The role of ecotoxicological evaluation in changing the environmental paradigm of wastewater treatment management

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ABSTRACT

Wastewater Treatment Plants (WWTP) discharge complex effluents raising special concern and direct toxicity assessment can be an added value strategy in WWTP environmental protection. The EU project "Integrated approach to energy and climate changes: changing the paradigm of wastewater treatment management" was set up for a WWTP discharging into Tagus estuary (Portugal). The main objectives of the project are to implement a tool to optimize the management of WWTP in terms of energy efficiency and environmental impact and to reduce the environmental costs of the treatment process. Setting up adequate ecotoxicological methodologies and selecting a battery of ecotoxicity tests to be used in the characterization of WWTP wastewaters are the aim of this study. The results of a battery of tests demonstrated not only that the treatment efficiently reduced wastewater toxicity, but also that the use of an ecotoxicological approach can contribute to the environmental management of the Treatment Plant.

INTRODUCTION

The major problem in controlling wastewater discharges is related to its environmental toxicity. Ecotoxicological evaluation became as relevant to the protection of ecological systems as chemical specific evaluation after the implementation of the Water Framework Directive – WFD, 2000/60/EC [1]. Wastewater Treatment Plants (WWTP) discharge complex effluents to the receiving waters raising special concern. Direct toxicity assessment can be an added value strategy when we face complex effluents for which many chemicals cannot be quantified and/or interactive effects are likely to be significant, e.g. [2,3]. In many countries ecotoxicity tests are in use for wastewater management [4] or Best Available Techniques compliance [5]. Ecotoxicological evaluation has advantages to protect biological treatment plants from toxic influents [6] and to monitor the effectiveness of WTTP [2, 7-11].
The EU project (2010-2012) "Integrated approach to energy and climate changes: changing the paradigm of wastewater treatment management" (LIFE08 ENV/P/000237) was set up for a WWTP discharging into Tagus estuary (Portugal). The main objectives of the project are to implement a tool to optimize the management of WWTP in terms of energy efficiency and environmental impact and to reduce the environmental costs of the treatment process.The aim

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of the ecotoxicological survey is the evaluation of the effectiveness of the treatment process in reducing the toxicity and the protection of the biological treatment through the setting up of adequate ecotoxicological methodologies and the selection of a battery of tests to be used in the characterization of WWTP wastewaters.
To characterize the wastewater in the different treatment phases, a battery of tests with organisms bearing different functions at the ecosystem level was used. Acute aquatic toxicity tests were performed using *Vibrio fischeri*, *Pseudokirchneriella subcapitata*, *Thamnocephalus platyurus*, *Daphnia magna* and *Lemna minor* as test organisms. Knowing that the ecological relevance of species and exposure time is questionable in routine ecotoxicological evaluation, the results of such an approach should help building an adequate testing strategy for the ecotoxicological effects of WWTP discharges. The first results of this program are reported and discussed.

MATERIAL AND METHODS

Wastewater Treatment Plant

The WWTP under study is located in Loures (Portugal), receives domestic and industrial wastewaters and discharges into Tagus estuary. It has the capacity to treat an average flow of 54 500 m^3/day corresponding to a population equivalent of about 213 500 inhabitants.
This WWTP was commissioned in 1989 with a secondary level of treatment. Between 1998 and 1999, has undergone improvement works which include the additional line of treatment of liquid and solid phases being endowed with a tertiary treatment with final disinfection. The intervention also included the installation of a deodorization system and a process of anaerobic digestion of biosolids and therefore production of biogas.

Wastewater Sampling

Wastewater 1h-composite samples were collected during two sampling campaigns (April 2010 and January 2011) with different strategies and periodicities:

- 2010 Campaign - samples collected at the input (A) and after primary treatment (B) in different days of the week (Monday, Tuesday and Friday) at 10h, 14h and 23h.
- 2011 Campaign – samples collected every 3h at the input (A), after primary treatment (B) and after secondary treatment (C) from Friday at 10h to Saturday at 13h.

A total of 47 samples were collected. Each sample was divided into subsamples, kept frozen (-20ºC) for ecotoxicological analysis for no more than 1 month.

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: Bacteria 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 [12];
- Alga test: Alga toxicity was assessed by measuring the growth inhibition of *Pseudokirchneriella subcapitata* exposed for 72 hours. A miniaturized test in microtitration plates was carried out according to ISO 8692: 2004 [13]. The inoculum used was available in algal beads with immobilized cells [14]. Optical density (OD 670

nm) of algae suspensions was determined. To select the best methodology for this kind of samples, 2010 campaign samples were filtered by 0.45µm pore size membranes and 2011 campaign samples were decanted;

- ThamnoTox test: Crustacean toxicity was assessed by determining the mortality of *Thamnocephalus platyurus* exposed for 24 hours according to ThamnoToxKit F^{TM} test procedure [15];
- 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 [16]. 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 [17]. 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).

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}-15min for Microtox test by using Microtox Omni^{TM} software (Azur Environmental, 1999).
- EC_{50}-72h for Alga test, LC50-24h for ThamnoTox test and EC_{50}-48 h for Daphnia test by using Tox-Calc^{TM} software (version 5.0, Tidepool Scientific software, 2002);
- EC_{50}-7d for Lemna test by using Biostat 2.0 software (LemnaTec, 2001);

Aiming to include all raw data for statistical analysis, EC_{50} values not determined due to low effect levels were considered as 100%. Data obtained are also presented as percentage inhibition at the highest tested concentration.

The tests sensitivity was assessed by Slooff's index [18]: 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, Alga, ThamnoTox, Daphnia and Lemna tests.

Toxicity removal of the two treatment units (primary and secondary) was calculated using values of inhibition at the highest tested concentration for samples before and after treatment as:
Toxicity Removal = I_{In}-I_{Out} / I_{In} x 100

RESULTS AND DISCUSSION

Different response ranges for the input wastewater samples *versus* primary treated wastewater samples can be observed for the 2010 Campaign (Table 1) on three of the tests: for Microtox [1.1 % < EC_{50} <17.2 %] and [2.8 % < EC_{50} < 42.6 %] respectively; for Daphnia [28.0 % < EC_{50} < 90 %] and [67.0 % < EC_{50} < 90 %] respectively; for ThamnoTox [28.1 % < LC_{50} < 41.1 %] and [33.0 % < LC_{50} < 46.6 %] respectively. For Alga and Lemna tests, EC_{50} are either 90% or higher, revealing no toxicity and not distinguishing treated from untreated samples.

Table 1. EC_{50}-t and LC_{50}-t values from ecotoxicological tests of wastewater samples of the 2010 campaign (A – WWTP input; B – after primary treatment)

		Microtox	Alga	Daphnia	ThamnoTox	Lemna
	Sample	EC_{50}-15 min	EC_{50}-72h	EC_{50}-48h	LC_{50}-24h	EC_{50}-7d
A	**Mon-10h**	17.2	>90	>90	35.4	<90
	Mon-14h	5.2	>90	>90	33.0	>90
	Mon-23h	3.1	>90	67.0	28.1	90,0
	Tues-10h	7.2	>90	>90	37.0	<90
	Tues-14h	7.9	>90	>90	35.4	>90
	Tues-23h	2.2	>90	52.0	29.4	>90
	Fri-10h	5.6	>90	28.0	37.9	>90
	Fri-14h	2.3	>90	53.0	39.7	>90
	Fri-23h	1.1	>90	74.0	41.1	>90
B	**Mon-14h**	42.6	>90	>90	36.2	>90
	Mon-23h	9.0	>90	90.0	33.0	90.0
	Tues-10h	34.9	90.0	>90	54.8	<90
	Tues-14h	20.8	>90	>90	36.2	<90
	Tues-23h	5.6	>90	67.0	42.5	>90
	Fri-10h	6.0	>90	67.0	46.6	>90
	Fri-14h	8.8	>90	90.0	44.5	>90
	Fri-23h	2.8	>90	>90	43.5	>90

Analyzing the results for Microtox and Daphnia tests obtained in the different days of the week the highest toxicity was measured on Friday. A peak in toxicity was also obtained for Microtox test in all samples collected at 23h. This is in accordance with Chapman (2007) [19] that stresses that difficulties in obtaining representative samples arise in WWTP effluents, whose composition is highly variable, and repeated testing is required.

Results of ecotoxicity tests presented as percentage inhibition at the highest tested concentration (Figure 1), excluding ThamnoTox test that showed 100% effect for all samples, show that the pattern of inhibition can be different also along the day according to the test organism:

- After primary treatment the inhibition of the bacteria luminescence gets higher along the day;
- In the same day the inhibition of the mobility in *Daphnia* can go from 0 to 100%, both for input and after primary treatment samples;
- At the input the growth inhibition of *Lemna* usually decreases along the day and the week;
- The alga test shows growth inhibition between 18 and 50% for all samples with no pattern along the day or the week. These low inhibition values can be linked with the inclusion of filtration in the test procedure in this campaign.

The alga and the plant test results seem to express simultaneously growth inhibition due to wastewater contaminants and interferences from factors like shading and nutrient concentration. The alga test is not considered the most appropriate test for nutrient rich wastewaters because of the complex relationship of inhibition and promotion of algae growth [5].

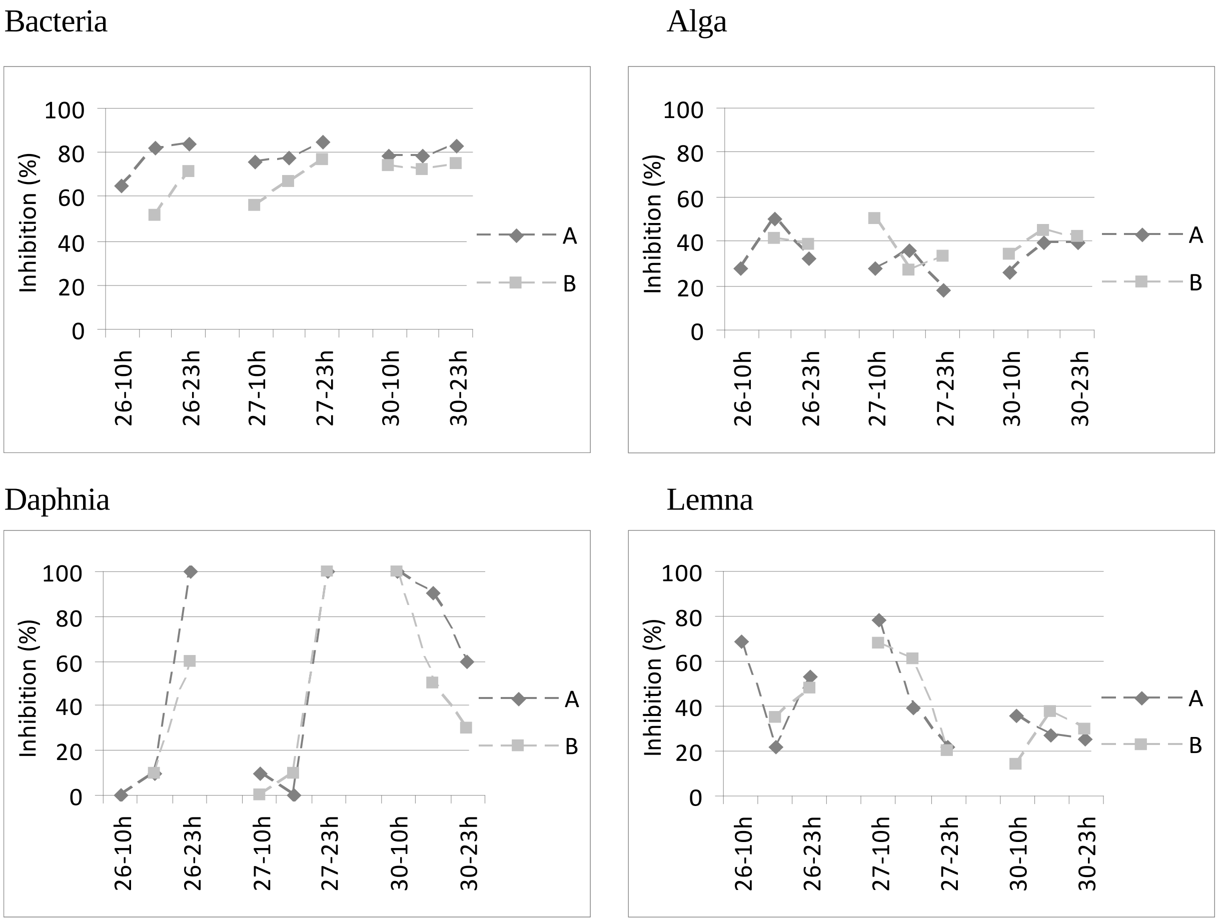

Figure 1. Inhibition effect in test organisms at the highest tested concentration in ecotoxicological tests of wastewater samples of the 2010 campaign (A – WWTP input; B – after primary treatment)

Results of the 2011 Campaign (Table 2) show differences in ranges for the same tests when comparing input wastewater with primary and secondary treated wastewater samples: for Microtox [3.1 % < EC_{50} < 31.0 %], [2.9 % < EC_{50} < 22.0 %] and EC_{50} higher than 90 %, respectively; for Daphnia [33.0 % < EC_{50} < 90 %], [35.0 % < EC_{50} < 90 %] and EC_{50} higher than 90 %, respectively; for ThamnoTox [30.8 % < LC_{50} < 74.9 %], [35.4 % < LC_{50} < 57.0 %] and [65.6 % < LC_{50} < 83.1 %] respectively. For the Alga test, EC_{50} values range from 9.0% for a sample of primary treated effluent to values higher than 90% obtained for input samples and for the majority of secondary treated samples. For Lemna test, EC_{50} are either 90% or higher, revealing no toxicity and not distinguishing treated from untreated samples, except for the input sample on Friday at 10h.

On the basis of EC_{50} we can distinguish input and after secondary treatment samples, with different values in the tests for input samples and with EC_{50} higher than 90% for all treated samples in Microtox, Daphnia and Lemna tests and the majority of treated samples in Alga test. Also for Thamnotox, EC_{50} values show detoxification of wastewater.

Table 2. EC_{50}-t and LC_{50}-t values from ecotoxicological tests of wastewater samples of the 2011 campaign (A – WWTP input; B – after primary treatment; C – after secondary treatment)

		Microtox	Alga	Daphnia	ThamnoTox	Lemna
Sample		EC_{50}-15 min	EC_{50}-72h	EC_{50}-48h	LC_{50}-24h	EC_{50}-7d
A	**Fri-10h**	26.3	50.1	>90	36.2	50,0
	Fri-13h	5.4	16.9	80.0	41.6	<90
	Fri-16h	3.5	25.8	59.0	40.6	>90
	Fri-19h	4.1	42.5	33.0	32.2	>90
	Fri-22h	2.8	47.0	59.0	32.2	>90
	Sat-1h	3.1	>90	52.0	39.7	>90
	Sat-4h	5.7	29.1	90.0	30.8	>90
	Sat-7h	31.0	>90	52.2	74.9	>90
	Sat-10h	24.2	64.4	>90	60.2	>90
	Sat-13h	3.9	42.2	62.2	47.7	>90
B	**Fri-10h**	22.0	30.2	>90	47.7	<90
	Fri-13h	12.7	22.2	>90	40.6	>90
	Fri-16h	9.1	64.5	>90	44.5	>90
	Fri-19h	7.1	48.4	59.0	37.9	>90
	Fri-22h	3.5	40.2	67.0	35.4	>90
	Sat-1h	4.2	25.3	69.5	39.7	>90
	Sat-4h	2.9	61.4	35.0	54.8	>90
	Sat-7h	6.7	9.0	54.8	36.2	>90
	Sat-10h	4.6	47.9	69.4	43.5	>90
	Sat-13h	14.9	54.3	>90	57.0	>90
C	**Fri-10h**	>90	>90	>90	65.6	<90
	Fri-13h	>90	>90	>90	65.6	>90
	Fri-16h	>90	>90	>90	82.5	>90
	Fri-19h	>90	>90	>90	65.6	>90
	Fri-22h	>90	24.1	>90	69.5	>90
	Sat-1h	>90	>90	>90	71.7	>90
	Sat-4h	>90	27.0	>90	75.8	>90
	Sat-7h	>90	>90	>90	78.5	>90
	Sat-10h	>90	>90	>90	80.3	>90
	Sat-13h	>90	>90	>90	83.1	>90

In general along these 28 hours monitoring program, the lowest EC_{50} values in sites A and B, corresponding to higher toxicity, were obtained for wastewater samples collected between Friday at 19h and Saturday at 4h.

Results of ecotoxicity tests presented as percentage inhibition at the highest tested concentration (Figure 2) confirm the results obtained in the 2010 campaign showing that the pattern of inhibition can be different according to the test organism and makes evidence for secondary treatment efficiency:

- After secondary treatment the inhibition of the bacteria luminescence and *Daphnia* mobility get very low;
- The inhibition of *Daphnia* mobility can go in 28 hours from 0 to 100% at the input and from 15% to 100% after primary treatment;

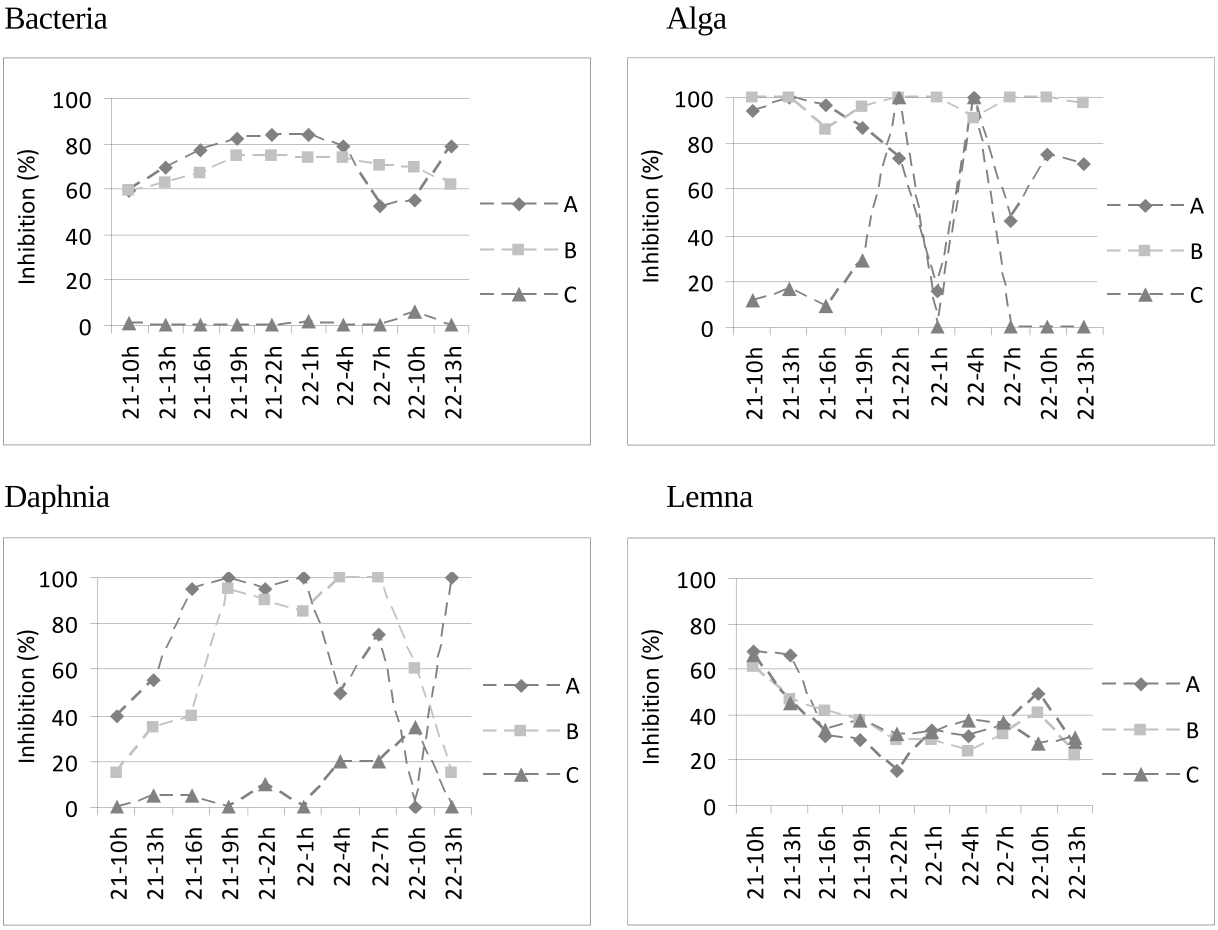

Figure 2. Inhibition effect in test organisms at the highest tested concentration in ecotoxicological tests of wastewater samples of the 2011 campaign (A – WWTP input; B – after primary treatment; C – after secondary treatment)

- The growth inhibition in *Lemna* is higher on Friday morning decreasing along the sampling period for all sampling sites;
- The alga test shows growth inhibition between 15.7 and 100% with variation along the sampling period for the input samples, high values for the primary treated samples and lower values for the secondary treated samples. No pattern of effect can be seen.

ThamnoTox test presented the highest effect [63 % and 100% mortality] in all samples, seeming to be the lowest discriminative test. Nevertheless analyzing mean mortality values for A, B and C sites allows verifying that percentage effect gets lower after secondary treatment.

Slooff's sensitivity index shows that the bacterium *Vibrio fischeri* is the most sensitive species, and allows to establish the following gradient of test sensitivity: Microtox > ThamnoTox > Alga > Daphnia > Lemna, from the corresponding Slooff's index values 0.2 < 0.7 < 1.0 < 1.2 < 1.5.
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 [10, 20, 21].. 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 [22, 23].

According to the wastewater classification proposed by Tonkes *et al.* (1999) [24], that considers samples with an EC_{50} value for the most sensitive species higher than 100% as non toxic, between 10% and 100% as slightly toxic and lower than 10% as toxic, at the WWTP input 74% of the samples are toxic and 26% are slightly toxic, after primary treatment 67% of samples are toxic and 33% are slightly toxic and after secondary treatment all the samples are non toxic.

Toxicity removal was obtained for both treatment levels when considering *Vibrio fischeri* luminescence inhibition, *Pseudokirchneriella subcapitata* growth inhibition and *Daphnia magna* inhibition of mobility. For primary treatment the mean toxicity removal values were 9% for the bacteria and 11% for the crustacean and for secondary treatment the mean toxicity removal values were 99% for the bacteria, 65% for the alga and 87% for the crustacean. Tyagi *et al.* (2007) [23] found that the mean percentage removal in toxicity for *D. magna* after primary and secondary treatment were 29% and 76%, respectively.

When assessing effects in WWTP and controlling complex wastewaters, it is important to consider effects at different trophic levels due to differences in relative sensitivity of the organisms. In a previous work, Mendonça *et al.* (2009) [25] proposed a test battery to monitor WWTP wastewaters including tests with a bacterium, an alga and a crustacean.
What needs to be stressed is the holistic approach that ecotoxicological assessment is able to perform. As stated by Lofrano & Brown (2010) [26] "…with greater understanding of the impact of the wastewater on the environment and more sophisticated analytical methods, advanced treatment is becoming more common". Despite the fact that the adoption of this approach is still not global, the validity of the use of acute tests to drive environmental improvement has been demonstrated [27].

CONCLUSIONS

The acute toxicity of the samples analyzed shows to be dependent on the WWTP treatment level and the species tested. Microtox, Alga and Daphnia tests were able to distinguish the two levels of treatment and to assess toxicity removal efficiency. *Vibrio fischeri*, the bacterium used in the Microtox test, was the most sensitive species in WWTP samples evaluation.
These results demonstrated not only that the treatment efficiently reduced wastewater toxicity toward the selected test organisms, but also that the use of an ecotoxicological approach can contribute to the environmental management of the Treatment Plant.

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