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Environmental Management of Wastewater Treatment Plants – the Added Value of the Ecotoxicological Approach

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1. Introduction

Pollution control has been changed by advances in scientific knowledge, because there is a connection of environmental contamination with the ability to measure it. With greater understanding of the impact of wastewater on the environment and more sophisticated analytical methods, advanced treatment is becoming more common (Lofrano & Brown, 2010).

The assessment of biological effects of wastewater discharges in the ecosystems is today considered relevant and ecotoxicological tests identifying the ecological hazard are useful tools for the identification of environmental impacts. Direct toxicity assessment, making use of ecotoxicological tests, can play an important role in supporting decision-making, either regulatory driven or on a voluntary basis.

Within the Integrated Pollution Prevention and Control Directive - IPPC, 2008/1/EC (European Commission [EC], 2008), the Direct Toxicity Assessment concept has been included as a suitable monitoring tool on effluent in several Best Available Techniques (BAT) Reference Documents. Also, in Water Framework Directive – WFD, 2000/60/EC (EC, 2000), direct toxicity assessment of Wastewater Treatment Plant (WWTP) effluents can contribute to attain or keep ecological quality objectives in water masses. So, for EU countries to comply with good ecological status, ecotoxicity evaluation of WWTP effluents is extremely relevant.

In many countries ecotoxicity tests are already in use for wastewater management (Power & Boumphrey, 2004; Tinsley et al., 2004; United States Environmental Protection Agency [USEPA], 2004; Vindimian et al., 1999). Bioassays are also used for wastewater surveillance and BAT compliance by authorities in Germany (Gartiser et al., 2010a). A global evaluation of wastewaters should include ecotoxicological tests to complement the chemical characterization, with advantages especially in the case of complex wastewaters (Mendonça et al., 2009). This approach has advantages particularly to protect biological treatment plants from toxic influents (Hongxia et al., 2004), to monitor the effectiveness of WWTP (Cêbere et al., 2009; Daniel et al., 2004; Emmanuel et al., 2005; Libralato et al., 2006; Metcalf & Eddy, 2003) and in the impact assessment of complex wastewaters. Bioassays are considered a suitable tool for assessing the ecotoxicological relevance of complex organic mixtures (Gartiser et al., 2010b).

As it is often referred (e.g. Metcalf & Eddy, 2003; Movahedian et al., 2005; Teodorović et al., 2009), physico-chemical parameters alone are not sufficient in obtaining reliable information on treated wastewater toxicity and toxicity tests must be performed in combination with routine chemical analysis. The prediction of toxicity from chemical data is considered limited and the better coincidence between the toxicity and chemical-based assessments were achieved when information from all tests in a test-battery was assembled (Manusadžianas et al., 2003).

In the framework of Life Cycle Assessment (LCA) comprehensive analysis of WWTP is evaluated for the physico-chemical characterization of the wastewaters as well as the inventory of inputs and outputs associated with the global process (Hospido et al., 2004). In a recent work Life Cycle Impact Assessment was done using emerging pollutants quantification to rank potential impacts in urban wastewater (Muñoz et al., 2008). A step forward in this approach would be to use ecotoxicological indicators.

In the last ten years and in the framework of European and National contracts developed in Lisbon area (Portugal) studies were conducted on the integrated evaluation of the ecotoxicological and physicochemical parameters of wastewaters from treatment plants receiving domestic and industrial effluents. The evaluation of ecotoxicological data from four of these WWTP was the main aim of this study. Data from acute tests with different species (bacteria, algae, crustaceans and plants) are discussed.

2. Material and methods

2.1 Wastewater treatment plants

The characteristics of the four WWTP that receive domestic and industrial wastewaters are presented in Table 1. These systems differ from each other, namely in the magnitude of flows (the daily flow goes from 16 000 m³/day to 155 000 m³/day), the treatment level implemented (from preliminary treatment to tertiary treatment) and the site of discharge (river, estuary or coastal area).

	WWTP 1	WWTP 2	WWTP 3	WWTP 4
Population equivalent	130 000	700 000	800 000	250 000
Flow (m³/day)	16 000	70 000	155 000	54 500
Treatment type	secondary	tertiary	preliminary	tertiary
Discharge	River	River	Sea	Estuary

Table 1. General information on the Wastewater Treatment Plants (WWTP)

2.2 Wastewater sampling

Wastewater samples were collected with different strategies and periodicities in the different Treatment Plants:

- WWTP1 and WWTP2 – Influent and effluent 24h-composite samples collected seasonally in November, March, September and December 2003/2004;
- WWTP3 – Effluent 24h-composite sample collected monthly from 2006 to 2009;
- WWTP4 – Influent and effluent 1h-composite samples collected in different days of the week (Monday, Tuesday and Friday) at 10 h, 14h and 23h in April 2010.

As presented in Figure 1, sampling point for WWTP1 was after secondary treatment, for WWTP2 after tertiary treatment, for WWTP3 after preliminary treatment and for WWTP4 after primary treatment.

Each sample was divided into subsamples, kept frozen (-20°C) for ecotoxicological analysis for no more than 1 month.
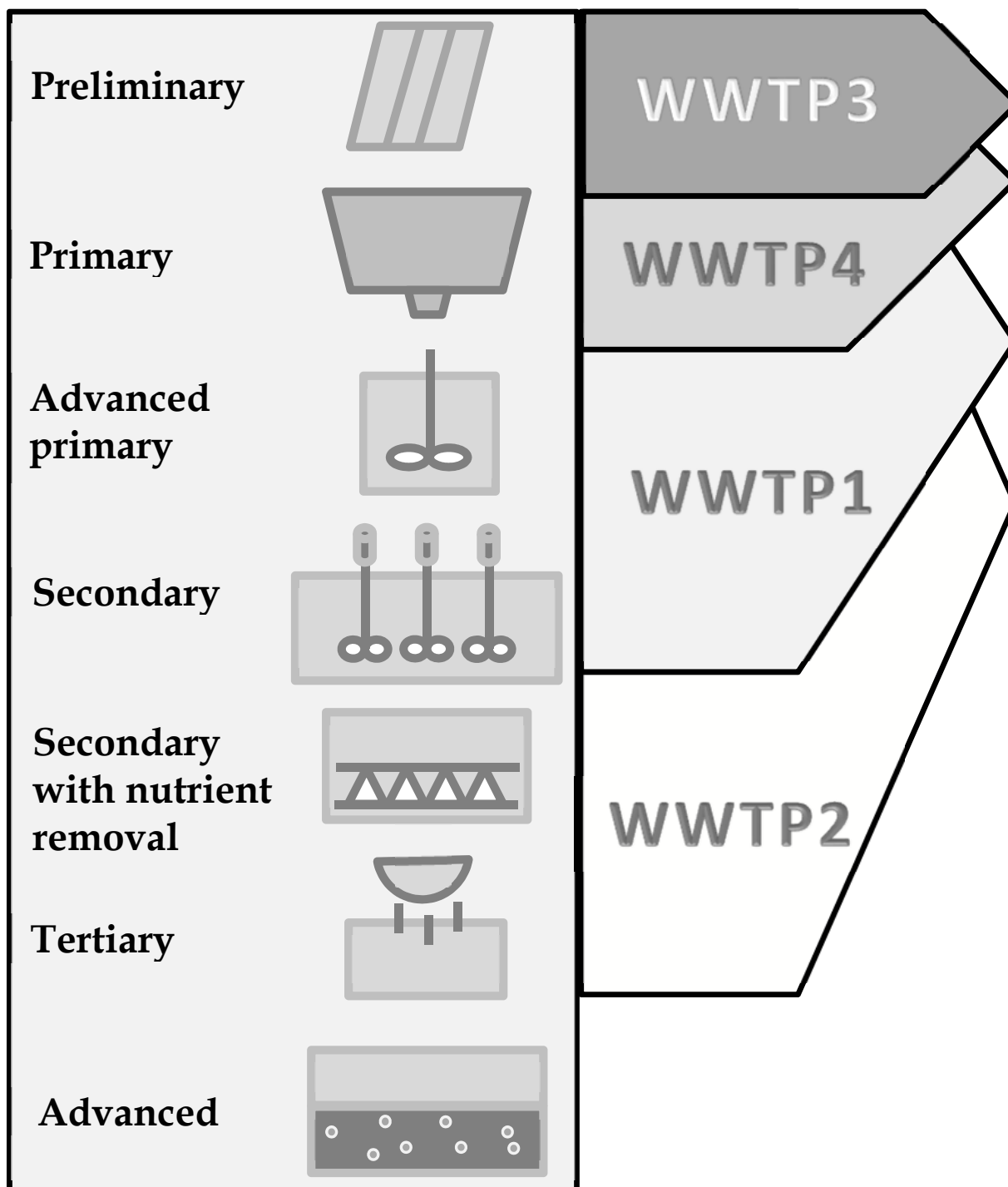

Fig. 1. General Scheme of WWTP treatment process and identification of the level of treatment analyzed in each Treatment Plant.

2.3 Ecotoxicity tests

Ecotoxicological evaluation of the samples was performed using *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Thamnocephalus platyurus*, *Daphnia magna* and *Lemna minor* as test organisms, to assess acute aquatic toxicity, according to the following methods:

- Microtox test: Bacterial toxicity was assessed by determining the inhibition of the luminescence of *Vibrio fischeri* (strain NRRL B-11177) exposed for 15 minutes (Microtox® Test, Microbics, Carlsbad, U.S.A.). The test was performed according to the basic test procedure (Microbics, 1992);
- AlgalTox test: Algal toxicity was assessed by measuring the growth inhibition of *Pseudokirchneriella subcapitata* exposed for 72 hours, according to AlgalToxKit F™ test procedure (Microbiotests, 2004) that follow the OECD guideline 201 (Organisation for Economic Co-operation and Development [OECD], 1984). Optical density (OD 670 nm) of algae suspensions was determined;
- ThamnoTox test: Crustacean toxicity was assessed by determining the mortality of *Thamnocephalus platyurus* exposed for 24 hours according to ThamnoToxKit F™ test procedure (Microbiotests, 2003);
- Daphnia test: Crustacean toxicity was also assessed by determining the inhibition of the mobility of *Daphnia magna* (clone IRCHA-5) exposed for 48 hours, according to ISO 6341:1996 (International Organization for Standardization [ISO], 1996). Juveniles for testing were obtained from cultures maintained in the laboratory;
- Lemna test: Plant toxicity was assessed by determining the growth inhibition of *Lemna minor* (clone ST) exposed for 7 days, according to ISO 20079: 2005 (ISO, 2005). Plants for testing were obtained from cultures maintained in the laboratory. Total frond area was used as growth parameter, quantified by an image analysis system – Scanalyzer (LemnaTec, Würselen, Germany).

All samples were tested with Microtox, Daphnia and Lemna tests. For WWTP1, WWTP2 and WWTP4 samples, AlgalTox and ThamnoTox tests were also performed.

2.4 Data analysis

For each toxicity test EC_{50}-t or LC_{50}-t, the effective concentration (% v/v) responsible for the inhibition or lethality in 50% of tested population after the defined exposure period (t), was calculated:

- EC_{50}-72h for AlgalTox test, LC_{50}-24h for ThamnoTox test and EC_{50}-48 h for Daphnia test by using Tox-Calc™ software (version 5.0, Tidepool Scientific software, 2002);
- EC_{50}-7d for Lemna test by using Biostat 2.0 software (LemnaTec 2001);
- EC_{50}-15 min for Microtox test by using Microtox Omni™ software (Azur Environmental, 1999).

To obtain a direct interpretation between values and toxicity, ecotoxicity test results are in this work presented in Toxic Units (TU), calculated as $TU = 1/EC_{50} \times 100$. Aiming to include all raw data for TU calculation and for statistical analysis, EC_{50} values not determined due to low effect levels were considered as 100%. For data analysis, values lower than 1 TU were considered as 0.5 TU.

The tests sensitivity was assessed by Slooff's index (Slooff, 1983): each single test result (expressed as EC_{50} or LC_{50}) is divided by the arithmetic mean of all test results for each sample, and the geometric mean of these ratios for each test is calculated. The smaller value stands for the more sensitive test. The Slooff's index was calculated for Microtox, AlgalTox, ThamnoTox, Daphnia and Lemna tests.

Pearson correlations were determined for WWTP3 using statistical analysis software (JMP®
5.0.1) for the 48 samples on the following 4 variables:

- Wastewater flow (pers. comm.);
- Ecotoxicological data from Microtox, Daphnia and Lemna tests.

3. Results and discussion

Aiming to assess direct toxicity of samples from four WWTP we evaluated data from acute
tests with different species: bacteria, algae, crustaceans and plants. The results are presented
in Tables 2 to 5.

Results obtained for WWTP1 (Table 2) show clearly that influent and effluent samples have
different toxicity levels to the species tested, except for Lemna that shows no toxicity both
for influent and effluent samples.

	Sample	Microtox	AlgalTox	ThamnoTox	Daphnia	Lemna
Influent	**Nov 03**	27.0		3.8	<1	<1
	Mar 04	19.2	5.0	2.3	1.4	
	Sep 04	5.6	<1	7.1	4.8	<1
	Dec 04	11.5	1.8	1.7	2.4	<1
Effluent	**Nov 03**	<1		<1	<1	<1
	Mar 04	1.9	<1	2.2	<1	<1
	Sep 04	<1	<1	<1	<1	<1
	Dec 04	<1	<1	<1	<1	<1

Table 2. Values for ecotoxicological tests in Toxic Units (TU) obtained for WWTP1 influent
and effluent samples

For WWTP2 (Table 3), influent and effluent samples have also different toxicity levels to the
species tested, except for AlgalTox that shows no toxicity both for influent and effluent
samples. The effluent samples show in this case no toxicity in all the tests performed.

	Sample	Microtox	AlgalTox	ThamnoTox	Daphnia	Lemna
Influent	**Nov 03**	17.2		3.7	1.2	1.1
	Mar 04	62.5	<1	3.0	1.4	1.4
	Sep 04	47.6	<1	2.0	1.8	1.1
	Dec 04	83.3	<1	1.6	2.5	<1
Effluent	**Nov 03**	<1		<1	<1	<1
	Mar 04	<1	<1	<1	<1	<1
	Sep 04	<1	<1	<1	<1	<1
	Dec 04	<1	<1	<1	<1	<1

Table 3. Values for ecotoxicological tests in Toxic Units (TU) obtained for WWTP2 influent
and effluent samples

For WWTP3 (Table 4), effluent samples have different toxicity levels to the species tested, with Microtox having the higher TU values along the four years. No significant correlations were obtained between toxicity test results and corresponding daily discharge flow.

	Microtox				Daphnia				Lemna			
	2006	**2007**	**2008**	**2009**	**2006**	**2007**	**2008**	**2009**	**2006**	**2007**	**2008**	**2009**
Jan	16.3	14.5	5.9	33.3	3.2	1.4	2.4	1.5	1.6	<1	<1	<1
Feb	4.6	13.2	6.4	10.8	2.9	1.0	1.3	<1	1.2	<1	1.6	<1
Mar	2.2	10.4	15.6	11.6	1.4	2.0	2.9	1.8	<1	<1	1.3	1.0
Apr	8.1	8.4	14.9	10.9	1.4	1.9	2.6	2.5	1.4	<1	<1	<1
May	27.8	14.7	14.5	12.5	4.6	3.1	4.8	1.7	1.6	1.1	<1	1.0
Jun	32.3	16.4	13.2	10.3	7.1	2.6	2.1	1.2	<1	1.4	<1	1.0
Jul	13.5	25.0	19.2	22.2	6.6	2.2	1.2	<1	<1	1.1	1.1	1.0
Aug	14.5	12.2	19.2	4.1	3.2	3.1	3.6	2.2	<1	1.2	1.0	1.1
Sep	25.6	12.7	20.4	5.1	8.1	3.2	1.6	1.5	<1	1.4	<1	<1
Oct	17.5	7.8	31.3	10.0	2.6	1.5	1.5	1.3	1.1	<1	<1	<1
Nov	18.9	13.0	83.3	15.4	3.4	3.1	2.9	4.3	<1	1.3	1.0	1.1
Dec	16.7	4.7	71.4	9.4	3.2	1.4	3.2	<1	1.2	1.4	<1	<1

Table 4. Values for ecotoxicological tests in Toxic Units (TU) obtained for WWTP3 effluent samples.

No time pattern for effluent toxicity was observed in WWTP3. Between October 2008 and January 2009, the effluent samples were particularly toxic to the bacteria, with 83.3 TU in November 2008 (Figure 2).

For WWTP4 (Table 5), the difference in toxicity levels is not so clear between untreated and treated wastewater samples although for Microtox the range of values is higher for the untreated samples [5.8 TU - 93.5 TU] versus treated samples [2.3 TU – 35.8 TU].

During the week monitoring, the highest TU value was obtained on Friday night for Microtox. A peak in toxicity was obtained for Microtox in all samples collected at 23h. This is in line with Chapman (2007) that concludes that difficulties in obtaining representative samples arise in WWTP effluents, whose composition is highly variable, and repeated testing is required.

Analyzing the mean TU values obtained in the different tests, Microtox test shows higher values in all WWTP, followed by the crustacean tests. Low toxicity values were obtained in the plant and algae tests (Figure 3).
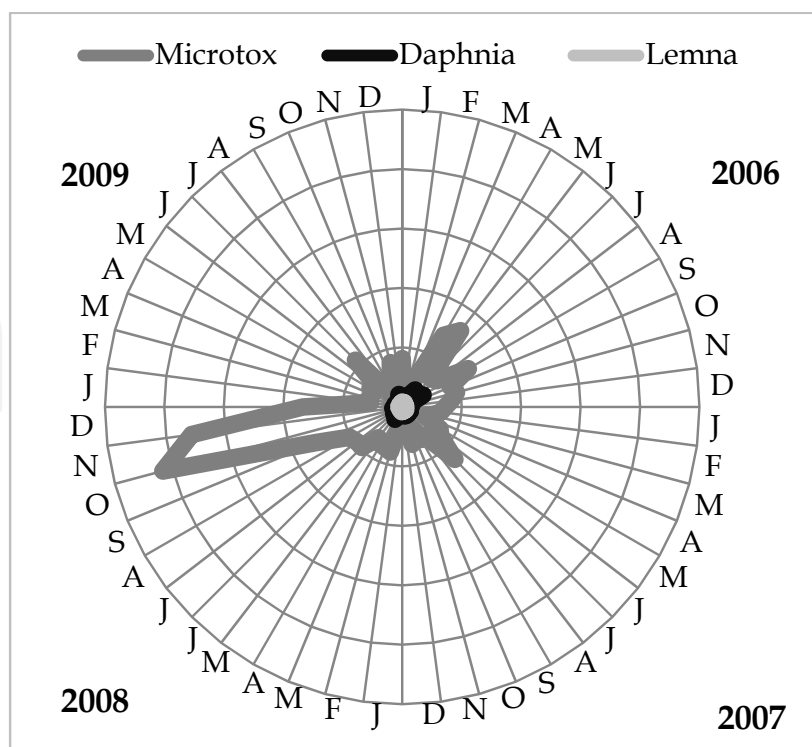
Fig. 2. Distribution of sample toxicity in Toxic Units (TU) for WWTP3 monthly samples from 2006 to 2009.

	Sample	Microtox	AlgalTox	ThamnoTox	Daphnia	Lemna
Influent	**Mon-10h**	5.8	<1	2.8	<1	1.3
	Mon-14h	19.4	<1	3.0	<1	<1
	Mon-23h	32.7	<1	3.6	1.5	1.1
	Tues-10h	13.9	<1	2.7	<1	1.3
	Tues-14h	12.6	<1	2.8	<1	<1
	Tues-23h	46.5	<1	3.4	1.9	<1
	Fri-10h	17.9	<1	2.6	3.6	<1
	Fri-14h	43.9	<1	2.5	1.9	<1
	Fri-23h	93.5	<1	2.4	1.4	<1
Effluent	**Mon-14h**	2.3	<1	2.8	<1	<1
	Mon-23h	11.1	<1	3.0	1.1	1.1
	Tues-10h	2.9	1.1	1.8	<1	1.3
	Tues-14h	4.8	<1	2.8	<1	1.3
	Tues-23h	17.8	<1	2.4	1.5	<1
	Fri-10h	16.6	<1	2.1	1.5	<1
	Fri-14h	11.4	<1	2.2	1.1	<1
	Fri-23h	35.8	<1	2.3	<1	<1

Table 5. Values for ecotoxicological tests in Toxic Units (TU) obtained for WWTP4 influent and effluent samples
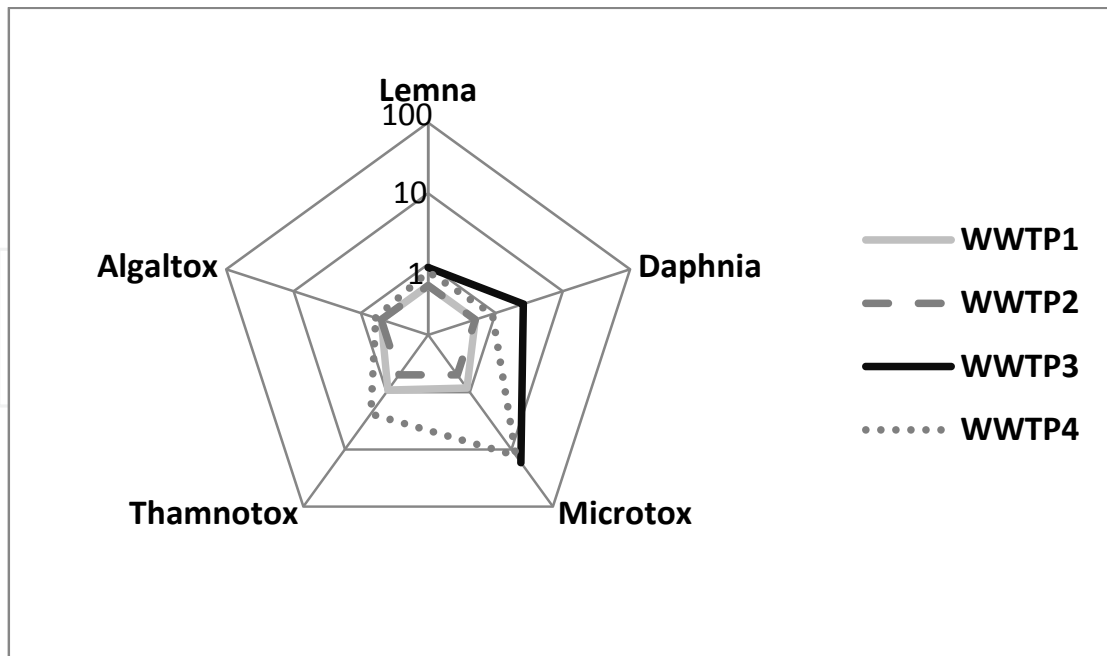
Fig. 3. Mean Toxic Units (TU) values for the tested species and for all effluent samples.

The acute toxicity is dependent on the treatment level of the studied WWTP and the species tested (Figure 3). TU values for Microtox and ThamnoTox are higher in the case of WWTP3 and 4, with preliminary and primary levels of treatment, respectively. The used tests are able to distinguish the different levels of treatment, with the exception of AlgalTox.

From data presented in Figure 4, toxicity removal was obtained for all the WWTP where input and output wastewaters were monitored. For WWTP4 – primary treatment – removal values were in the range 15-60%. For the WWTP with secondary (WWTP1) and tertiary (WWTP2) levels of treatment toxicity removal evaluated by both crustaceans is similar, only the bacteria achieve to detect higher efficiency (100%) with the tertiary treatment. Tyagi et al. (2007) found that the mean percentage removal in toxicity for *D. magna* after primary, secondary and tertiary treatment were 29%, 76% and 100%, respectively. Also Movahedian et al. (2005) reinforces that toxicity removal increases with the level of treatment (e.g. 8% for preliminary treatment and 38% for primary treatment).

A wastewater classification adapted from Tonkes et al. (1999) to the TU values, is as follows: samples with less than 1 TU are considered non toxic; between 1 and 10 TU are considered slightly toxic; with more than 10 TU are considered toxic. Values higher than 10 TU were obtained for Microtox test in 69% of the samples tested. Values between 1 and 10 TU were obtained for 79% of the samples for ThamnoTox and 74% of the samples for Daphnia. No toxicity to the alga and to the plant was registered for the majority of samples, respectively 90% and 65%.

Slooff's sensitivity index calculated for this group of acute test results shows that the bacterium *Vibrio fischeri* is the most sensitive species, and allows to establish the following gradient of test sensitivity, Microtox > ThamnoTox > Daphnia > AlgalTox > Lemna, from the corresponding Slooff's index values 0.2 < 0.7 < 1.0 < 1.4 < 1.6.

The sensitivity of Microtox test and the reliability of this test in monitoring toxicity of treatment plant wastewaters have also been observed by other authors (Araújo et al., 2005; Libralato et al., 2006; Lundström et al., 2010b). Related to the crustacean toxicity several authors concluded that *Daphnia magna* acute test can be a useful analytical tool for early

warning system to monitor the different operational units of wastewater treatment plants (Movahedian et al., 2005; Tyagi et al., 2007) or to use in toxicity identification evaluation procedures (Hongxia et al., 2004). Also a study with a copepod as test organism showed that conventionally treated sewage effluent resulted in the most negative effects leading to the conclusion that additional treatments created effluents with less negative impacts (Lundström et al., 2010a).
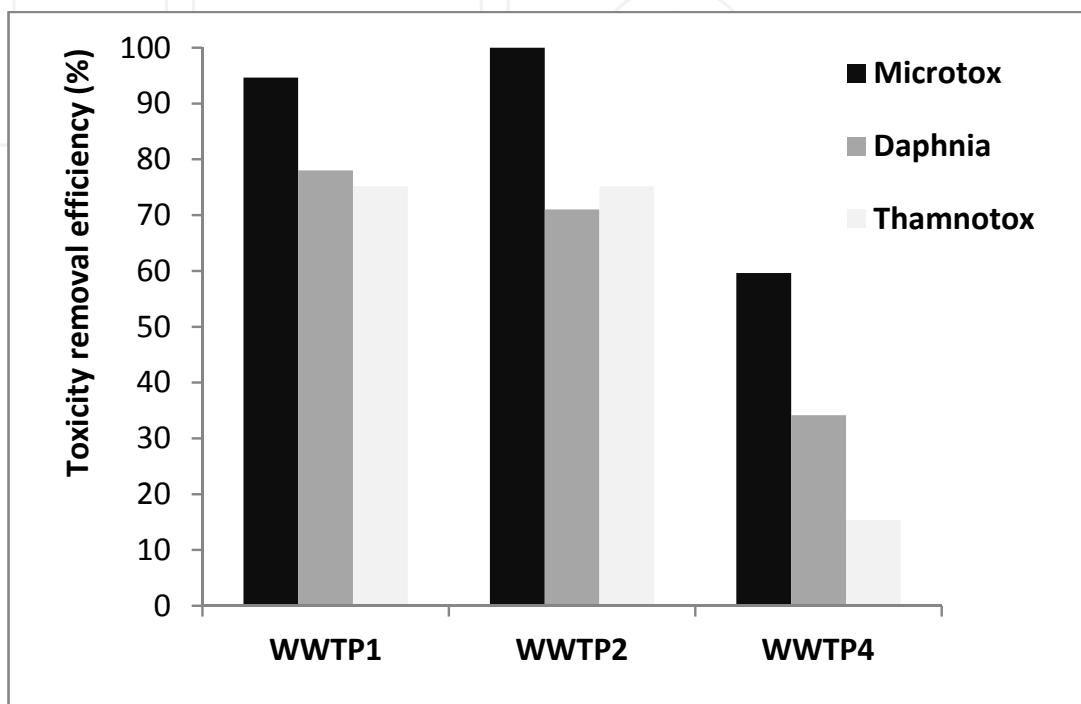

Fig. 4. Toxicity removal efficiency evaluated in WWTP 1, 2 and 4, for Microtox, Daphnia and ThamnoTox tests.

Though we found low sensitivity of *Lemna minor* in WWTP toxicity evaluation, the ecotoxicological assessment of pharmaceutical and food industries effluents using *Lemna minor* as a test organism was considered suitable by Radić et al. (2010) that demonstrated the relevance of *Lemna* as a sensitive indicator of water quality. In nutrient rich wastewaters, although the algae test can be sensitive, it might not be the most appropriate test because of the complex relationship of inhibition and promotion of algae growth often observed (Gartiser et al., 2010a).

When using the wastewater classification for the most sensitive species, in this study the bacteria *V. fischeri* used in the Microtox test, and considering all the WWTP under study, the distribution of toxicity level of treated samples in percentage is in accordance with the treatment process level implemented (Figure 5). For a tertiary treated effluent 100% samples are non toxic and for a preliminary treated effluent 75% are toxic.

Concerning WWTP systems and considering the relative sensitivity of the organisms used in wastewater testing and the importance to consider effects at different trophic levels, the test battery proposed in a previous work (Mendonça et al., 2009) for characterization of WWTP discharges included tests with a bacterium, an alga and a crustacean to monitor this type of wastewaters. For a screening only one test with the most sensitive species, Microtox, was proposed.
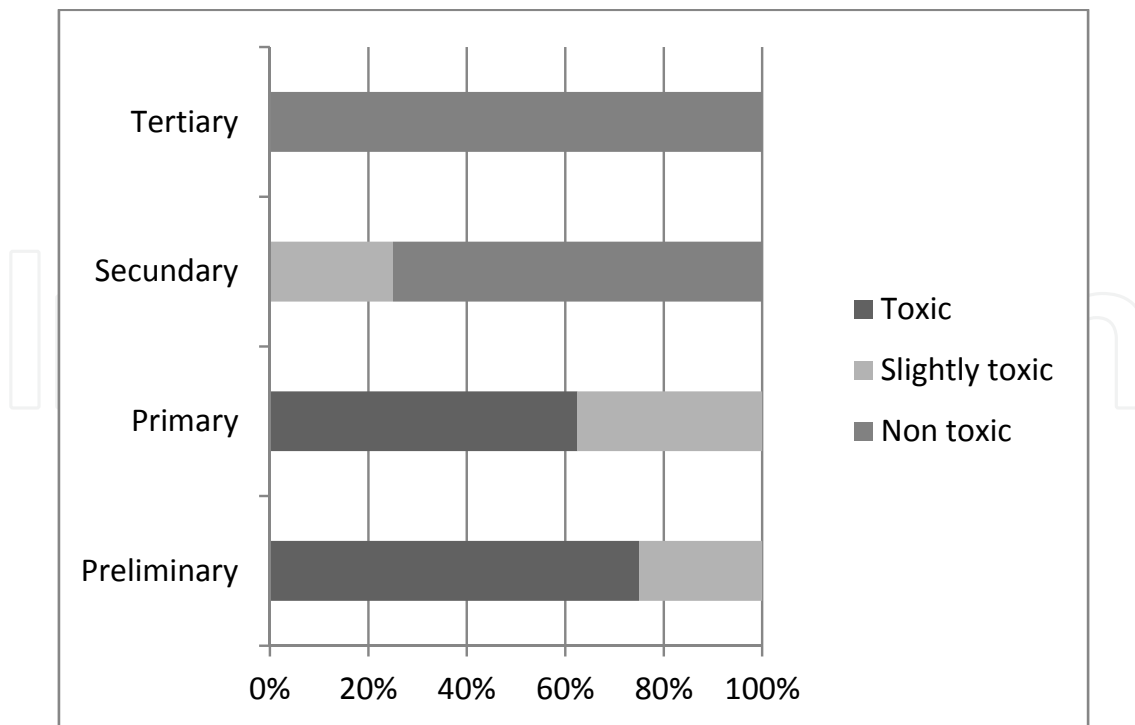
Fig. 5. Distribution of treated samples according to toxicity level for the more sensitive species - Microtox, and Wastewater Treatment Plant process level.

Once secondary and tertiary treatment are employed, the prevention of eutrophication became the next goal for wastewater treatment, requiring the removal of nitrogen, phosphorous or both (Lofrano & Brown, 2010).

On the other hand, little is known about the potential interactive effects of organic wastewater contaminants, namely steroids and hormones present in municipal effluents, when in complex mixtures that may occur in the environment and about their effect on human health (Filby et al., 2007). Chronic toxicity test and endocrine disruption assay of WWTP effluent samples indicated that, in a long term, potential population effects could arise in the receiving waters (Mendonça et al., 2009). Kontana et al. (2008) in an ecotoxicological assessment of municipal wastewater using several test organisms including *Vibrio fischeri* and *Daphnia magna*, observed a decrease of ecotoxicological responses for all bioassays but also the induction of immune response after tertiary treatment, pointing to the need of using sensitive biomarkers if wastewaters are intended for reuse.

Considering ecotoxicity testing as an integral part of the toolbox to investigate the environmental impacts of effluents but knowing that it can be complex, time consuming and expensive, a tiered approach is recommended when defining a realistic assessment strategy (European Centre for Ecotoxicology and Toxicology of Chemicals [ECETOC], 2004; OSPAR Convention for the Protection of the marine Environment of the North-East Atlantic [OSPAR], 2007). The validity of the use of acute tests to drive environmental improvement has been demonstrated, but methodologies for chronic toxicity need further development.

4. Conclusion

This work shows that wastewater acute toxicity is dependent on the treatment level of the WWTP and the species tested. The bacterium *Vibrio fischeri*, the test organism in Microtox test, proved to be the most sensitive species in wastewater ecotoxicological evaluation.

The distribution of treated samples according to the toxicity level to the most sensitive species clearly reveals the treatment process level implemented. All the used tests, with the exception of AlgalTox test, are able to distinguish the different levels of treatment and to assess toxicity removal efficiency.

The ecotoxicological approach proves to have an added value to hazard and risk assessment of discharges to the receiving waters and environmental management of the Wastewater Treatment Plant can use this tool with advantages. Even if a preliminary treatment in the WWTP is associated with the discharge in a submarine outfall, environmental monitoring including toxicological parameters proves to be important.

The inclusion of these ecological relevant data in the assessment of the grey water footprint for point sources of water pollution, like WWTP, can be the next step to have good indicators of the degree of water pollution.

5. Acknowledgment

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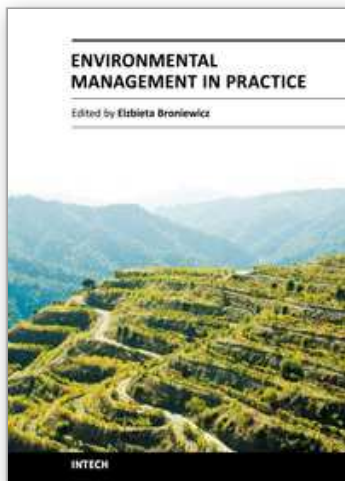
Environmental Management in Practice

Edited by Dr. Elzbieta Broniewicz

In recent years the topic of environmental management has become very common. In sustainable development conditions, central and local governments much more often notice the need of acting in ways that diminish negative impact on environment. Environmental management may take place on many different levels - starting from global level, e.g. climate changes, through national and regional level (environmental policy) and ending on micro level. This publication shows many examples of environmental management. The diversity of presented aspects within environmental management and approaching the subject from the perspective of various countries contributes greatly to the development of environmental management field of research.

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