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Ecotoxicological evaluation of the wastewater treatment process of the sewage treatment plant of Thessaloniki, Greece

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Abstract

The LUMISTox® toxicity test was employed to assess the removal of municipal wastewater toxicity during the biological treatment with activated sludge in the wastewater treatment plant of Thessaloniki, Greece. Possible associations of toxicity data with chemical parameters of organic pollution of wastewaters, namely BOD_5, COD, DOC, SS and persistent organic pollutants (POPs) were also investigated. Toxicity and chemical parameters were concurrently measured at three sampling points of the treatment plant, the entrance of the unit (raw wastewater, RW), the effluent of the secondary sedimentation tank (SSE), and the final sewage sludge (FS).

Substantial reduction of toxicity was observed from RW to SSE (mean ± S.D. of bioluminescence inhibition 36 ± 9.4% and 13 ± 4.0%, respectively) indicating removal of toxicants during primary and secondary clarification, also suggesting that a large part of the toxicity measured is attributed to the biodegradable fraction of the organic content of wastewater.

Significant positive correlations were observed between % inhibition values and wastewater parameters (BOD, COD, SS). In sludge, correlations were in general poor. Negative strong correlation was observed between $EC_{20/15}$ and TOC suggesting that the organic content of sludge contributes to the toxicity measured. Toxicity was positively correlated with the concentrations of certain POPs in RW, while weaker negative correlations were observed in SSE. Correlations in sewage sludge were less significant.

It was concluded that toxicity testing is a useful tool supplementing chemical analyses in the evaluation of the potential hazard from effluent discharges and disposal of waste sludge.
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Keywords: LUMISTox®; Bioluminescence; Wastewater; Sewage sludge; Persistent organic pollutants

1. Introduction

Wastewater treatment plants (WWTPs), especially those located in industrial areas, face discharges containing a complex mixture of various organic and inorganic substances [1–5]. In Europe, recent guidelines [6,7] have been based in the detection of specific pollutants included in a list of priority organic pollutants [8]. Despite the fact that the effluents of WWTPs contain these pollutants at trace levels, they appear to have toxic effect to living organisms and therefore classic chemical analysis seems to be inadequate for their characterization. Thus, use of bioassayled marine environmental monitoring approaches can complete the characterization of such discharges. Several bioassays can be used to examine the toxicity of wastewater [9]. Organisms used as bio-indicators include the bioluminescence emitting marine bacterium *Vibrio Fisceri* (LUMISTox®, Microtox®, Toxalert®), the aquatic microorganism *Daphnia Magna* (Daphnokit F™), the *Selenastrum capricornutum* (Algaltoxkit F™), the *Nitellopsis obtuse* (Charatox), the *Thamnocephalus platyurus* (Thamnotoxkit F™), the *Tetrahymena thermophila* (Protoxkit F™), etc. [1,10–14].

Persistent organic pollutants (POPs) constitute a wide group of compounds which are either intentionally produced, such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs), or unintentionally or accidentally formed as byproducts of industrial or other human activities, for instance dibenzo-*p*-dioxins and furans (PCDDs/Fs) and polycyclic aromatic hydrocarbons (PAHs). Some POPs belong to more than one source category [15]. These compounds are characterized

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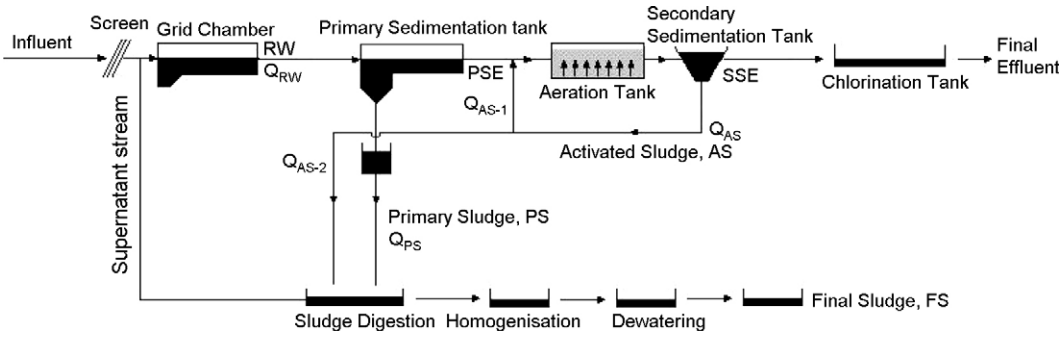

Fig. 1. Flow chart of the WWTP of Thessaloniki.

by pronounced persistence against chemical/biological degradation, high environmental mobility, strong tendency for bioaccumulation in human and animal tissues, significant impacts on human health and the environment even at extremely low concentrations. Their low biodegradability makes them refractory to the biological treatment of wastewater [3]. PCBs and OCs were interdicted in the USA and Europe in mid 70 s; however, some of them were still being used till lately in developing countries. In 2001, the Stockholm Convention on POPs banned the production and use of these chemicals worldwide.

This study aimed at investigating the toxicity of the wastewaters and sludge of the WWTP of Thessaloniki, a plant that receives mainly urban wastewaters, and to compare this data with other WWTPs around the world. Another objective of this study was to examine whether this toxicity is attributed to POPs by correlating toxicity with POPs and typical chemical parameters of wastewaters (BOD, COD, TOC, SS). Finally, it aimed at evaluating the wastewater treatment process, in terms of toxicity, by assessing the difference in toxicity between the inlet and the outlet of the plant.

The evaluation of the levels of toxicity of the specific wastewaters is of great importance for the area of Thessaloniki, since the outlet of the plant is being disposed in the Thermaikos gulf, only few kilometres away from the delta of Axios river, an area protected by the Ramsar convention, and where, extensive cultivation of mussels takes place.

2. Materials and methods

2.1. Plant description

The WWTP of the city of Thessaloniki serves about 1 million residents by treating daily 120 000–150 000 m^3 of raw wastewaters. About 5–10% of the total flow is contributed by industry. The plant also receives the greatest part of the local urban runoff, which is maily composed of atmospheric deposition, and traffic-related emissions deposited on the road surface. The treatment process includes screening, grid removal, primary sedimentation without use of chemical coagulants, conventional activated sludge treatment and effluent disinfection using chlorine gas (Cl_2). The treated wastewater is discharged in Thermaikos Gulf via a channel. Sewage sludge (primary plus excess activated) is anaerobically digested, thickened, and dewatered [16]. The greatest amount of this sludge is deposited in a municipal landfill, while its use as soil amendment is also under consideration by the local authorities. The flow chart of the plant is shown in Fig. 1.

2.2. Sampling

Sampling was conducted during the period November 2001–December 2002. Twenty-four hours composite, flow-proportioned samples of wastewater were collected from the influent of the plant (raw wastewater, RW), and the effluent of the secondary sedimentation tanks (SSE), and grab samples of the final sludge (FS). All samples were collected in brown glass vessels with Teflon caps, pre-cleaned with acetone and *n*-hexane, and were kept refrigerated (4 °C). The toxicity measurements were carried out within 24 h from sampling, while the storage period for extraction/ analysis of POPs did not exceed 10 days.

2.3. Sample processing and analysis

The LUMISTox® acute toxicity test was performed according to the standard procedures for liquid and solid samples outlined in Lange [17]. The instrumentation used included a LUMISTox 300 luminometer, a LUMISTherm incubator, and the non-pathogenic bacteria *Vibrio Fisceri* LCK 480 (liquid dried), all obtained from Lange GmbH, Duesseldorf, Germany. Briefly, wastewater samples were filtered through a 0.45 μm polysulphone membrane and their pH and salinity values were adjusted to 7.0 ± 0.2 and 2–4%, respectively. Aqueous sludge elutriates were obtained according to the "Dr. Lange LUMISterra" procedure [17]. Briefly, 10 g of fresh material was mixed with 40 ml 2% NaCl solution in Milli-Q water and vigorously agitated on a magnetic stirrer for 30 min. After the suspension was settled, the liquid phase was separated by filtration through a 0.45 polysulphone membrane and pH and salinity were adjusted as above [18].

Initially a screening procedure was conducted to estimate the toxicity of each sample [17–19]. Samples that inhibited bacterial luminescence by more than 15% were subsequently tested to quantify their acute toxicity in terms of EC_{20} (%) or EC_{50} (%), that is the effective concentration (%) of tested solution causing a 20% or 50% reduction of bioluminescence, respectively. The reduction of bacteria bioluminescence was measured after 15 and 30 min incubation times. EC values were calculated by the instrument software. One blank (Milli-Q

water containing 2% NaCl) was measured with each sample. Control solutions ($K_2Cr_2O_7$: 4.0 mg/L and NaCl: 7.5%) were also used to verify the quality of bacteria and of the reagents.

The experimental procedure employed for sample processing and analysis for POPs has been described in detail elsewhere [20]. Briefly, wastewater and sludge samples were liquid–liquid and ultrasonically extracted, respectively, using *n*-hexane and cleaned-up through solid phase extraction Florisil cartridges. Purified extracts were solvent exchanged to iso-octane and analyzed by gas chromatography coupled to electron capture detection (GC–ECD). The gas chromatography system employed was a HP-5890 series II chromatograph, equipped with a DB-5 column (50 m × 0.32 mm × 0.17 μm) and an on-column injection port. Carrier and make-up gas was nitrogen (99.999% purity). The system was calibrated using the Pesticide-Mix 33 (Dr Ehrenstorfer, standard mixture) which contained 7 PCBs (IUPAC #s 28, 52, 101, 118, 153, 138 and 180) and 19 OCs (Hexachlorobutadine, Dichlobenil, Quintozene, Heptachlor, Aldrin, Isobenzan, Isodrin, Heptachlor-exo-epoxide (HexE), Heptachlor-endo-epoxide, a-Endosulfan, *p*,*p*′-DDE, Dieldrin, Endrin, *p*,*p*′-DDD, *p*,*p*′-DDT, α-HCH, β-HCH and γ-HCH). Reagent blanks, duplicate samples and the method of standard additions were employed for the data quality control. The certified reference material, CRM-481 (industrial soil containing PCB-101, 118, 128, 149, 153, 156, 170 and 180) obtained from BCR, Brussels, Belgium was also used for method validation. Recoveries obtained from the analysis of the CRM 481 ranged between 90% and 105% for all contained analytes.

The total organic carbon content of sludge (TOC) was determined in dried subsamples by the Wakley–Black method, adopted and modified by Jackson [21]. The dissolved organic carbon content of wastewater (DOC) was measured by a Shimadzu TOC-V_{CSH} Analyzer in subsambles filtrated through 0.45 μm nitrate cellulose membranes (Schleicher&Schuell). Other classical wastewater parameters such as BOD_5, COD and SS were measured according to the standard methods for the analysis of water and wastewater [22].

Table 1
Summary statistics of bioluminescense inhibition ($N = 13$)

	Mean	S.D.	Median	Maximum	Minimum
RW	36	9.4	33	60	25
SSE	13	4.0	14	20	6.0
FS	59	15	64	85	35

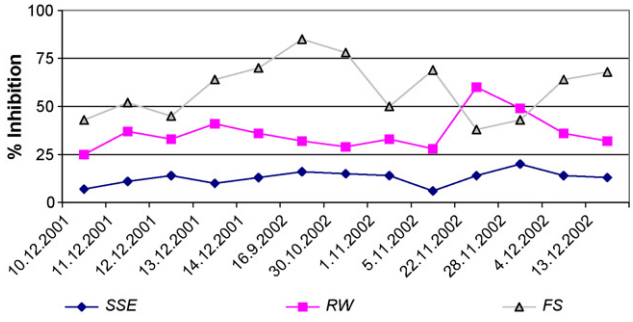

Fig. 2. Bacteria bioluminescence inhibition (%) caused by raw wastewater (RW), secondary sedimentation effluent (SSE) and final sludge (FS).

3. Results and discussion

3.1. Toxicity of wastewater and sludge samples

Summary statistics of bacterial bioluminescense inhibition (incubation time 15 min) are presented in Table 1. All samples examined caused bioluminescence inhibition, and, as expected, the sewage sludge elutriates exhibited the highest values (35–85% versus 25–60% in RW and 6–20% in SSE).

In Fig. 2, a comparison between the inhibition of bioluminescence caused by the RW and the respective SSE samples is presented. As seen, in all days the toxicity of RW was higher that that of SSE, indicating that toxicants are removed during primary and secondary clarification, also suggesting that a large part of the toxicity measured is attributed to the biodegradable fraction of the organic content of wastewater. Bioluminescence

Table 2
$EC_{20/50}$ values for 15 and 30 min incubation time

	RW				SSE				FS			
	$EC_{20/15}$ (%)	$EC_{20/30}$ (%)	$EC_{50/15}$ (%)	$EC_{50/30}$ (%)	$EC_{20/15}$ (%)	$EC_{20/30}$ (%)	$EC_{50/15}$ (%)	$EC_{50/30}$ (%)	$EC_{20/15}$ (%)	$EC_{20/30}$ (%)	$EC_{50/15}$ (%)	$EC_{50/30}$ (%)
10 December 2001	16	12	ND	ND	ND	ND	ND	ND	7.2	9.1	90	90
11 December 2001	14	ND	51	42	ND	ND	ND	ND	6.5	4.4	75	69
12 December 2001	19	ND	45	29	ND	ND	ND	ND	8.5	6.1	90	90
13 December 2001	13	10	41	27	ND	ND	ND	ND	7.3	3.2	43	23
14 December 2001	22	ND	ND	31	ND	ND	ND	ND	2.4	2.2	4.6	4.4
15 September 2002	19	8.0	ND	ND	90	12	ND	ND	0.65	0.79	2.6	4.1
30 October 2002	ND	ND	ND	ND	ND	ND	ND	ND	3.9	2	13	10
01 November 2002	50	38	ND	ND	ND	ND	ND	ND	5	3.6	90	90
05 November 2002	19	15	62	57	ND	ND	ND	ND	2.1	1.8	12	15
22 November 2002	13	12	36	34	ND	ND	ND	ND	6.1	2.6	90	90
28 November 02	ND	ND	ND	ND	19	16	40	39	10	1.9	45	70
04 December 02	ND	ND	ND	ND	ND	ND	ND	ND	2.3	1.9	90	90
13 December 02	14	ND	ND	ND	ND	ND	ND	ND	90	90	90	90

ND: not determined.

Table 3
Toxicity of various wastewaters, as reported in literature

	% inhibition	$EC_{50/30}$	Instrument	Type of wastewater	Reference
Untreated textile	100	0.54–0.62	Microtox	Textile industry	[37]
Porto influent	100	28.2 ± 1.47	Microtox	Textile industry	[37]
Porto effluent	29	–	Microtox	Textile industry	[37]
Piera influent	100	4.27 ± 0.31	Microtox	Urban WWTP	[37]
Piera effuent	<20	–	Microtox	Urban WWTP	[37]
Igualada influent	100	0.81 ± 0.04	Microtox	Industrial WWTP	[37]
Igualada effluent	68	75 ± 5.3	Microtox	Industrial WWTP	[37]
Igualda influent	81.2–81.5	25.8	Toxalert 100	Industrial WWTP	[25]
Igualada effluent after primary settlement	97.8	5.4	Toxalert 100	Industrial WWTP	[25]
Igualada effluent	39.3–42.6	n.m.	Toxalert 100	Industrial WWTP	[25]
La Llagosta influent	51.9–53.5	74.7	Toxalert 100	Industrial WWTP	[25]
La Llagosta effluent after primary settlement	66.3–66.5	59.3	Toxalert 100	Industrial WWTP	[25]
La Llagosta effluent	35.0	n.m.	Toxalert 100	Industrial WWTP	[25]
Calaf influent	50	100 ± 9.8	Microtox	Industrial and urban WWTP	[37]
Calaf effuent	<20	–	Microtox	Industrial and urban WWTP	[37]
Porto influent	100	13 ± 0.07	Microtox	Textile industry WWTP	[37]
Sweden	20–100	–	Toxalert	Tannery WWTP	[38]
Barcelona	100	–	Toxalert	Tannery WWTP	[38]
Barcelona influent	70	–	Toxalert	Industrial and urban WWTP	[38]
Barcelona effluent	15	–	Toxalert	Industrial and urban WWTP	[38]
Berlin	0–22	–	Toxalert	Cement industry WWTP	[38]
Basf 1	n.m.	–	Toxalert	Industrial WWTP	[38]
Basf 2	n.m.	–	Toxalert	Industrial WWTP	[38]
Manresa. Spain	21–36	–	Toxalert	Industrial and urban WWTP	[39]
Terrassa. Spain	55.8	–	Toxalert	Industrial and urban WWTP	[39]
Myslenice influent	–	10.1	Microtox	Small municipal WWTP	[40]
Myslenice effluent	–	>98	Microtox	Small municipal WWTP	[40]
Plaszow influent	–	12.5	Microtox	Large municipal WWTP	[40]
Plaszow effluent 1	–	78.1	Microtox	Small municipal WWTP	[40]
Plaszow effluent 2	–	>98	Microtox	Small municipal WWTP	[40]
Nowa Huta influent	–	>98	Microtox	Metallurgical–industrial WWTP	[40]
Nowa Huta effluent	–	>98	Microtox	Metallurgical–industrial WWTP	[40]
Camacari, Brasil—influents	–	0.43–5.25%	Microtox	Industrial WWTP	[31]
Camacari, Brasil–effluents	–	6.58–>82%	Microtox	Industrial WWTP	[31]
Ozone-treated wastewater. Thessaloniki	$-5 < I\% < -15$	–	Microtox	Urban WWTP	[24]

n.m.: not measured.

inhibition never reached 100% as has been observed by other investigators (Table 3), nor dropped to negative values (indicating stimulation of bacteria according to Wong et al. [23]) as reported by Anasontzis et al. [24]. Farre et al. [25] noticed an increase in the % bioluminescence inhibition between raw influent and primary sedimentation effluent, attributable to the addition of chemicals during primary sedimentation step. In the present study, toxicity was not measured in primary sedimentation effluents, also because there is no use of chemical coagulants in the primary sedimentation tank at the WWTP of Thessaloniki, so such an observation could not be verified.

Effective concentration values (EC_{20} and EC_{50}) for 15 and 30 min incubation times could be calculated only in some of the wastewater samples. EC values exceeding 90% cannot be calculated by the software of LUMISTox®. EC values higher than 100% have been reported by other researchers although they have no physical meaning [14,26]. Sludge elutriates exhibited measurable EC values in all sampling days (Table 2). Based on the EC values of Table 2, toxicity unit (TU) and the toxicity index (TI) values were calculated from the formula $TU/TI = (EC_{50}/EC_{20}) - 1 \times 100$ [27]. Persoone et al. [28] proposed that samples can be classified as non-toxic when TU = 0 (Vengris et al. [29] proposed TU < 0.4); slightly toxic when 0 < TU < 1, toxic when 1 < TU < 10, very toxic when 11 < TU < 100, and extremely toxic when 100 < TU. In the present study, assuming that samples for which EC values could not be calculated have TU > 1, 7 out of the 13 RW samples (54%) were classified as slightly toxic and 6 (46%) were classified as toxic. In addition, 12 out of the 13 SSE samples (92%) were classified as slightly toxic and only 1 (8%) was classified as toxic. Similar TU levels in wastewater samples have been reported by other investigators (0-471 TU [13]; 0-237 TU [30]). Although the above classification has been proposed for aquatic samples, it was here performed on the aqueous elutriates of sewage sludge samples. Higher toxicity was observed for sewage sludge in comparison to wastewaters, since 6 samples (46%) were slightly toxic, 5 (38%) were toxic and 2 (15%) were very toxic. The higher toxicity of sludge in comparison to wastewater is attributable to the fact that a myriad of organic and inorganic wastewater contaminants are removed due to sorption on particles and accumulated in wasted sludge.

The wastewater toxicity of the WWTP of Thessaloniki can be compared with the toxicity of other WWTPs (Table 3). As seen, literature values for bioluminescence inhibition vary

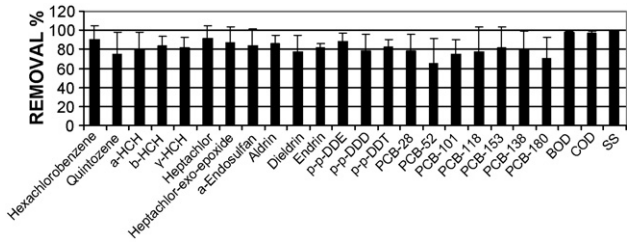

Fig. 3. Removal of POPs and typical pollution parameters during the wastewater treatment process.

between −15% and 100%, suggesting that toxicity depends greatly on the type of wastewater each plant receives. In general, the % bioluminescence inhibition and the EC values reported in literature reveal higher toxicity than that observed in the WWTP of Thessaloniki.

3.2. POPs concentrations in the WWTP of Thessaloniki

The total (dissolved plus adsorbed) concentrations of the 26 targeted POPs and their removal rates are presented in detail in [2]. A summary of these data is given in Table 4. Briefly, 22 POPs were found at detectable concentrations at the various treatment stages. Most abundant pollutants were PCB-52; PCB-101; PCB-180 and HexE. For all POPs, for all sampling days, concentrations in RW were higher than SSE ones. Table 4 presents also the concentration levels of typical pollution parameters of wastewater and sludge.

3.3. Toxicity reduction during the treatment process

The removal efficiency of chemical pollution parameters and toxicity during the wastewater treatment process was evaluated by using the general formula:

$$R\% = [(C_{RW} - C_{SSE}) \times 100]/C_{RW}$$

where C_{RW} and C_{SSE} are the concentrations or the bioluminescence inhibition in untreated effluent and the secondary sedimentation effluent, respectively.

The removal efficiency of POPs and conventional wastewater parameters are shown in Fig. 3. As seen, the removal efficiency was 98% for BOD, 97% for COD, and 99% for SS. Lower values were obtained for DOC (67%) and individual POPs (65–91%) [2].

Table 4
Summary data of POPs concentrations encountered at various stages of Thessaloniki WWTP

POPs	RW (mean) ($ng\,l^{-1}$)	SSE (mean) ($ng\,l^{-1}$)	FS (mean) ($ng\,l^{-1}$) (dw)
Hexachlorobutadine	ND	ND	ND
Dichlobenil	ND	ND	ND
Hexachlorobenzene	20	1.7	6.8
Quintozene	60	14	20
Isobenzan	ND	0.23	15
a-Endosulfan	51	2.7	6.4
α-HCH	39	6.2	5.0
β-HCH	26	6.3	8.2
γ-HCH	1.4	0.57	10
Aldrin	10	ND	ND
Isodrin	ND	ND	ND
Dieldrin	27	8.9	15
Endrin	1.8	2.8	ND
Heptachlor	46	6.4	40
Heptachlor-exo-epoxide	330	25	270
Heptachlor-endo-epoxide	ND	ND	ND
p,p′-DDE	12	0.23	27
p,p′-DDD	22	6	78
p,p′-DDT	6.9	ND	ND
PCB-28	4.8	3.3	6.8
PCB-52	390	110	160
PCB-101	260	45	91
PCB-118	15	5.9	30
PCB-153	14	0.98	22
PCB-138	11	2.9	22
PCB-180	340	74	210
∑PCBs	1000	250	550
BOD ($mg\,l^{-1}$)	650	11	
COD ($mg\,l^{-1}$)	1400	48	
SS ($mg\,l^{-1}$)	1100	11	22%
DOC ($mg\,l^{-1}$)	72	19	
TOC (% O.M.[a])			40

[a] O.M.: organic matter.

The removal of toxicity varied between 48.3% and 78.6% (mean: 63.9%; median: 61.1%; S.D.: 10%). Araujo et al. [31] observed higher toxicity reduction (75.6–99.6%, mean value: 92.7%) in the WWTP of Camacari (Brasil), although the COD removal was lower than those in the Thessaloniki WWTP. This may be attributed to differences in the toxic load composition of wastewater in the two WWTPs. Note that the WWTP of Camacari is receiving industrial wastewater with higher toxic load and organic content in comparison to the Thessaloniki

Table 5
Correlation coefficients between toxicity and chemistry data (conventional parameters of organic pollution) for RW and SSE

	% inhibition	$EC_{20/15}$	$EC_{20/30}$	$EC_{50/15}$	$EC_{50/30}$	DOC	BOD	COD	SS
% inhibition	1								
$EC_{20/15}$		1							
$EC_{20/30}$			1						
$EC_{50/15}$		**0.997**		1					
$EC_{50/30}$	*−0.514*	**0.995**		**0.994**	1				
DOC						1			
BOD	**0.730**	**−0.642**		*−0.607*	*−0.577*	*0.514*	1		
COD	**0.730**	**−0.643**		*−0.607*	*−0.577*	*0.512*	**0.999**	1	
SS	**0.751**	*−0.590*		*−0.592*	*−0.560*	*0.518*	**0.983**	**0.983**	1

Correlation coefficients significant at the 95% are italicized, correlation coefficients significant at the 99% are bold-faced.

Table 6
Correlation coefficients between toxicity and chemistry data (conventional parameters of organic pollution) for FS

	% inhibition	$EC_{20/15}$	$EC_{20/30}$	$EC_{50/15}$	$EC_{50/30}$	SS	TOC
% inhibtion	1						
$EC_{20/15}$	**−0.795**	1					
$EC_{20/30}$	**−0.660**		1				
$EC_{50/15}$	**−0.835**		**0.756**	1			
$EC_{50/30}$	**−0.863**	*0.519*	**0.737**	**0.997**	1		
TSS	*−0.559*		**0.673**			1	
TOC	*0.586*	**−0.804**					1

Correlation coefficients significant at the 95% are italicized, correlation coefficients significant at the 99% are bold-faced.

WWTP that receives municipal wastewater. Effluents, generally, present variable combination and their toxic effects vary dependent on the interaction of their components [32].

3.4. Correlation analysis between toxicity and wastewater parameters

Tables 5 and 6 present Pearson linear correlation coefficients for wastewater and sludge samples, respectively. In wastewater samples, strong positive correlations were observed for bioluminescence inhibition with BOD, COD and SS (0.730, 0.730 and 0.751, respectively). EC values were accordingly negatively correlated with these parameters. The significant correlation found for toxicity with BOD and COD suggests that a large part of the toxic load of wastewater is due to the organic load. Significant correlations between EC and COD have been observed by other investigators [31,33]. Nonetheless, absence or even poor correlation has been found in other cases [31,34,35] suggesting that in complex samples, where a big number of pollutants can be found, COD may not provide good correlations with the high toxicity [36].

In sludge samples, strong negative correlation was observed between $EC_{20/15}$ and TOC (−0.804) suggesting significant contribution of TOC to the overall toxicity of sludge. Statistically significant, yet weaker positive correlation was also observed between $EC_{20/30}$ and TSS (0.673).

The results of the correlation analysis between toxicity data and POPs (Table 7) are quite controversial. In RW, % bioluminescence inhibition exhibited significant positive correlation with six POPs, suggesting that these pollutants may play important role to the measured toxicity of untreated wastewater. However, in SSE, the % bioluminescence inhibition exhibited negative correlation with POPs. Such a result could only be considered as random, indicating that the concentrations of individual POPs in treated wastewater are quite low to affect their toxicity. Finally, in the final sludge, correlations with toxicity were insignificant for almost all POPs and only PCB-138 exhibited correlation significant at the $P < 0.01$ level. The lack of correlation may be a consequence of the fact that the hundreds of different chemicals accumulated in sludge act individually or interacting with each other, thus resulting in antagonistic and synergistic effects, which are difficult to predict. In addition, sludge contaminants that are below detection limit for chemical analyses, may still have a toxicological effect, which is again difficult to predict. PCBs and other sludge contaminants (PAHs, non-ylphenol and non-ylphenol carboxylates) have been reported to be the most toxic fractions contributing to the toxicity of sludge [1]. Previous examination of the ecotoxicity of the sludge from the Thessaloniki's WWTP indicated significant correlation of the LUMISTox® acute toxicity of sludge elutriates with PAHs, but correlation with PCBs was less significant [41].

It is important to note that acute toxicity measurements may not reflect the spectrum of toxicity, or the hazard, associated with the exposure to a chemical contaminant. Contaminants may have carcinogenic or mutagenic effects at doses that produce no evidence of acute toxicity. For this purpose, tandem use of acute toxicity and genotoxicity screening tests are suggested to broaden the scope of environmental risk assessment. Given that POPs are long-living environmental contaminants

Table 7
Correlation coefficients between % bioluminescence inhibition and POPs in the WWTP of Thessaloniki

	% inhibition RW	% inhibition SSE	% inhibition FS
Hexachlorobutadine			
Dichlobenil			
Quintozene	**0.91**	**−0.80**	
Hexachlorobenzene	**0.68**		
a-HCH		**−0.66**	
b-HCH	**0.81**	**−0.66**	
γ-HCH			
Isobenzan			
Heptachlor			
Heptachlor-exo-epoxide		**−0.70**	
Heptachlor-endo-epoxide			
a-Endosulfan		**−0.83**	
Aldrin			
Isodrin			
Dieldrin	**0.65**	**−0.76**	
Endrin			
0,10			
p,p′-DDE	**0.79**		
p,p′-DDD		**−0.73**	
p,p′-DDT			
PCB-28			*−0.60*
PCB-52	**−0.76**		
PCB-101	**0.88**	**−0.80**	
PCB-118			
PCB-153			*0.57*
PCB-138		**−0.83**	**0.72**
PCB-180		*−0.56*	

Correlation coefficients significant at the 95% are italicized, correlation coefficients significant at the 99% are bold-faced.

with strong tendency for bioconcentration and biomagnification, the use of wet chronic bioassays on eukaryotes would also be useful in order to assess the effects of these pollutants on a multi-generational basis.

4. Conclusions

Wastewater and sludge samples collected from the Thessaloniki municipal WWTP exhibited bioluminescence inhibition that varied between 6% for SSE and 85% for FS individual samples. In all sampling dates, % inhibition was higher in the RW than in the SSE samples, thus verifying the effective operation of the WWTP. Negative inhibition was never observed. Toxicity reduction varied between 48.3% and 78.6%. EC_{20} and EC_{50} values could be calculated for all FS samples and only for a few RW and SSE samples, due to relatively low toxicity. Significant correlation was observed between % inhibition and wastewater parameters, such as BOD, COD and SS. In FS samples, correlations found, were in general poor, however good negative correlation between TOC and $EC_{20/15}$ was observed, suggesting that the organic content of sludge contributes to the measured toxicity. Correlation between bioluminescence inhibition and POPs concentrations was found to be quite poor, and only in RW some POPs may contribute to the measured toxicity.

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References

[1] M. Farre, D. Barcelo, Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis, TRAC–Trends Anal. Chem. 22 (5) (2003) 299–310.
[2] M. Karvelas, A. Katsoyiannis, C. Samara, Occurrence and fate of heavy metals in the wastewater treatment plant of Thessaloniki, Environ. Sci. Poll. Res., Spec. (3) (2002) 194–195.
[3] A. Katsoyiannis, C. Samara, Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: occurrence and removal, Water Res. 38 (11) (2004) 2685–2698.
[4] M. Blanchard, M.J. Teil, D. Ollivon, L. Legenti, M. Chevreuil, Polycyclic aromatic hydrocarbons and polychlorobiphenyls in wastewaters and sewage sludges from the Paris area (France), Environ. Res. 95 (2) (2004) 184–197.
[5] T. Pham, S. Proulx, PCBs and PAHs in the Montreal urban community (Quebec, Canada) wastewater treatment plant and in the effluent plume in the St. Lawrence river, Water Res. 31 (8) (1997) 1887–1896.
[6] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy (2000) Official Journal of the European Communities.
[7] Decision No 0455/2001/EC of the European Parliament and the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC. (2001) Official Journal of the European Communities.
[8] M.D. Hernando, A.R. Fernandez-Alba, R. Tauler, D. Barcelo, Toxicity assays applied to wastewater treatment, Talanta 65 (2005) 358–366.
[9] R. Chandra, P.K. Pandey, A. Srivastava, Comparative toxicological evaluation of untreated and treated effluent with *Nostoc Muscorum* L. (Algal assay) and Microtox bioassay, Environ. Monit. Assess. 95 (2004) 287–294.
[10] E. Emmanuel, Y. Perrodin, G. Keck, J.M. Blanchard, P. Vermande, Ecotoxicological risk assessment of hospital wastewater: a proposed framework for raw effluents discharging into urban sewer network, J. Hazard. Mater. A117 (2005) 1–11.
[11] F. Kargi, I. Konya, COD, *para*-chlorophenol and toxicity removal from *para*-chlorophenol containing synthetic wastewater in an activated sludge unit, J. Hazard. Mater. 132 (2–3) (2006) 226–231.
[12] E. Bizani, K. Fytianos, I. Poulios, V. Tsiridis, Photocatalytic decolorization and degradation of dye solutions and wastewaters in the presence of titanium dioxide, J. Hazard. Mater. 136 (1) (2006) 85–94.
[13] L. Manusadzianas, L. Balkelyte, K. Sadauskas, I. Blinova, L. Pollumaa, A. Kahru, Ecotoxicological study of Lithuanian and Estonian wastewaters: selection of the biotests and correspondence between toxicity and chemical based indices, Aqua. Toxicol. 63 (2003) 27–41.
[14] S. Monarca, D. Feretti, C. Collivignarelli, L. Guzzella, I. Zerbini, I. Bertanza, R. Pedrazzani, The influence of different disinfectants on mutagenicity and toxicity of urban wastewater, Water Res. 34 (17) (2000) 4261–4269.
[15] K. Breivik, R. Alcock, Y.F. Li, R.E. Bailey, H. Fiedler, J.M. Pacyna, Primary sources of selected POPs: regional and global scale emission inventories, Environ. Pollut. 128 (2004) 3–16.
[16] M. Karvelas, A. Katsoyiannis, C. Samara, Occurrence and fate of heavy metals in the wastewater treatment process, Chemosphere 53 (2003) 1201–1210.
[17] B. Lange, Lumistox 300 Operating Manual, Dusseldorf, Germany, 1998, 1–42.
[18] K. Zabetoglou, D. Voutsa, C. Samara, Toxicity and heavy metal contamination of surficial sediments from the bay of Thessaloniki (Northern aegean sea) Greece, Chemosphere 49 (2000) 17–26.
[19] D. Papadopoulou, C. Samara, Polycyclic aromatic hydrocarbon contamination and lumistox solvent extract toxicity of marine sediments in the north Aegean Sea, Greece, Environ. Toxicol. 17 (2002) 556–566.
[20] A. Katsoyiannis, C. Samara, Determination of selected persistent organic pollutants in sewage and sewage sludge from the wastewater treatment plant of Thessaloniki, Greece, in: Proceedings from the 2nd European Conference on Pesticides and Related Organic Micropollutants in the Environment, 2002, pp. 93–99.
[21] M.L. Jackson, Soil Chemical Analyses, Prentice Hall, 1958.
[22] APHA-AWWA-WPCF. Standard Methods for the Examination of Water and Wastewater, Washington, DC (1989).
[23] Y.S. Wong, F.Y. Tam, P.S. Lau, X.Z. Xue, The toxicity of marine sediments in Victoria Harbour, Hong Kong, Mar. Pollut. Bull. 31 (4) (1995) 464–470.
[24] G. Anasontzis, M. Petala, B. Tsiridis, P. Samaras, A. Zouboulis, G. Sakellaropoulos, M. Lazaridou-Demetriadou, Toxicological study of the ozone-disinfection of secondary effluents, in: Proceedings of the 12th Seminar for the Environmental Protection, 2002, pp. 67–74.
[25] M. Farre, G. Kloter, M. Petrovic, M.C. Alonso, M.J.L. de Alde, D. Barcelo, Identification of toxic compounds in wastewater treatment plants during a field experiment, Anal. Chim. Acta 456 (2002) 19–30.
[26] S. Galassi, L. Guzzella, V. Croce, Screening organic micropollutants in surface waters by SPE extraction and ecotoxicological testing, Chemosphere 54 (2004) 1619–1624.
[27] J.B. Sprague, B.A. Ramsay, Lethal effects of mixed copper and zinc solutions for juvenile salmon, J. Fish Res. Bd. Can. 22 (1965) 425–432.
[28] G. Persoone, M. Goyvaerts, C. Jansen, W. De Coen, M. Vangheluwe, Cost-effective acute hazard monitoring of polluted waters and waste dumps with the aid of Toxkits, Final Report, Comission of European Communities, Contact ACE 89/BE 2/D3, 1993. 600.
[29] T. Vengris, R. Binkienė, R. Butkienė, O. Nivinskienė, V. Melvydas, L. Manusadžianas, Microbiological degradation of a spent offset-printing developer, J. Hazard. Mater. 113 (1–3) (2004) 181–187.
[30] T. Kusui, C. Blaise, Ecotoxicological assessment of Japanese industrial effluents using a battery of small-scale toxicity tests, in: S.S. Rao (Ed.), Impact Asseessment of Hazardous Aquatic Contaminants, Ann Arbor Press, Michigan, USA, 1999, pp. 161–181.

[31] C.V.M. Araujo, R.B. Nascimento, C.A. Oliveira, U.J. Strotmann, E.M. da Silva, The use of Microtox® to assess toxicity removal of industrial effluents from the industrial district of camacari (BA Brazil), Chemosphere 58 (2005) 1277–1281.
[32] E.M. da Silva, A.M.V.M. Soares, O.M.F. Sobral, I.M.C.A. Lopes, J.F.J.S. Correia, E.M.D.C. Marchante, C.B.A. Chastinet, A.J.M. Moreno, Ecotoxicological responses of isolated mitochondrial systems to complex effluents are they worthwhile? Chemosphere 37 (1998) 2695–2701.
[33] R. Guerra, Ecotoxicological and chemical evaluation of phenolic compounds in industrial effluents, Chemosphere 44 (2001) 1737–1747.
[34] M. Nohava, W.R. Vogel, H. Gaugitsch, Evaluation of the luminescent bacteria bioassay for the estimation of the toxicological potent of effluent water samples-comparison with data from chemical analyses, Environ. Int. 21 (1995) 33–37.
[35] C.Y. Chen, C.L. Lu, An analysis of the combined effects of organic toxicants, Sci. Total Eniviron. 289 (2002) 123–132.
[36] R. Boluda, J.F. Quintamilla, J.A. Bonilla, E. Saez, M. Gamon, Application of the Microtox® test and pollution indices to the study of water toxicity in the Albufera Natural Park (Valencia Spain), Chemosphere 46 (2002) 355–369.
[37] M. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barcelo, Determination of drugs in surface water and wastewater samples by liquid chromatography–mass spectrometry: methods and preliminary results including toxicity studies with vibrio fischeri, J. Chromatogr. A 938 (2001) 187–197.
[38] M. Castillo, M.C. Alonso, J. Riu, M. Reinke, G. Kloeter, H. Dizer, B. Fischer, P.D. Hansen, D. Barcelo, Identification of cytotoxic compounds in European wastewaters during a field experiment, Anal. Chim. Acta 426 (2001) 265–277.
[39] M. Farre, M.J. Garcia, L. Tirapu, A. Ginebreda, D. Barcelo, Wastewater toxicity screening of non-ionic surfactants by Toxalert and Microtox bioluminescence inhibition assays, Anal. Chim. Acta 427 (2001) 181–189.
[40] M. Pardos, C. Benninghoff, C. Guegnen, R. Thomas, J. Dobrowolski, J. Dominik, Acute toxicity of Polish (waste) water with a microplate-based Hydra attenuata assay: a comparison with the Microtox test, Sci. Total Environ. 243 (244) (1999) 141–148l.
[41] I. Mantis, D. Voutsa, C. Samara, Assessment of the environmental hazard from municipal and industrial wastewater treatment sludge by employing chemical and biological methods, Ecotoxicol. Environ. Saf. 62 (2005) 397–407.