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The relationship between whole effluent toxicity (WET) and chemical-based effluent quality assessment in Vojvodina (Serbia)

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Abstract The relationship between whole effluent toxicity (WET) and chemical-based effluent quality assessment across a range of effluent types was examined for the first time in Serbia. WET was determined by *Daphnia magna* acute tests, while chemical-based toxicity was taken as theoretical for concentrations of priority chemicals and effluent quality assessment based on the valid Serbian regulations. A poor correlation was found between WET and chemical-based effluent quality assessment: positive toxicity tests were found, in general, in cases where samples satisfied the requirements of mandatory effluent monitoring. Statistically insignificant correlation between the predicted and observed toxicity indicated that the presence of priority substances accounted to the overall toxicity only to a certain degree, most probably due to a rather short list of priority pollutants regularly analysed in effluents. Current monitoring requirements neglect hazards that derive from potentially present toxicants and unpredictable toxicity of complex mixtures, which led to poor correlation between the WET and chemical-based results in this study.

Keywords WET · Acute tests · *Daphnia magna* · Priority pollutants

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Introduction

Environmental regulations rely on two approaches to quantify the toxicity of industrial wastes and effluents for the purpose of setting acceptable discharge levels. Chemical-based approach enabled scientists to link individual compounds with harmful ecological effects and to develop priority substances lists to restrict or eliminate discharge of toxic chemicals into the environment (Sarakinos et al. 2000). However, this approach can be applied only after the identification of chemicals present in an effluent and assessment of their potential toxicity, although industrial effluents, particularly those that are treated only partially, as it is still case in Serbia, are generally complex and poorly characterised mixtures of a large number of chemicals. The second approach, the whole effluent toxicity testing (WET), is an integrative tool that measures the toxic effect of an effluent mixture as a whole and accounts for uncharacterised sources of toxicity and for possible

interactions between or among individual chemicals (Chapman 2000).

In their review of international trends in the use of bioassays for effluent management, Power and Boumphrey (2004) showed that many European countries use acute and chronic toxicity tests, as well as tests of mutagenicity, biodegradation, or bioaccumulation, as a part of a whole effluent assessment. According to the cited review, the longest tradition and most developed system in mandatory effluent toxicity assessment in licensing and/or compliance monitoring can be found not only in the USA, Canada and Germany but also in France, Northern Ireland, Norway and Sweden, while many other countries have already adopted guidelines for WET approach or have intentions to introduce WET as mandatory requirement under various regulations. The advantages of this approach were also recognised by the European Oslo and Paris (OSPAR) Commission (OSPAR 2000), which includes bioassays in its recent proposal for effluent monitoring.

In spite of the fact that WET approach has been recognised as a useful complimentary tool and included in effluent management strategy in many countries, it still represents the topic quite intensively discussed in recent studies. There are many unsolved problems and opened questions, such as: (a) the relationship between the results of WET and chemical-based effluent quality assessment (Rodriguez et al. 2006; Mendonca et al. 2007; Picado et al. 2008; Boillot et al. 2008; Ra et al. 2008); (b) the contribution of individual toxicants to overall toxicity of the effluent (Sarakinos et al. 2000); (c) the interaction between potentially present toxicants and other compounds in the effluent, and consequently, the bioavailability of toxicants (Cedergreen et al. 2008; Ra et al. 2006b; Kramer et al. 2004); (d) the problem of toxicity identification evaluation in complex mixtures (Brack 2003; Gutiérrez et al. 2008; Caffaro-Filho et al. 2008; Eunhee et al. 2008); and (e) the increasing need to find, establish and standardise alternative methods and end-points, including the application of genomic based tools in WET approach, which would be more sensitive to priority pollutants and emerging substances (Barata et al. 2008; Kwon et al. 2008).

Serbian environmental legislation is ambient-standard based: valid regulations are developed to ensure that the discharge does not violate numeric ambient standards and narrative criteria outside the mixing zone, exclusively evaluated via water quality assessment based on physicochemical parameters. Whole effluent toxicity testing is not required by any environmental regulation in Serbia. Therefore, the purpose of the presented study was to examine the relationship between whole effluent toxicity and chemical-based effluent quality assessment across a range of effluent types. The data from this study provide a unique opportunity to compare mandatory chemical-based effluent quality assessment in Serbia and WET results in order to establish the industry-specific trends in toxicity. Furthermore, comparing theoretical toxicity, which is estimated from individual chemicals, with the measured WET permitted an evaluation of the extent to which priority substances accounted for WET in complex effluents. Finally, the results of this study could be used in efficiency analysis of the valid environmental regulations in Serbia.

Methods

Effluent sampling and chemical analyses

Effluent samples—taken as composites over 2 h as prescribed by OGRS (1983)—were collected from industrial facilities and publicly owned treatment plants located along the shores of the River Danube and the Danube–Tisza–Danube canal system in the Serbian province of Vojvodina. Effluent sources types are listed in Table 1.

All effluent samples were analysed for pH, chemical oxygen demand (COD), total suspended solids (TSS) and concentration of ammonium ion (NH_4), while majority of samples were analysed for electroconductivity (EC), surfactants, biological oxygen demand, greases and oils. Additional analyses for priority and specific pollutants (metal cations, nitrates, nitrites, phosphates, total N, cyanide, phenol, sulphides, sulphates) were performed on case-specific basis, according to requirements of OGRS (1983), as shown in Table 2.

Table 1 Sample types and abbreviations

Industry type	Abb.	Industry type	Abb.	Industry type	Abb.
Small manufacture	SM1	Metal processing	M1	Textile	T 1
Small manufacture	SM2	Metal processing	M2	Textile	T 2
Small manufacture	SM3	Metal processing	M3	Chemical	Ch
Small manufacture	SM4	Metal processing	M4	WWTP	TP1
		Metal processing	M5	WWTP	TP2
		Metal processing	M6		

WWTP Waste water treatment plant

All parameters were analysed using standard methods and procedures according to APHA et al. (1995).

Daphnia magna culture and toxicity tests

The laboratory culture of *D. magna NSV* originates from the population of some 40 specimens of *D. magna* that were taken out of a natural lake located in the Province of Vojvodina (Serbia) in 1974 and that have been kept ever since in the Ecotoxicology Laboratory of the Faculty of Sciences, Novi Sad. Therefore, the culture was named *D. magna NSV*.

The population of *D. magna NSV* was grown, as explained earlier (Teodorovic and Planojevic 2008) in cultures of parthenogenetic females, in cohorts of different ages, at 20 ± 2°C, in 1-l borosilicate glass test vessels. The daphnids were cultured in a photoperiod of 16-h light/8-h dark, with light at about 800 lux. Reconstituted hard water was prepared in compliance with the ISO standard guide (ISO 1996). Water had been aerated up to 100% oxygen saturation several hours before organisms were introduced. Daphnids were cultured at a density of 10–15 adults per litre. Algae (*Chlorella vulgaris*, laboratory strain) were cultured in the laboratory on Algae broth 148 (Difco Laboratories; Detroit, Michigan, USA), at 25 ± 2°C and used as daphnid food. As recommended by the US Environmental Protection Agency (USEPA 2002), daphnid cultures were fed with algae on a daily basis—1 ml (cell density $> 1 \times 10^7$ cells/ml), and three times a week, their diet was supplemented with 1 ml of YCT mixture—1:1:1 suspension of 5 g yeast/l (commercial yeast for human consumption), 5 g of dried cereal leaves/l (wheat was grown in water culture in the laboratory) and 5 g/l of digested fish flakes (commercial fish pellets No. 1 for aquaria fish).

WET tests were conducted as single-species acute multi-concentration tests on *D. magna* according to standardised USEPA (2002) procedures. For most effluents, a preliminary test was performed first by preparing five concentrations of an effluent, from 0.01% to 100%, and adding two control batches (only reconstituted water that was used also as a dilutant). This test used two replicates per each treatment. Tests lasted for 48 h, during which daphnids were not fed. Afterwards, a definitive 48-h multi-concentration test was run with effluent concentrations being selected to include concentrations causing zero and 100% daphnid mortality in the preliminary test. This test included 5–7 effluent dilutions (with a dilution factor of 0.3–0.8, but usually 6.25%, 12.5%, 25%, 50% and 100% effluent) and two sets of control batches containing only reconstituted water. Four replicates were prepared for each treatment. Mortality in control batches was up to 20%.

Statistical analyses and data processing

The measured effluent toxicity was expressed as 48 h TU_a according to the following equation:

$$48\,\text{h}\,TU_a = \frac{100}{48\,\text{h}\,LC_{50}} \tag{1}$$

Toxic unit acute (TU_a) is a reciprocal value of the lethal concentration—LC_{50}—i.e. effluent concentration that causes 50% of organisms to die by the end of the acute exposure period of 48 h. Lethal concentrations were estimated by regression models: Probit, Spearman–Karber and graphic, using TesTox software (Teodorovic and Mauric 2003).

Theoretical toxicity of an effluent was also expressed in toxic units TU_i and calculated as follows:

$$TU_i = \text{conc}_i F_{\text{tox}\,i} \tag{2}$$

Table 2 The results of chemical analyses, theoretical toxicity (T TU), empirical toxicity (48 h TU_a) and chemical characterisation (F_{ch}) of selected effluent samples

Parameter	Ftox	SM1	SM2	SM3	SM4	M1	M2	M3	M4	M5	M6	T1	T2	WWTP1	WWTP2	CH1
pH		7.8	7.7	7.9	8.1	7.2		8.4	8.2	6.9	7	7.7	7.3			7.9
COD (mg O_2/l)		300	110	438	468	400	50	180	120	600	120		1200	760	40	170
BOD_5(mg O_2/l)		1.76	37	272	292	95	19	33	–	250	52			125	12	
TSS (mg/l)		37	223	26	28	26	2225	87	–	58	39	104	141	0.51	170	76
Surfactants (mg/l)		1.95	2	9.4	1.45	2.04	0.6292	0.86	0.09445	0.07	0.08	0.66	5.01	0.1	0.58	0.08
EC (μS/m^2)		1675	240	2.2	2.64	640				870	1140					2264
Total N (mgN/l)		300	1.4	188	54.7											
Kjeldal N (mgN/l)		0.195	0.15	12	1.75								2			
Greases and Oils (mg/l)	1	155	143	1.033	0.9408	28.8	290	0.23	115.7	74.1	93.4	148		0.06	1.2	136.9
Mineral Oils (μg/l)	1														64	
NH_4^+ (mgN/l)	0.1	0.5	1.25	5.7	8.04	2.5	8.75			2	3	12.3	14	7.5	10	1.75
NO_3^- (mgN/l)	0.005	5	1									< 2	2	< 2	2	14
NO_2^- (mgN/l)	0.5	0.15	0.1									–	0.2	< 0.2	< 0.2	0.4
PO_4^{3-} (mgP/l)	0.02	< 1	1.34			1.34						2.68				
H_2S (mg/l)	0.6														7.14	
S^{2-} (mg/l)	0.6														7.14	
SO_4^2 (mg/l)	0.001													61	70	
Phenols (mg/l)	0.03													0.13	0.11	
Cl^- (mg/l)	0.1													60	57	
CN^- (mg/l)	0.5													0.28	< 0.03	
Cu (mg/l)	2					0.67	11.17			–	0.23					
Zn (mg/l)	2.5					0.94	62.5	0.41	0.04	12.78	11.7					
Fe (mg/l)	0.15					0.12	1.55			0.2	3.86					
Pb (mg/l)	0.1					0.14				0.17	0.11					
$Cr_{(tot)}$(mg/l)	0.2						0.084	0.6	0.2							
Ni (mg/l)	0.2							3.6	0.63							
Fch		0.5	0.5	0.89	0.26	0.39	4.3	0.51	0.55	0.68	0.72	0.87	0.47	0.6	2.03	3.59
T TU		2.19	2.16	2.37	2.03	1.52	2.67	0.32	2.07	2.03	2.09	2.17	0.18	1.01	1.05	2.14
48 h TUa		6.07	6.06	0	1.73	7.09	1.21	1.35	0	60.20	74.29	4.78	1.39	2.78	0	0

where $F_{\text{tox i}}$ is the weighing factor assigned to chemical i. Each priority contaminant was assigned a toxic weighing factor, F_{tox}, calculated as the inverse of its mean LC_{50} so that:

$$F_{\text{tox}} = (\text{mean } 48\,\text{h}\,LC_{50})^{-1}\,(\text{mg/l}) \tag{3}$$

The LC_{50} values were obtained from USEPA AQUIRE database (USEPA 2008). Total theoretical toxicity was then calculated as the logarithm of the sum of toxic units of each detected priority pollutant in effluent as follows:

$$\text{T TU} = \log\left(\sum \text{conc}_i F_{\text{tox i}}\right) \tag{4}$$

Theoretical toxicity of the effluent is the sum of TUs of its constituent chemicals. This numerical score can then be used to compare relative toxicity of different industrial sectors, as well as to identify the predominant toxicants in effluent. Similarly to total theoretical toxicity unit, a chemical factor F_{ch} has been introduced—a single value based on mandatory chemical analyses that evaluates the effluent quality. Chemical factor is calculated as follows:

$$F_{\text{ch}} = \frac{\sum \text{conc}_i \text{MCL}_i^{-1}}{k} \tag{5}$$

where k stands for number of water quality parameters analysed in a sample and MCL_i for the maximum contaminant i level according to the valid Serbian regulations. Since environmental regulations are ambient-standard based, it means that MCL was derived from the ambient standards for priority toxicants and hazard substances in mixing zones (OGRS 1982). It was assumed that if calculated F_{ch} is less than 1, the effluent quality complies with the regulations, and vice versa. Despite the fact that priority pollutant list in ambient waters sets limitation for 223 substances (OGRS 1982) and that official surface water classification sets additional limits for 15 more basic water quality parameters (OGFRY 1978), a routine compliance effluent monitoring (OGRS 1983) consists of analysis of 10–15 basic and industry-specific water quality parameters. So, when compared to, for example, Priority List of the Canadian Ministry of Environment (Sarakinos et al. 2000), only 18 priority pollutants (listed in Table 2) were analysed via mandatory monitoring in 15 samples discussed in this paper.

Results and discussion

The results of chemical analyses of effluent samples that were evaluated in this study, as well as calculated theoretical toxicity (T TU), empirical toxicity (48 h TU_a) and chemical characterisation (F_{ch}) are presented in Table 2. The highest values of chemical factors, expressed as F_{ch} (calculated according to Eq. 5), were recorded in M2 and Ch (4.3 and 3.59, respectively). However, as shown in Table 2, the overall assessment based on chemical-specific approach (non-compliance expressed as $F_{\text{ch}} > 1$) indicated only three out of 15 samples as those violating environmental standards. On the other hand, theoretical toxicity (T TU), calculated according to Eq. 4, based on the presence of priority pollutants (T TU > 1) predicted toxicity in 13 out of 15 samples. The highest value was calculated for sample M2—2.67. However, empirical toxicity expressed as 48 h TU_a (calculated according to Eq. 1) observed in 11 out of 15 samples, reached high values, particularly in samples M6 (74.29) and M5 (60.2).

The regression analysis of the observed whole effluent toxicity (expressed as 48 h TU_a—calculated according to Eq. 1) and plotted against chemical characterisation according to the valid Serbian regulations (expressed as F_{ch}—calculated according to Eq. 5) revealed no significant relationship between the two methods of effluent quality assessment ($Y=1.23-0.01X$; $R=-0.18$; $R^2=0.03$; $p>0.05$; $n=15$; Fig. 1).

The percentage of agreement between overall WET and results of chemical compliance monitoring was also examined (Fig. 2). Four possible outcomes were considered: (1) WET information for a facility indicated unacceptable toxicity and compliance chemical monitoring detected unacceptable effluent quality (agreement on unacceptable effluent quality); (2) WET information for a facility indicated no toxicity and compliance chemical monitoring detected acceptable effluent quality (agreement on acceptable effluent quality); (3) WET information for a facility indicated unacceptable toxicity while compliance chemical

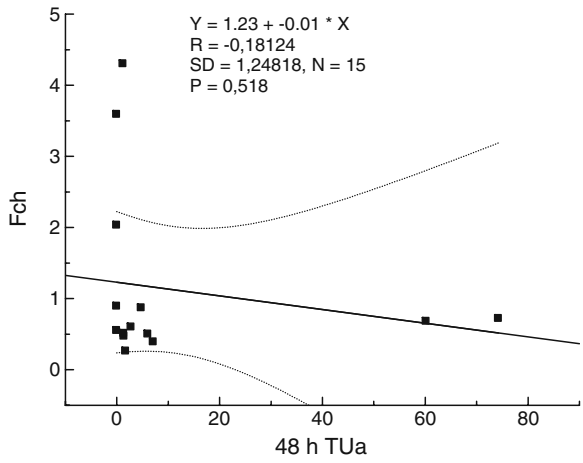

Fig. 1 Regression analysis and plot with corresponding confidence intervals of the relationship between the whole effluent toxicity (48 h TUa) and chemical characterisation according to the valid Serbian regulations (F_{ch})

monitoring detected acceptable effluent quality (disagreement on unacceptable effluent quality); (4) WET information for a facility indicated no toxicity while compliance chemical monitoring detected unacceptable effluent quality (disagreement on acceptable effluent quality).

We recorded poor agreement between WET and chemical characterisation of effluents: 80% of samples resulted with disagreement while for only 20% of them, there was an agreement between two effluent quality measurements (Fig. 2).

It should be emphasised that even in 67% of samples, we observed disagreement on unacceptable effluent quality—WET failed, while compliance monitoring passed. In only one sample (7%), we recorded agreement on unacceptable effluent quality—both WET and compliance monitoring failed.

The regression analysis of the observed whole effluent toxicity (48 TU_a—calculated according to Eq. 1) plotted against theoretical toxicity (toxic units T TU—calculated according to Eq. 4) revealed no significant relationship between the two toxicity measures ($Y = 1.06 + 5.91X$, $R = 0.19$, $R^2 = 0.04$, $p > 0.05$, $n = 15$; Fig. 3).

Detailed analysis of residual toxicity—difference between the observed (expressed as 48 h TU_a calculated according to Eq. 1) and theoretical toxicity (expressed as T TU calculated according to Eq. 4) is plotted in Fig. 4. A positive residual denotes that WET was greater than predicted toxicity for a given effluent, whereas a negative residual denotes the opposite result. It is obvious that the smallest difference was observed in effluent from waste water treatment plants (0.36) and textile industry (0.42). There were two groups of exceptional samples whose WET did not correspond to toxicity estimates based on the presence and concentration of priority contaminants: chemical industry (−2.14) with a negative value of residual toxicity and the other

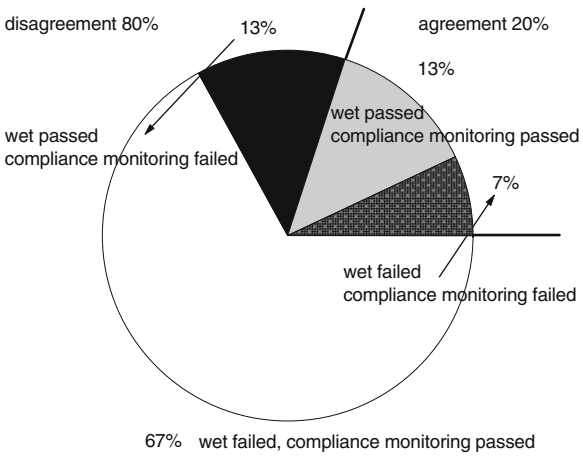

Fig. 2 Percentage of agreement between the WET test and chemical characterisation based on compliance monitoring

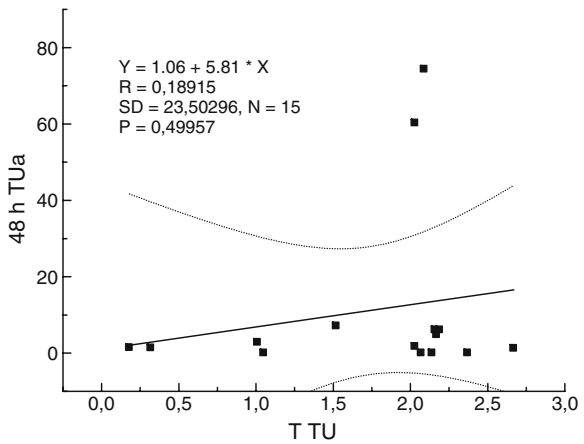

Fig. 3 Regression analysis and plot with corresponding confidence intervals of the relationship between whole effluent toxicity (48 h TUa) and theoretical toxicity (T TU)

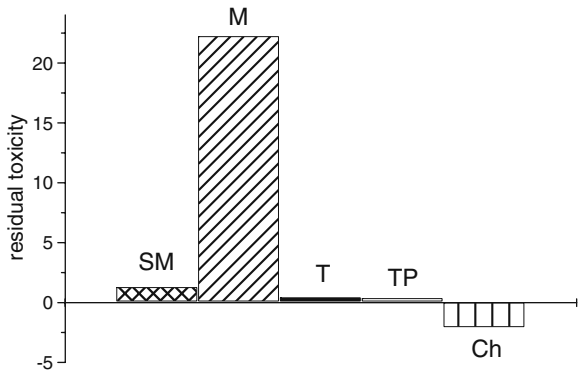

Fig. 4 Mean residual whole effluent toxicity by categories of industrial activities: *SM* small manufacture, *M* metal processing industry, *T* textile industry, *TP* waste water treatment plant, *Ch* chemical industry

group consisting of effluents whose WET was greater than predicted: small manufacture (1.28) and metal processing industry (22.24).

In addition, no correspondence between theoretical toxicity (toxic units T TU—calculated according to Eq. 4) and chemical characterisation was recorded (regression equation $Y = 0.19 + 0.54X$; $R = -0.33$, $R^2 = 0.11$, $n = 5$; Fig. 5).

In our study, no correlation was found between the observed WET and chemical characterisation according to the valid Serbian regulations. The results of chemical analyses of the effluent

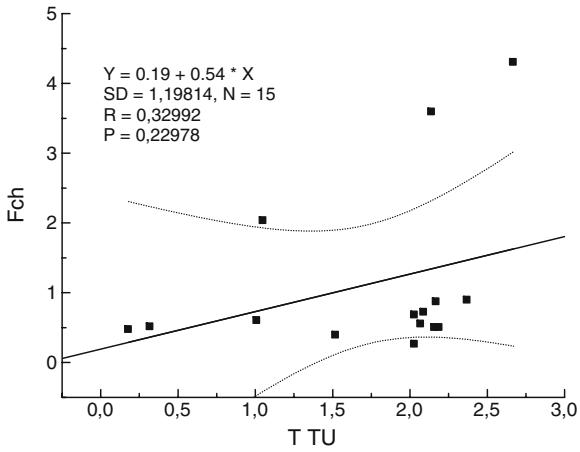

Fig. 5 Regression analysis and plotting with corresponding confidence intervals of the relationship between theoretical toxicity (T TU) and chemical characterisation according to the valid Serbian regulations (F_{ch})

samples assessed in our study showed that high content of suspended matter and high organic load (high values of TSS and COD) as well as high contents of grease and oils, apparently related to insufficient treatment, are still the major characteristics of almost all studied effluents. Toxicity was observed in 11 out of 15 samples, while non-compliance based on chemical characterisation was recorded in three samples. Only one sample (M2) with the observed toxicity (48 h TU = 1.21) also violates the effluent discharge regulations, due to high content of Zn (62.5 mg/l). Other two cases of non-compliance with effluent regulations, WWTP2 (due to 7.14 mg/l H_2S) and Ch (1,369 mg/l total greases and oils) represent two out of four samples with no observed toxicity (48 h $TU_a = 0$). Another two non-toxic effluents (48 h $TU_a = 0$), SM3 and M4, are in compliance with effluent regulations. However, in ten out of 15 samples, toxicity (48 h TUa > 1) was observed in effluents, which are in compliance with effluent regulations. The similar pattern, though using fish and algae for toxicity testing of various effluents was reported by Di Marzio et al. (2005)—they also observed severe toxicity of effluents containing individual parameters within ELV. Poor correlation between toxicity (assessed using Microtox) and COD values in untreated mixture of industrial wastewaters and high correlation in case of treated effluent was observed by Araujo et al. (2005), whereas Hernando et al. (2005) tested correlation between TOC and toxicity (*Vibrio fishery*, *D. magna* and *Selenastrum capricornum*) in order to assess the efficiency of WWTP. As they found none, they concluded that chemical measurements and toxicity test should be seen as complimentary methods in effluent control. Limitations of predicting toxicity from chemical data was also observed in ecotoxicological study of industrial and urban wastewaters in Estonia and Lithuania (Manusadzianas et al. 2003). Whole effluent toxicity tests are increasingly used to monitor compliance of permitted discharges. By using an in situ bioassessment approach, Maltby et al. (2000) demonstrated receiving water toxicity and ecological degradation that were consistent with the results of WET tests performed on the point source discharge and concluded that systematic approach provides a comprehensive and

ecologically relevant database for assessing the ecological risk posed by point source discharges, so that by applying a set of complementary diagnostic tools, resource managers and dischargers would gain greater confidence in the permit limits set for effluents and the methods used to monitor compliance. Quite a large number of specific research studies of ecotoxicity of industrial effluents using conventional toxicity tests combined with chemical quality assessment, were conducted world-wide, such as, for instance, in Portugal (Mendonca et al. 2007), Spain (Rodriguez et al. 2006), UK (Tinsley et al. 2004), Italy (Guerra 2001), Netherlands (Tonkes et al. 1999), Turkey (Sponza 2002), Lithuania and Estonia (Manusadzianas et al. 2003), Argentina (Gomez et al. 2001; Di Marzio et al. 2005), Brasil (Araujo et al. 2005) and South Korea (Ra et al. 2006a). The conclusion from all the cited studies, which stands for our research as well, is that clear environmental benefit can be delivered by targeting the use of effluent bioassays at catchments with well defined water quality problems, where ecotoxicity from complex effluents is expected to be a contributing factor, despite compliance with chemical limits.

Another concern of this study was the presence of selected priority contaminants and their contribution to the observed toxicity. In our study, we used a simple additive approach: T TU of an effluent was calculated as the logarithm of the sum of theoretical toxicity of individual constituent (TU_i). The problem of predictability of overall toxicity of complex mixtures according to individual toxicity of the present priority pollutants was excessively discussed, so far. Two most commonly used models to predict joint effects of mixtures are concentration addition and independent action. Recently, (Ra et al. 2006b) a combined two-step prediction using the former two was suggested. The concept of concentration addition assumes a similar toxicological mode of action for mixture components. The alternative concept of independent action assumes a dissimilar action of mixture components that interact with different target molecule sites, leading to a common toxicological endpoint via distinct chains or reactions within an organism. With respect to risk assessment procedures, Altenburger et al. (2000) suggested that concentration addition gives a valid estimation of the overall toxicity for complex effluents, particularly if components are of similar mechanisms of action. However, it has been suggested that the concentration addition model overestimates the mixture toxicity, while competing notion of independent action is more appropriate for mixtures of dissimilarly acting chemicals (Backhaus et al. 2000). Still, as the similarity of components is often unknown for mixtures found in the environment, it could be concluded that concentration addition may give a realistic worst-case estimation of mixture toxicities for risk assessment procedure. A recent comprehensive review (Cedergreen et al. 2008) showed that none of the models proved significantly better than others. Moreover, half of the experiments could not be correctly described with neither of the two models. Thus, the authors concluded that neither model could be selected over the other on the basis of accuracy alone.

In our study, the regression analysis of the observed effluent toxicity (WET) plotted against theoretical toxicity (theoretical toxic units T TU) revealed no significant relationship between the two toxicity measures. However, it should be noted that although correlation coefficient was not significant, regression line was still positive, indicating that priority pollutants measured by regular compliance chemical-based monitoring of effluent quality do contribute, at least to some extent, to overall measured toxicity. In WWTP and textile industry effluents, the difference between predicted and exhibited toxicity was negligible, meaning that although limited in number of parameters, effluent monitoring programme still covers all potentially toxic or hazardous substances. The findings were to some extent surprising, at least for textile industry, as several of regularly occurring priority pollutants, like Cr, AOX, halogen compounds, dyes (Sponza 2002) are not routinely monitored in textile effluents according to the valid Serbian regulations. Moreover, they obviously do not reach lethal concentrations for selected test species. Chemical industry (−2.14) challenges the assumption of additivity of toxicity, or, rather, the results question the toxicity factors assigned to some priority contaminants. Namely, relatively high T TU values (theoretical toxicity) in this effluent derives

from high content of oil and greases and ammonium ion, which, actually, did not cause any direct effect on survival of selected test species. Yet, these results must be taken with a certain degree of reserve, as WET test were conducted on a single species, using single end-point. It could well be that both effluent types (textile and chemical industry) would have proved toxic if a battery of tests species and more end-point were used, as suggested by Castillo et al. (2000).

The other group of effluents showing non-standard properties consists of those with WET greater than predicted: small manufacture (1.28) and metal (22.24). In other words, even the addition model could not predict the overall quality of these extremely toxic effluents, as the measured WET is by far greater then the sum of theoretical toxicity values of the analysed pollutants. Similar pattern was observed in the study by Benfenati et al. (2003) where an additive approach in terms of the contribution made by different constituents was assumed: the predicted toxicity of complex mixtures (landfill leachate and textile effluent) containing metals as well as various organic contaminants was by far lower than the measured toxicity both in case of *Microtox* and *D. magna* acute test, as the predicted toxicity accounted for only 15–27% of the observed toxicity (mostly associated with presence of metals) meaning that other unidentified substances appear to be contributing to the toxicity of the mixture. When mechanisms of toxic action are more specific, or when there is more than one mode of toxic action (as it is the case with most trace metals), one can assume neither that joint toxic action of a mixture will be strictly additive nor that theoretical toxicity will necessarily reflect WET (Sarakinos et al. 2000).

In our study, we calculated values of both used metrics (chemical factor and theoretical toxicity) on overall metal concentration, as this is exactly the parameter used in effluent characterisation according to the valid Serbian regulations, although it is a well known fact that bioavailability and toxicity of metals are not directly related to total metal concentration. Toxicity predictions for trace metals are most meaningful when toxicity is evaluated using metal ion, instead of total metal, concentrations (Sarakinos et al. 2000). In addition, it has been proven that bioavailability (and thus toxicity) of metal ions directly depends on several environmental factors, such as water hardness, pH value (Barata et al. 1999) and DOM (Kramer et al. 2004). For more accurate prediction of toxicity of bioavailable metals, particularly for risk assessment procedures, Biotic ligand model BLM was proposed (De Schamphelaere and Janssen 2002). Metal toxicity to aquatic biota is a complex function of chemical characteristics of water and biology of the target organism. However, in view of the lack of simple tools to address these relationships, regulatory frameworks, including the one currently in force in Serbia, usually set protective criteria on the basis of total or dissolved metal concentrations. There are only several exceptions that we are aware of, such as, for example, the correction by hardness to derive metal water quality criteria and water effect ratio approach used by the USEPA (1994) and water quality standards for total Zn and dissolved Cu based on total water hardness according to Council Directive 78/659/EEC.

Generally, in cases when total metal concentrations were used for toxicity prediction, the toxicity tends to be over-estimated, as only a smaller part of metals in mixture proves to be actually bioavailable and, therefore, could be associated with toxicity of metal containing mixture. Therefore, it was to be expected that all metal—containing effluents in our study follow such pattern. However, only in two out of six metal-containing samples theoretical toxicity exceeded the observed: in M2 and M4. Extremely high concentration of Zn (62.5 mg/l) and Cu (11.17 mg/l) in M2 contributed the most to high T TU value (2.67—the highest T TU in the study). Still, the observed toxicity was considerably lower—48 h TUa 1.21, proving that total metal concentration measured as mandatory parameter cannot be linked directly to bioavailability and toxicity. It is, therefore, rather surprising to find metal-containing effluents in the group of samples with positive value of residual toxicity: the highest discrepancy between theoretical and empirical toxicity recorded in our study was observed in case of metal processing industry effluents: in four out of six samples containing metals observed toxicity was greater that theoretical. The extreme empirical toxicity was recorded only in samples labelled M5 and M6

(60.2 and 74.29 TU_a)—samples with high content of Zn (11.7–12.78 mg/l) and Pb (0.11–1.17 mg/l) but still in compliance with effluent regulations.

Due to a rather outdated concept of mandatory effluent monitoring in Serbia (a limited number of priority pollutants, most attention paid to basic water quality parameters), as well as inadequate way of derogation and expression of environmental quality standards (most typical in case of total metal concentration), in case of positive outliers (when measured toxicity was much higher then predicted), it was difficult to determine whether this was due to positive interactions among chemicals or to the presence of unknown chemicals. This means that uncharacterised toxic substances in industrial effluents and interactions between chemicals, both synergistic and antagonistic, have a significant effect on the toxicity of complex effluents.

Conclusions

The results of this study, although only preliminary (screening) as WET was estimated on a single species only, using a single end-point, indicate that effluent quality assessment and compliance monitoring according to the valid environmental regulations in Serbia tend to underestimate the effluent toxicity-measured WET was higher than predicted by chemical-based approach. Additionally, the correlation found between WET and chemical-based effluent quality assessment was poor. Positive toxicity tests were, in general, found in cases where samples satisfied the requirements of mandatory effluent monitoring. Since Serbian environmental legislation is ambient-standard based, the limitations for effluent discharges into the surface water do not even exist. Valid regulations are developed to ensure that the discharge does not violate numeric ambient standards and narrative criteria outside the mixing zone. Such monitoring requirements rely mostly on basic water quality parameters, apparently neglecting the potential hazard that derives from toxicants assumed to be present in toxic amounts, as well as toxicity of mixtures, which, in the end, led to pour correlation between the WET and chemical-based results in this study. The use of effluent bioassays in regular mandatory monitoring, particularly in cases where ecotoxicity from complex effluents is known to be a contributing factor, despite compliance with chemical limits could be beneficial for receiving ecosystems and together with chemical analyses of effluents and hydrological characterisation and biomonitoring of receiving waters contribute to more comprehensive and rational environmental protection practice. Screening single species WET test, potentially with *D. magna* as the most common aquatic test, could represent an initial step in the process of upgrading the mandatory effluent assessment in Serbia.

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