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TOXICITY ASSESSMENT OF HOSPITAL WASTEWATER BY THE USE OF A BIOTEST BATTERY

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Abstract: In the paper toxicity assessment of hospital wastewaters samples was performed using direct-contact tests consisting of five species, which represent three different trophic levels of the food chain. IC_{50} or EC_{50} values were estimated for each tested organism: *Pseudokirchneriella subcapitata* $IC_{50/72h}$ 18.77%, *Daphnia magna* $EC_{50/48h}$ 20.76%, *Thamnocephalus platyurus* $EC_{50/24h}$ 22.62%, *Artemia salina* $EC_{50/24h}$ 59.87% and *Vibrio fisheri* $EC_{50/15min}$ 46.17%. Toxic potential of hospital wastewater was described using a system of wastewater toxicity classification. The toxic units (TU) values estimated for each test indicate that hospital wastewaters are toxic (Class III). The variable results of the tests' sensitivity confirmed the need of application of microbiotests battery with organisms of different trophic levels.

INTRODUCTION

Recently, an increasing attention has been paid to the occurrence of pharmaceuticals in wastewaters, surface waters and ground waters [5]. Hospital wastewaters are a source of antibiotics, anaesthetics, disinfectants, heavy metals, AOX (Adsorbable Organic Halogens), iodised X-ray contrast media and cytostatic agents [6]. One of their important features is that amount of compounds released into the environment depends on pharmaceuticals transformation and removal rates [12, 13]. Hospitals are a significant source of these compounds due to their diagnostic, laboratory and research activities, simultaneously drugs are also excreted in non-metabolized form by patients [10]. Nevertheless, it is important to notice that hospitals are not the only source of pharmaceuticals in surface waters. On the one hand the increasing amount of antibiotics and other drugs used outside of hospitals can be observed, and, on the other hand, their removal from waste waters by conventional systems is inefficient [3]. Numerous data indicate that hospital wastewaters are characterized by 15 times higher ecotoxicological potential than municipal wastewaters. According to WHO reports, hospital sludge is composed of biodegradable domestic waste waters in 80%. The remaining fraction includes great amount of unbiodegradable and high toxic risk pollutants. Environmental risk assessment of hospitals effluents often needs to be performed using quantitative structure – activity relationships because experimental ecotoxicological data are only available for very low percentage of pharmaceuticals used in hospitals. Standard physical and chemical characteristics of sewage treatment plant effluent do not reflect real environmental hazard. As single compounds, despite several exceptions, pharmaceuticals indicate no or moderate toxic effect, however components of micropollutants mixture often influence each other so the evaluation of real toxic effect could be feasible [12]. Thus, the purpose of this study was to assess the toxic potential of hospital wastewaters by using a battery of biotests in order to make the determination of their ecological status and as a complement to the standard analytical methods.

MATERIALS AND METHODS

Samples collection

The experiments were performed with wastewater samples from St Joseph's Hospital for Phthisiatry and Lungs Diseases located in Pilchowice (Upper Silesian region, the south of Poland). The hospital has a total capacity of 129 beds and produces approximately 105 m^3 of wastewater daily. The wastewater generated in patients lavatories is collected in a septic tank from which it is conveyed to hospital wastewater treatment plant including biological trickling filter and using disinfection process. After the treatment the effluents are released into the river. The hospital wastewater from the septic tank was sampled twice before any treatment activities. The first sampling was done in September 2009 (series I, II). The second sampling was done in December 2009 (series III, IV). For the testing the samples were filtered and stored at -20^{o}C.

Toxicity tests

Toxicity assessment of hospital wastewaters samples was performed using direct-contact tests consisting of 5 species, which represent three different trophic levels of the food chain (Tab. 1). The 72 h growth inhibition test of green algae *Pseudokirchneriella subcapitata*, the 48 h mortality of crustaceans *Daphnia magna*, the 24 h mortality of crustaceans *Thamnocephalus platyurus* and *Artemia salina* bioassays were conducted following the Standard Operational Procedures of the respective toxkits, i.e. Algaltoxkit F™ [9],

Table 1. Biotoxicity tests used in validation study

Tropic Level	Organisms	Type of test	Biotoxicity testing	End Points	Controls
Bacterial	*Vibrio fischeri*	microbiotest	bioluminescention inhibition	$EC_{50/5min}$ $EC_{50/15min}$	*RW(2%NaCl)
Micro invertebrates	*Artemia salina*	microbiotest	mortality/24 h	$EC_{50/24\ h}$	**DW(2%NaCl)
	Daphnia magna	standards OECD202	mortality/48 h	$EC_{50/\ 48h}$	**DW
	Thamnocephalus platyurus	microbiotest	mortality/24 h		**DW
Plants	*Pseudokirchneriella subcapitata*	standards OECD201	Growth inhibition /72h	IC_{50}	**DW

*RW - reconstituted water; **DW - distilled water

Daphtoxkit F^{TM} magna [4], Thamnotoxkit F^{TM} [8], Arttoxkit M^{TM} [7]. *Vibrio fischeri* test was performed according to Microtox manual [1].

Algal growth inhibition test [9]

The test was carried out with *Pseudokirchneriella subcapitata,* according to the standard protocol [6]. Four algal tests with three replicates were conducted. The cell density was adjusted to 10 000 cell/ml. Each test consisted of five filtered effluent dilutions and a control group. The test flask was incubated on a shaker under continuous illumination. After 72 h the inhibitory effect was measured using spectrophotometer Jenway 1200 at 640 nm. The data obtained from the test was IC_{50} value – the Inhibition Concentration of a wastewater sample that causes an inhibition of test organism growth by 50%.

Daphnia magna immobilization test [4]

The test was carried out with neonates (< 24h). Five test dilutions were prepared in a 50% dilution series with three replicates of five animals. The test volume was 10 ml per well. The animals were not fed during the experiment. The duration of each test was 48 h. After an exposure, the number of immobilized daphnids for each dilution was recorded. The test was performed four times. The data obtained from the test was EC_{50} value – the Effective Concentration of a wastewater sample that causes a mortality of test organism by 50%.

Thamnocephalus platyurus and *Artemia salina* toxicity test [7, 8]

Tests were performed according to the MicroBioTest Standard Operational Procedure. Newly hatched organisms were used for the test. Five test dilutions were prepared in a 50% dilution series. Each sample was tested in triplicate of five animals in disposable multiwall test plates. The test volume was 1 ml per well. After 24 h the number of dead crustaceans was recorded. The *Thamnocephalus platyurus* and *Artemia salina* acute toxicity test was performed four times. The data obtained from the test were EC_{50} values – the Effective Concentration of a wastewater sample that causes a mortality of test organism by 50%.

Vibrio fischeri luminescence inhibition – Microtox [1]

Microtox test uses a luminescent marine bacterium *Vibrio fisheri*, which was purchased in a lyophilized form from Azur Environmental (Carlsbad, US). The test was carried out with Microtox 500 Analyzer. Reagents and samples of hospital effluents were handled according to the WET (Whole Effluent Toxicity) described in the Microtox manual [1]. The test exposes organisms to wastewaters samples and measures the percentage of bioluminescence inhibition after 5 and 15 minutes. The data obtained from the test were EC_{50} values – the Effective Concentration of a wastewater sample that causes a reduction in the light output of test organism by 50%. All calculations were conducted by means of standard Microtox software (MicrotoxOmni).

Toxicity assessment according to Persoone *et al.* 2003 [11]

The results of toxicity test were examined for environmental relevance by calculating Toxicity Units (TU). The TU of an effluent is the inverse of its EC_{50} (IC_{50}) values:

$TU = 100/EC_{50}$

Table 2. Wastewater toxicology classification scale proposed by Persoone *et al*., 2003 [11]

Toxic Unit TU_a	Class	Toxicity
$TU_a < 0,4$	I	Non toxic
$0.4 \leq TU_a < 1.0$	II	Low toxic
$1.0 \leq TU_a < 10.0$	III	Toxic
$10.0 \leq TU_a < 100.0$	IV	Very toxic
$TU_a > 100.0$	V	Extremely toxic

RESULTS AND DISCUSSION

In order to evaluate hospital wastewater ecotoxicity, five different aquatic organisms were used. Tables 3–7 present toxicity according to all the tested organisms.

Table 3. Hospital wastewater toxicity according to *Pseudokirchneriella subcapitata*

Sample concentration [%]	Toxic Effect [%]				Average ± SD
	Series I	Series II	Series III	Series IV	
6.25	33.97	3.94*	3.00*	16.42	14.33 ± 14.44
12.5	54.20	20.21	9.60	50.64	33.66 ± 22.13
25.0	74.08	48.82	30.45	74.15	56.88 ± 21.27
50.0	94.50	90.58	67.17	99.00	87.81 ± 14.18
100.0	100.00	100.00	100.00	100.00	100.00 ± 0.00

*no statistically significant difference according to control ($p < 0.05$; t Student test)

Table 4. Hospital wastewater toxicity according to *Daphnia magna*

Sample concentration [%]	Toxic Effect [%]				Average ± SD
	Series I	Series II	Series III	Series IV	
6.25	0.0*	5.0*	5.0*	0.0*	2.5 ± 2.88
12.5	5.0*	0.0*	15.0	10.0	7.5 ± 6.45
25.0	35.0	45.0	40.0	35.0	38.8 ± 4.75
50.0	100.0	100.0	100.0	95.0	98.7 ± 2.50
100.0	100.0	100.0	100.0	100.0	100 ± 0.00

*no statistically significant difference according to control ($p < 0.05$; t Student test)

Table 5. Hospital wastewater toxicity according to *Thamnocephalus platyurus*

Sample concentration [%]	Toxic Effect [%]				Average ±SD
	Series I	Series II	Series III	Series IV	
6.25	6.67*	0.00*	6.67*	0.00*	3.33 ± 3.84
12.5	13.33	13.33	6.67*	13.33	11.66 ± 3.33
25.0	33.33	46.67	60.00	53.33	48.33 ± 11.38
50.0	80.00	100.00	93.33	100.00	93.33 ± 9.48
100.0	100.00	100.00	100.00	100.00	100.00 ± 2.88

*no statistically significant difference according to control ($p< 0.05$; t Student test)

Table 6. Hospital wastewater toxicity according to *Artemia salina*

Sample concentration [%]	Toxic Effect [%]				Average ± SD
	Series I	Series II	Series III	Series IV	
6.25	0.00*	0.00*	6.67*	0.00*	1.67 ± 3.33
12.5	0.00*	0.00*	0.00*	0.00*	0.00 ± 0.00
25.0	6.67*	0.00*	0.00*	0.00*	1.67 ± 3.33
50.0	6.67*	13.33	0.00*	0.00*	5.00 ± 6.38
100.0	100.00	100.00	100.00	100.00	100.00 ± 0.00

*no statistically significant difference according to control ($p < 0.05$; t Student test)

Table 7. Hospital wastewater toxicity according to *Vibrio fischeri*

Sample concentration [%]	Toxic Effect [%]			Average ± SD
	Series I	Series II	Series III	
6.25	9.40	8.70	9.30	9.13 ± 0.31
12.5	18.70	17.50	16.50	17.58 ± 0.89
25.0	39.10	36.50	39.10	38.28 ± 1.21
50.0	62.30	59.60	67.90	63.29 ± 3.43
100.0	89.60	86.70	90.60	88.99 ± 1.67

*no statistically significant difference according to control ($p < 0.05$; t Student test)

The highest toxic effect from all the tested organisms was observed for *Pseudokirchneriella subcapitata*. The obtained EC_{50} value was 18.77% and even low effluent dilutions revealed high toxic effect causing decrease in algal growth (Fig.1). In the case of crustaceans *Daphnia magna* and *Thamnocephalus platyurus* estimated EC_{50} values were similar to those of algae, respectively 20.76% and 22.62%. The lowest effect was observed for *Artemia salina* EC_{50} 59.77% and *Vibrio fischeri* EC_{50} 46.17%. Different results were obtained by Berto *et al.*, 2009 [2], who also tested toxicity of raw hospital effluent due to algae *Scenedesmus subspicatus* and *Daphnia magna*. The test with algae showed a biphasic response: increase in algal growth in low effluent dilutions followed by a decrease in algal growth, with LOEC of 16%. Contrary to our studies, the hospital raw wastewater was much more toxic according to *Daphnia magna*, LOEC 4%. Current studies indicate that all the tests present a linear response according to sample dilutions (Fig. 1).

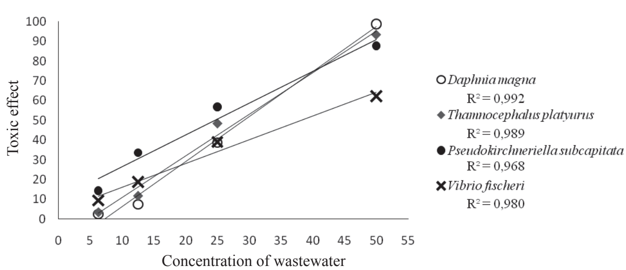

Figure 1. Relationship between concentration of hospital wastewater samples and generated toxic effect according to tested species

The data of potential toxicity of hospital wastewater were described using a system of toxicity classification proposed by Persoone *et al.* [11]. Toxic units and toxic wastewater classification were calculated on the basis of toxicity tests and they are both presented in Table 8. The TU values estimated for each test indicate that hospital wastewaters belong to Class III.

Table 8. Biotoxicity tests results in comparison with wastewater toxicity classification

Type of test	Organisms	End Point	Toxic Unit	Toxicity Class [Persoone et al.,2003]
		EC_{50} (LC_{50}) [%]	TU_a	
MICROTOX®	*Vibrio fischeri*	46.17	2.16	III toxic
Arttoxkit F™	*Artemia salina*	59.87	1.67	III toxic
Daphtoxkit F™	*Daphnia magna*	20.76	4.81	III toxic
Thamnotoxkit F™	*Thamnocephalus platyurus*	22.62	4.42	III toxic
Growth Inhibition Test	*Pseudokirchneriella subcapitata*	18.77	5.32	III toxic

The variable results of the tests' sensitivity confirmed the need of the application of microbiotests battery with organisms of different trophic levels. However, the highest toxic response was obtained for *Pseudokirchneriella subcapitata*, which is to be considered as a basic point in the trophic chains of aquatic ecosystems and introduction of hospital wastewaters into the natural environment could lead to inhibition of its growth [2].

CONCLUSION

The aim of this study was to estimate the ecotoxicological potential of hospital wastewater. Five bioassays were used in order to investigate the effect of different aquatic organisms: bioluminescent marine bacterium, algae and crustaceans. The results of the work showed that the hospital wastewater samples are very toxic according to the applied species. Among the test organisms, *Pseudokirchneriella subcapitata* was the most sensitive in detecting toxicity of samples, while sensitivity of *Daphnia magna* and *Thamnocephalus platyurus* as well as *Vibrio fischeri* and *Artemia salina* was comparable. The results of the present study has proved that it is possible to carry out the ecotoxicological risk assessment of hospital wastewater by the use of bioassays battery.

It is well established that hospital wastewaters are a complex mixture containing chemical compounds, such as pharmaceuticals, active substances, pigments, disinfectants, etc. and in many cases toxic effects induced by those substances are observed in non-target classes of organisms. The interactive effects between pharmaceuticals, disinfectants and surfactants should be taken into account in legislation concerning hospital effluents. It is often the case that one toxicant can aid bioaccumulation processes or cause a synergistic or antagonistic effect on another toxicant in the cells of a test organisms. According to this knowledge it is necessary to broaden chemical analysis, performed on routinely with a battery of biotests to estimate the environmental hazards associated with the pollutants present in hospital effluents.

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ZASTOSOWANIE BATERII BIOTESTÓW DO OCENY TOKSYCZNOŚCI ŚCIEKÓW SZPITALNYCH

Do oceny potencjału toksykologicznego ścieków szpitalnych zastosowano baterię biotestów z wykorzystaniem pięciu gatunków reprezentujących trzy różne poziomy troficzne. Wartości wskaźników ekotoksykologicznych wyznaczone w poszczególnych testach wyniosły: test inhibicji wzrostu glonów *Pseudokirchneriella subcapitata* $IC_{50/72h}$ 18,77%, testy toksyczności ostrej z wykorzystaniem skorupiaków *Daphnia magna* $EC_{50/48h}$ 20,76%, *Thamnocephalus platyurus* $EC_{50/24h}$ 22,62%, *Artemia salina* $EC_{50/24h}$ 59,87% oraz test toksyczności chronicznej z wykorzystaniem bakterii morskich *Vibrio fischeri* $EC_{50/15min}$ 46,17%. Dla każdego testu obliczono jednostki toksyczności TU, na podstawie których analizowane próby ścieków szpitalnych zaklasyfikowano jako toksyczne. Szeroki zakres otrzymanych wartości wskaźników ekotoksykologicznych wyznaczonych dla poszczególnych organizmów testowych, potwierdza konieczność stosowania rozbudowanej pod względem różnorodności organizmów baterii biotestów.