro systems, including spleen cell proliferation as well as cytokine production and (iii) the comparison between chlorination and ozonation concerning the above parameters.

**METHODS**

**Wastewater samples collection**

Secondary municipal wastewater samples (15 L) were collected from the secondary sedimentation tank of a treatment plant within the area of Thessaloniki (Northern Greece). The plant is operating under the activated sludge
process and is performing biological nutrient removal, while disinfection is achieved by ozonation.

**Jar test, granular activated carbon, chlorination, ozonation and liquid sterilisation**

The examination of coagulation of municipal effluents was implemented by conducting laboratory-scale coagulation tests (jar tests). The applied coagulant was ferric chloride 0.1 M (FeCl₃·6H₂O, Fluka). The coagulation process consisted of a rapid agitation stage at 100 rpm for 2 min, followed by a second stage of slow agitation for 15 min at 50 rpm and a third step of sedimentation for 30 min. Activated carbon treatment was conducted through a Granular Activated Carbon (GAC, 830 NORIT) in a column of 30 cm height and 10 cm internal diameter. The contact time was 10 min. Chlorination was conducted by using sodium hypochloride solution of 11.5% Cl₂ and utilising 4 ppm of chloride under continuous rapid agitation for 10 min followed by slow agitation in order to remove residual chlorine. The choice of this process was based on both the typical chloride doses used in wastewater treatment plants for disinfection and to avoid any toxic responses derived from residual chloride (Abarnou & Miossec 1992). For the removal of residual chlorine slow agitation was used since in most cases there is no addition of chemicals to enhance the removal of residual chlorine in the wastewater treatment plants in Greece. Moreover, the addition of further chemical substances might have adverse effects on toxicity testing. Ozonation experiments were performed in a laboratory semibatch column reactor, which consisted of a 200 cm height cylindrical tube with a 4 cm internal diameter. A Schott ceramic porous diffuser was placed in the bottom of the reactor for ozone diffusion into the sample. Compressed and dried atmospheric air, at a flow rate of 3 L/min, was used for the production of ozone by an ozone generator (Model TOGC2A, Ozonia). The concentration of ozone in the feed gas was determined prior to the experiments and ozone dosage of 7.1 mg/L was applied. All experiments were performed at room temperature using 1 L sample, for reaction at the contact time of 5 min followed by 5 min of aeration in order to remove residual ozone. The conditions under which the ozonation experiments were performed were based on a previous study (Petala et al. 2008). Liquid sterilisation (LS) of ST and tertiary treated samples was performed in autoclave at 120°C and 1.1 atm, for 30 min. In total the following five combinations of tertiary treatment methods and types of disinfection were applied to the secondary treated effluent (ST): chlorination (Chl), ozonation (Ozon), coagulation-ozonation (Coag-Ozon), granular activated carbon-ozonation (GAC-Ozon) and coagulation-activated carbon-ozonation (Coag-GAC-Ozon).

**Physicochemical and microbiological parameters, endotoxin quantification**

Secondary and tertiary treated wastewater samples were analysed for biological oxygen demand (BOD), chemical oxygen demand (COD), nitrogen–ammonia, pH, orthophosphates and suspended solids (SS), according to Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF 1995). Bacteriological examination was performed using the multiple tube technique in a series of 10-fold dilutions, with five tubes per dilution, and quantified using the Most Probable Number (MPN). Lauryl sulphate broth (Merck KGaA, Germany) was used for the detection and enumeration of total coliforms (TC). E. coli confirmation was performed in EC broth (Merck KGaA, Germany) at 44.5°C in a water bath with the addition of Kovac’s reagent. Azide dextrose broth was used as a presumptive test followed by the confirmation test in kanamycin azide (KA) agar (Merck KGaA, Germany) for the detection and enumeration of enterococci (Zdragas et al. 2008).

The endotoxin concentrations were measured using the limulus amoebocyte lysate (LAL) from the certified European Endotoxin Testing Service CAMPREX, before and after sterilisation as recommended by the manufacturer.

**Toxicity testing**

The toxicity of wastewater samples was evaluated using the marine luminescence bacteria V. fischeri, originally in freeze-dried form and activated prior to use by a reconstitution solution. The changes in light emission of test organisms, obtained by their direct contact with the samples, were measured after exposure time of 30 min.
using the Microtox 500 Analyzer (SDI). For the determination of the toxic effects to *Daphnia magna* the Daphtoxkit F test by Microbiotests was used. The organisms were obtained in the form of ephippia, hatched for 3–4 days under adequate light and standard temperature. Five organisms were transferred to each plastic test well containing control medium and the samples. The toxic effect was evaluated as the percentage of nonviable/immobilised organisms after 24 h of exposure to the samples in the absence of light. In the microbiotest with the ciliate protozoan *Tetrahymena thermophila*, the growth inhibition of the ciliate protozoan was evaluated, after 24 h exposure in the undiluted wastewater sample, at 30°C in darkness. The test is based on the optical density measurement of the food substrate provided to the ciliates, in 1 cm disposable spectrophotometric cells.

Samples were tested for their phytotoxic properties, by using three plant organisms, i.e. *Alba sinapis*, *Shorgum saccharatum* and *Lepidium sativum* (Phytotoxkit supplied by Microbiotests) as test species. Ten seeds from each species were placed in flat shallow transparent test plates, composed of two compartments, the lower one able to maintain the reference OECD soil saturated to the water holding capacity. A control sample was also prepared using deionised water. All samples were prepared in triplicate and the average values are presented in the results. Test plates with the seeds were incubated for 3 d at 25°C. The inhibition of seed germination and of root growth was calculated by the following equation:

\[
I = \frac{A - B}{A} \times 100
\]

where:

- \( I \) = Inhibition (%)
- \( A \) = Mean seed germination or root length for the control soil
- \( B \) = Mean seed germination or root length for the examined mixture

**Mouse spleen cell culture**

Mouse spleen cells were cultured in RPMI-1640 complete medium containing 5 × 10⁻⁵ M 2-mercaptoethanol, 2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 24 mM NaHCO₃, supplemented with 5% foetal bovine serum (FBS). The same medium where indicated was also prepared using autoclaved ST or tertiary treated wastewater. Medium was also prepared as above using drinking water and served as control. For lymphoproliferation bioassay spleen cells were cultured in serial two-fold dilutions of the above medium, in triplicate, in 96-well microtitre plates, at a final cell density of 1 × 10⁶ cells/ml, in a total volume of 200 μl, at 37°C in 5% CO₂ atmosphere.

**Lymphoproliferation bioassay**

Splenocyte proliferation was determined 72 h after culture initiation as we previously described (Yiangou & Hadjipetrou-Kourounakis 1989). In all cultures 20 μl containing 0.4 μCi tritiated thymidine (³H-TdR) were added 18 h before cell harvesting and ³H-TdR incorporation was measured by liquid scintillation spectrophotometry. Results were then expressed as counts per minute (CPM).

**Conditioning medium (CM)**

Conditioning medium (CM) was prepared by culturing mouse spleen cells (5 × 10⁶ cells/ml) in RPMI-1640 complete medium containing 50% (v/v) treated or untreated wastewater for 48 h at 37°C and 5% CO₂ atmosphere. Thereafter, cell culture supernatants were harvested by centrifugation and passed through a 0.22 μm filter. The culture medium was prepared using autoclave sterilised secondary or tertiary treated wastewater, as described above.

**Bioassays to determine the interleukin-1 (IL-1) and interleukin-2 (IL-2) activity in CM**

The levels of IL-1 and IL-2 in the supernatants were assessed with bioassays as described from Paetkau et al. (1976) and Watson (1979) respectively. CM IL-1 activity was measured by the standard thymocyte proliferation assay, where thymocytes from 1–2-month-old rats were used. In particular 100 μl of serially diluted CM were added to triplicate wells of a 96-well culture dish. Equal volumes of 1 × 10⁵ rat thymocytes in 100 μl RPMI supplemented with 10% FBS were distributed into each well together with 20 μl RPMI containing 1.5 μg of Con-A. After 54 h incubation at...
37°C and 5% CO₂ air, the cells were pulsed with 0.4 μCi of \(^3\)H-TdR. Total \(^3\)H-TdR incorporation was measured 18 hours later as we previously described (Yiangou et al. 1993).

CM IL-2 activity was determined by measuring its effect on the proliferation of rat spleen blast cells which had been activated with Con-A. Briefly, 50 ml containing 2 × 10⁶ cells/ml spleen cell suspension in complete RPMI with 5% FBS and 2.5 μg/ml Con-A were distributed in tissue culture flasks. Cultures were incubated at 37°C, in 5% CO₂ and 48 h later the cells were collected, washed with complete RPMI with 10% FBS, containing 25 mM methylmannoside (to inactivate Con-A) and then resuspended in the above medium to concentration 0.5 × 10⁶ cells/ml. The cells were cultured in microtitre plates for 24 h in the presence of serial dilutions of the previously isolated supernatants. Then, 20 μl of 0.4 μCi of \(^3\)H-TdR were added to each well and cultured for another 18 h.

Both IL-1 and IL-2 cytokine levels were then determined by reciprocal analysis as we previously described (Yiangou & Hadjipetrou-Kourounakis 1989) and expressed as % of control.

### Statistical analysis

Statistical analysis was performed using the two-tailed Student's t-test and statistical significance was accepted for values of \(p < 0.05\).

### RESULTS & DISCUSSION

#### Physicochemical and microbiological parameters, endotoxin quantification

The physicochemical and microbiological parameters of the ST as well as tertiary treated effluents are shown in Table 1. More specifically, samples treated with Coag-Ozon, Coag-GAC-Ozon, GAC-Ozon, and Ozon eliminated the microbial load and decreased the nutrient and suspended solids concentrations. Disinfection applied with the chlorination method showed only a slight improvement of ST effluent chemical quality. On the other hand the application of ozonation contributed to the removal of nutrients and suspended solids. It should be underlined that suspended solids removal is considered as a side effect of ozonation treatment (Gottschalk et al. 2000). Previous studies reported that the application of coagulation, using FeCl₃ combined with ozonation, may contribute to the reduction of copper and zinc ions, suggesting reduction of toxicity when compared to secondary treated effluents (Petala et al. 2006). Chlorination as well as GAC were not able to reduce sufficiently endotoxin levels in ST effluents, while in the case of ozonation endotoxin levels were reduced up to 75%. Further reduction was observed when tertiary treatment with Coag-Ozon was applied. Sterilisation of the tertiary treated samples achieved by liquid sterilisation in an autoclave resulted in further reduction of endotoxin to almost zero levels. Chlorine and ozone may react with inorganic and organic compounds present in secondary treated wastewater resulting in the formation of disinfection by-products (DBPs) potentially toxic to the recipient ecosystem organisms (Paraskeva & Graham 2002). The data presented in Table 1 indicate that the application of coagulation, ozonation and liquid sterilisation proved to be the most appropriate treatment that efficiently reduced all physiochemical and microbiological parameters as well as endotoxin levels in ST municipal wastewater.

#### Toxicity testing

The results obtained from the battery of bioassays and phytotoxicity tests (root length growth) are shown in Figures 1 and 2 respectively. It is important to underline that great variation of effects was observed among the different species used. This fact indicates not only the different level of sensitivity in each species but also the necessity to apply a battery of toxicity tests in order to depict the whole range of effects on all trophic levels. ST effluent exhibited increased toxicity effects on all species, compared to the tertiary treated or disinfected samples. D. magna showed increased sensitivity to ST effluents than other species reaching up to 50% immobilisation/mortality. The food uptake of T. thermophila was least affected, while the bioluminescence of V. fischeri was inhibited by about 30% (Figure 1). Chlorination of the ST reduced the toxic responses of all organisms and plant species used (Figures 1 and 2). Ozonation of ST, with the exception of D. magna, reduced the toxic responses reaching up to negligible...
values. *T. thermophila* was not affected by any of the samples to which ozonation was applied. In line with previous data (Pessala et al. 2004), the results obtained from Microtox exhibited that in all cases where samples were treated with ozone stimulation responses on the bioluminescence of *V. fischeri* (hormesis) were observed. The presence of certain ions, such as sodium, potassium, chloride, glucose, and pollutants in sublethal concentrations, may induce the bioluminescence activity, resulting in possible masking of the toxic effect of samples (Dizer et al. 2002). The stimulatory effect of sublethal or low concentrations of toxic chemicals on organismal metabolism, referred to as hormesis, has been found to be common in the widely used *Vibrio fischeri* luminescence bioassay (Christofi et al. 2002). This response to a temporal disruption in homeostasis may have an advantage when adapting to low levels of biological stress, as this is sufficient to elicit overcompensation and, as a result, higher levels are less likely to affect the organism after this initial exposure (Stebbing 1997). Under these stressed conditions the organisms must repair the stress-induced damage to ensure survival, and so overcompensating such activities ensures that enough repair is completed to fulfil this, until homeostasis is once again reached (Calabrese et al. 1999).

The impact of ozone dosage and contact time on the residual ozone content was substantial, as the samples were

| Physicochemical and microbiological characteristics of the secondary and tertiary treated samples |
|---------------------------------|--------|--------|--------|--------|--------|
|                                 | ST     | Chl    | Ozon   | Coag-Ozon | GAC-Ozon | Coag-GAC-Ozon |
| **Total coliforms/dL**          | $4.3 \times 10^3$ | -      | -      | -      | -      | -      |
| **E. coli/dL**                  | $2.3 \times 10^3$ | -      | -      | -      | -      | -      |
| **Enterococci/dL**              | $1.5 \times 10^2$ | -      | -      | -      | -      | -      |
| **Endotoxin (Eu/mL)**           |              |        |        |        |        |        |
| Without sterilis.               | 171.9   | 142    | 41.7   | 7      | 50     | 31.1   |
| Sterilis.                       | 47.7    | 10.1   | 1.5    | 0.36   | 1.72   | 0.9    |
| **pH**                          | 7.5     | 6.1    | 7.3    | 6.9    | 7.1    | 6.7    |
| **Biological oxygen demand, BOD₅ (mg/L)** | 10      | -      | -      | -      | -      | -      |
| **Chemical oxygen demand, COD (mg/L)** | 70      | 59     | 5      | 3      | 2      | 2      |
| **NH₃-N (mg/L)**                | 8       | 7      | 4      | 3      | 3      | 3      |
| **PO₄ (mg/L)**                  | 14      | 14     | 6      | 1      | 3      | 1      |
| **Suspended solids, SS (mg/L)** | 20      | 20     | 15     | 0.2    | 0.2    | 0.2    |

Figure 1 | Inhibition effects on the organisms used in battery of tests (%).
Figure 2 | Phytotoxicity results, root length growth inhibition (%).
aerated in order to remove residual ozone. Leynen et al. (1998) reported that the mean 48 h EC50 value for *D. magna* exposed to ozonated cooling water was as low as 0.035 mg O3/L. Similarly in chlorination, slow agitation was applied in order to remove residual chloride.

The species tested exhibited variable sensitivity, as they showed different responses. *Daphnia magna* seemed to be the most sensitive species, while all the species tested in phytotoxicity showed negligible to moderate inhibition root length growth. This fact underlines the necessity of incorporating more sensitive bioassays in the integrated monitoring of reclaimed wastewater intended for reuse.

**Effect of ST and tertiary treated effluents on splenocyte mitogenic responses and cytokine production**

The lymphoproliferative assay revealed that culturing the mouse spleen cells in RPMI-1640 medium, prepared with sterilised in autoclave (1.1 atm for 30 min) ST effluent, resulted in increased proliferative responses, as these were determined by ³H-TdR incorporation (Figure 3). This finding confirms our previous studies (Kontana et al. 2008) and suggests that ST may contain mitogenic factors able to activate blastogenesis of splenocytes. The increased lymphoproliferative responses using ST effluent are due to certain constituents present in wastewaters, since serial two-fold dilutions (1/2–1/32) of the ST-LS medium resulted in gradual reduction of splenocyte responses (Figure 3). Maximum activation of the splenocytes was detected at the 50% effluent concentration, suggesting that the undiluted samples consist of components that potentially suppress the cells of the immune system (Figure 3). Disinfection of the ST effluents with ozone significantly reduced the mitogenic activity of splenocytes while disinfection with chlorination moderately reduced the above response. Additionally, the magnitude of mouse spleen cells, proliferative response is directly related to the levels of endotoxin present in the samples. Endotoxin activates *in vitro* several cell lines such as human myeloid cell lines; U937, HL-60, hpMN and hpPMN, (De et al. 2005) as well as human peripheral blood cells (Abdolkhaleg & Shokooh 2009). Thus, these lines may be used as indicator cells to detect endotoxin activity in completely disinfected and sterile wastewater samples.

It is well documented that the *in vitro* proliferative responses of splenocytes to mitogens such as Concanavalin-A or LPS are mediated by cytokines such as IL-1 and IL-2 (Vı́ctor & De la Fuente 2003). IL-1 and IL-2 activity was determined in Conditional Medium (CM) obtained from cell cultures in medium prepared with the ST, Chl, Ozon and the GAC-Ozon sample. ST-CM as well as Chl-CM exhibits increased IL-1 activity (Table 2) while IL-2 activity was not affected (Table 2), suggesting that physicochemical wastewater parameters mainly activate the B lymphocytes rather than the T lymphocytes. More specifically, one of the most important parameters for these responses may be the microbially derived endotoxin, which in the present study was detected at high levels (Table 1). On the other hand, in the samples subjected to ozonation no induction of either IL-1 or IL-2 production was observed, suggesting that this type of disinfection/treatment is much more effective since the obtained effluent contains low levels of molecules able to initiate mild immune response to mice immune cells (Table 2). Figure 3 shows that lymphoproliferative responses of splenocytes to Ozon are increased by about 2–4-fold while no IL-1 or IL-2 production was observed in response to the same effluent (Table 2). Thus lymphoproliferative bioassay compared to cytokine bioassays is more sensitive to detect immune cell activation agents present in wastewater. However, the cytokine bioassays included in our study provide information concerning the presence of
active molecules able to stimulate immune responses. However, ELISA enzyme-linked immunosorbent assay could be used to detect only cytokine concentration in conditioning medium.

Furthermore, the above data indicate that microorganism-derived endotoxin is a widely distributed emerging contaminant in municipal wastewater and thus very probable to enter the aquatic environment resulting in the induction of cell proliferation as well as production of proinflammatory cytokines. The findings in the present study strongly suggest that endotoxin should be considered in routine monitoring of municipal wastewater quality, in order to apply appropriate treatment techniques for its removal. Both ozonation and liquid sterilisation exhibited efficient reduction of endotoxin levels or enabled their inactivation.

Ozonation compared to chlorination as a method of disinfection of secondary treated wastewater samples is significantly more effective. Despite the elimination of the microbial load, ozonation leads to the substantial reduction of the endotoxin levels, spleen cell mitogenic responses and IL-1 production as well as the improvement of the chemical characteristics of the effluents.

CONCLUSIONS

A battery of bioassays and biomarkers is required to evaluate the effectiveness of tertiary treatment of municipal wastewaters. Microtox test was not sensitive for detecting the toxicity of tertiary treated and disinfected effluents; however, its hormesis response may indicate the adverse effects of possible disinfection by-products or sublethal concentrations of various contaminants. Therefore, Microtox could serve as a useful (and rapid) tool for screening purposes; however, it should be accompanied by more sensitive bioassays. Spleen lymphoproliferative responses and cytokine production were proved to be more sensitive to assess the treatment efficiency of the reclaimed wastewaters intended for reuse. Ozonation as well as liquid sterilisation by autoclave removed endotoxin from the ST effluents more efficiently than chlorination, improving the quality of reclaimed wastewaters. Endotoxin derived from microorganisms contaminates urban wastewater environment and should be considered in routine monitoring.

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