water

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Supplementary material

Evaluation of Ecotoxicity of Wastewater from the Full-Scale Treatment Plants

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Table S1. Ecotoxicity of wastewater - data selected for review.

Wastewater	Species	Method	Endpoint	Information Extracted	Reference
Influent and effluent wastewater from 12 WWTPs located in industrial parks in China	*Photobacterium phosphoreum* T3 spp.	Bioluminescence inhibition test.	Inhibition percentage	Inhibition degree of influents from 25.9% to 100%, while for effluents it varied from 18.5% to 91%. Generally, the decrease of bioluminescence inhibition was found. Only in SBR the increase of inhibition percentage was observed.	[1]
	Euglena gracilis	Growth inhibition test. Measurement of absorbance at 610 nm.	Degree of growth inhibition (%)	Degree of inhibition for influents and effluents varied from 42.8% to 77.3%. For 9 out 12 WWTPs no significant change in the degree of inhibition between influent and effluent was reported.	
	Tetrahymena thermophila (protozoa)	Growth inhibition test. Measurement of absorbance at 492 nm.	Degree of growth inhibition (%)	Degree of inhibition of influents and effluents was between 69.7–96.0% and 75.9–95.9%, respectively. For 10 out 12 WWTPs no change in the inhibition degree between influent and effluent was reported.	
	Daphnia magna straus	Immobilisation inhibition test. Determination of the number of immobilised individuals after 24 h and 48 h of exposure.	Degree of inhibition (%)	Degree of inhibition reached 100% after 48 h exposure in 4 out of 12 influents tested. A large variation of results of tests. Degree of inhibition for influents and effluents varied from several to 100%.	
Two types of wastewater from	*Vibrio fischeri*	Microtox® test made in agreement with DIN ISO	EC50	Influents were highly toxic (EC50<60%), while the	[2]

the company processing meat: (1) he washing wastewater, (2) the condensate wastewater. The wastewater were treated in SBRs.		11348-3 (1998). Measurement of the emission of light for 15 minutes with different dilutions of wastewater and a suspension of luminescent bacteria.		effluents from SBR were low or not toxic (EC50>82%). The correlation between the ammonia nitrogen and the toxicity of wastewater was found.	
Effluents from 75 WWTPs of 16 countries (European Union Member States and Switzerland) were tested.	Human breast carcinoma MVLN cells	The MVLN reporter gene assay. Human breast carcinoma MVLN cells stably transected under the control of estrogen receptor with firefly luciferase gene were used.	EEQ	About one third of municipal WWTP effluents contained EEQ greater than 0.5 ng/L EEQ. EEQ above 0.5 ng/l indicated on the importance of cities as the major contamination source.	[3]
Effluents (93 samples) from SBRs and MBRs treating domestic, municipal and industrial wastewater. WWTPs were located in Venice (Italy).	*Phaeodactylum tricornutum* Bohlin	The growth inhibition or stimulation was determined according to ISO 10253 method. *P. tricornutum* was exposed to increasing concentrations of samples for 72 ± 2 h. Cellular density was measured.	Toxicity Unit (TU_{50}); Biostimulation Unit (BU_{50})	91% of all samples showed a stimulation effect. Among them 7, 30, 31 and 7 samples with low, medium, high and very high effects, respectively, were detected. In general, 90% of samples showed from medium to very high stimulation or toxicity effects. Toxicity classification system based on inhibition and stimulation of microalgae growth was established.	[4]
51 samples of stormwater were taken from urban area of Sydney, Brisbane and Melbourne (Australia)	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* according to EN ISO 11348-3 (1998).	Toxicity Equivalent Concentration (TEQ)	TEQ ranged between 0.20 and 2.75 mg/l for most samples tested. The results were around or slightly higher than these obtained for the secondary treated WWTPs effluents. Highest effects similar to primary effluents.	[5]
	Pseudokirchneriella subcapitata	The combined algae test integrates the quantification of the inhibition of photosynthesis with specific and non-specific effects on the growth rate. Inhibition of photosynthesis was assessed after 2 h of	IPAM : Diuron equivalent concentrations (DEQ). Algal growth: TEQ	In most samples photosynthesis was a more sensitive endpoint than growth inhibition, Algal toxicity was dominated by herbicides in most samples.	

		exposure using I-PAM (imaging pulse-amplitude modulated) fluorometry and inhibition of growth rate after 24 h exposure.			
The antibiotic wastewater treatment plant treating wastewater from a drug manufacturer (Shijiazhuang City, China). 15 samples from five sampling points incl. SBR, biofilm reactor and secondary clarifier	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* according to ISO 21338 (2010).	Toxicity Unit (TU50) calculated upon EC50-15 min.	TU50 varied from 1.40 to 49.75% depending on the sampling point. The raw wastewater samples were highly toxic and the wastewater toxicity decreased during the treatment process. A significant, positive linear correlation between TU50 and COD, BOD5, TOC, $NH4^+$ and others.	[6]
Samples taken seasonally (4 times per year) for 4 years from two WWTPs with conventional activated sludge systems. Each time samples were taken from influent, secondary- and tertiary effluent.	*Pseudokirchneriella subcapitata*	Algaltoxkit F™ according to OECD method no. 201 (2011). Algae growth was determined by the measurement of optical density.	EC50 transformed to Toxic Units (TUs)	TUs>100 for the influents; TUs varied from 1.3 to above 100 for the secondary effluents. TUs varied from 0.4 to above 100 for the tertiary effluents. Algae were the most sensitive species out of four species tested in this work; Seasonal decrease of the toxicity of effluents in autumn and winter in comparison to spring and summer was observed.	[7]
	Daphnia magna	Daphtoxkit F™ according to OECD method no. 202, (2004). Neonates were icubated at the appriopriate conditions and after 24 h and 48 h the number of dead/immobilized neonates was calculated.	EC50 transformed to Toxic Units (TUs)	TUs>100 for the influents;. TUs varied from 0.4 to above 100 for the secondary effluents. TUs varied widely from below 0.05 to above 100 for the tertiary effluents The toxicity to *D. magna* (48 h) was at the same level as toxicity to *P. subcapitata* determined in this work.	
	Artemia salina	Methodology elaborated by US EPA (2002). Method based on determination of immobilization *of Artemia nauplii* after 24 and 48 h.	EC50 transformed to Toxic Units (TUs)	TU values varied from 2.6 to 5.8 for the influents. *A. salina* was not affected by secondary and tertiary effluents of either WWTP (TUs<0.1).	

				A. salina was the least sensitive indicator out of organisms used in the toxicity tests in this study.	
	Vibrio fischeri (bacteria NRRL B-11177)	Microtox® test Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri*.	EC50 transformed to Toxic Units (TUs)	TU values varied from 3.8 to 40.0 for the influents. TUs varied from below 0.1 to 1.8 for the secondary effluents and for the tertiary effluents. Toxicity of the effluents was usually lower in autumn and winter than in spring and summer. *V. fischeri* was less sensitive than *P. subcapitata* and *D. magna* but it is fast method for evaluation of wastewater quality.	
Samples of 21 sites including three WWTPs (Australia) were taken.	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria Vibrio fischeri according to EN ISO 11348-3 (1998).	Toxicity Equivalent Concentration (TEQ)	Decrease of toxicity was found after activated sludge treatment, reverse osmosis, advanced oxidation. Chloramination and microfiltration caused to increase of toxicity.	[8]
	Pseudokirchneriella subcapitata	The combined algae test integrates the quantification of the inhibition of photosynthesis with specific and non-specific effects on the growth rate. Inhibition of photosynthesis was assessed after 2 h of exposure using I-PAM (imaging pulse-amplitude modulated) fluorometry and inhibition of growth rate after 24 h exposure.	IPAM : Diuron equivalent concentrations (DEQ). Algal growth: TEQ	Decrease of toxicity was found after activated sludge treatment, reverse osmosis. Some few enhanced treatment (e.g. UV radiation, microfiltration, ozonation) did not alter the toxicity to microalgae.	
Samples of influent and effluent from the conventional WWTP (Zgierz, Poland). Long-term (13 months) and short-term (two	*Escherichia coli*; Activated sludge microorganisms	ToxTrak™Method 10017, HACH LANGE Manual. Method is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. The presence of toxic substances in the sample decreases the	Degree of Inhibition (DI), Toxicity Unit (TU)	DI for raw wastewater varied from 10.2 to 59.8%, whereas for the treated ones from 3.3 to 35.6%. Out of 25 samples of the effluent, two belonged to class III and 23 samples were ranked as class IV. The toxicity of the effluent	[9]

weeks) measurement campaigns were conducted.		rate of resazurin reduction, which can be measured colorimetrically.		was always lower than this of influent. The linear correlation between the toxicity of the influent and effluent was found. Lower toxicity of raw wastewater was observed in summer than in winter.	
Piggery wastewater effluent samples were collected from a farm in Stellenbosch (Western Cape province, South Africa).	*Pseudokirchneriella subcapitata*	ALGALTOXKIT F, in agreeement with ISO norm 8692 and OECD method no. 201. 72-h growth rate inhibition test.	Percentage inhibition (%); EC50	*P. subcapitata* exposed to 1% unfiltered piggery effluent did not show any toxicity. EC50-48 h values for 10 and 20% unfiltered piggery effluent were 49.3% and 13.9%, respectively. Unfiltered piggery effluent at concentration 10% and 20% can be regarded as toxic.	[10]
	Daphnia magna	DAPHTOXKIT F made in agreement with ISO norm 6341 and OECD method no. 202. 48-h mortality/immobilization effect test	Percent immobile (%); LC50	At concentration higher than 1% piggery effluent caused significant percentage immobility of *D. magna* after 24-h exposure. The different percentage concentrations of piggery effluent and a high-level dose of mixtures of veterinary pharmaceuticals can cause acute toxicity to *D. magna.*	
	Tetrahymena thermophila	PROTOXKIT F, adhered to OECD method no. 202. 24-h reproductive inhibition test.	Percentage inhibition (%); EC50	EC50 values varied from 4.81 to 52.39% depending on the concentration of piggery effluent. A relationship between the percentage concentrations of toxicants and the percentage growth inhibition of protozoa was found.	
Samples of the effluent from the WWTP in the region of the Western Cape (South Africa).	*Pseudokirchneriella subcapitata*	ALGALTOXKIT F, in agreeement with ISO norm 8692 and OECD method no. 201 72-h growth rate inhibition test.	Percentage inhibition (%); LC; TU	TU varied from 0.234 to 1.000 indicating on low acute toxicity. The WWTP effluent can be an ecological toxic risk to microalgae. Lower toxicity was observed in the summer than in winter and autumn.	[11]

	Daphnia magna	DAPHTOXKIT F in agreeement with ISO norm 6341 and OECD method no. 202. 48-h mortality/immobilization effect test.	Percentage mortality (%); LC; TU	Percentage of mortality after 48 h varied from 5% to 45%. TU varied from 0.944 to 1. *D. magna* was the least sensitive organism in this study.	
	Tetrahymena thermophila	PROTOXKIT F, adhered to OECD method no. 202. 24-h reproductive inhibition test.	Percentage inhibition (%); EC50; TU	TU varied from 84 to 89.6. *T. termophila* was the most sensitive organism in this study. The effluents showed high acute toxicity to protozoan.	
Influent and effluent samples from three WWTP in Bangkok (Thailand) treating hospital wastewater were taken. Conventional activated sludge systems were applied in all WWTPs. In two of them the effluents were chlorinated.	Algae: *Chlorella vulgaris* and *Scenedesmus quadricauda*	OECD method no. 201. 72-h growth rate inhibition test.	EC50; TU	The values of EC50 determined for *Ch. vulgaris* were from 13.83 to 17.16% (v/v) for the influents and from 41.33 to 51.60 % (v/v) for effluents. In the case of *S. quadricauda* these were from 9.81 to 13.63% (v/v) for the influents and from 45.8 to 87.1% (v/v) for effluents. All hospital wastewaters gave similar toxic levels to the test algae. Toxicity decreased after treatment. TU of the effluents was from 1.15 to 2.42. *S. quadricauda* was more sensitive than *C. vulgaris* to hospital wastewater.	[12]
	Microcrustacean: *Moina macrocopa*	OECD method no. 202. 48-h mortality/immobilization effect test.	LC50; TU	The values of LC50 were from 32.37 to 38.16% (v/v) for influents, while in the case of effluents it was from 45.91 to 59.25% (v/v). Treatment reduced the toxic effect on the test water flea. Chlorination did not give a negative effect on this organism.	
Effluent samples from two textile WWTPs (Ksar Hellal, Tunisia) were tesetd.	*Raphidocelis subcapitata*	72-h growth rate inhibition test in agreement with standard UNI EN ISO 8692:2005.	Inhibition percentage	Inhibition percentage varied from 0 to 69.3% Good bioindicator for testing toxicity of the textile wastewaters.	[13]
	Lemna minor	7-d growth inhibition rate test in agreement with ISO SO/WD 20079 (2001).	Inhibition percentage;	Inhibition percentage varied from 10.1±4.2% to 52.7±25%. *L. minor* was less sensitive	

				than other bioindators used in this study.	
	Cucumis sativus and *Lepidium sativum*	72-h seeds germination and early growth tests according to method UNICHIM N.1651 (ISO 2003).	Inhibition percentage	Inhibition percentage of germination varied from 20.0±1.6% to 100±0%. Inhibition percentage of root elongation varied from 77.0±1.2% to 100±0%. Both plants were sensitive organisms to the textile wastewater.	
	Daphnia magna	24-h mortality/immobilization effect test in agreement with UNI EN ISO 6341:2012.	Mortality; TU	One effluent exhibited 100% mortality, while the second one did not cause any mortality. Good bioindicator for testing toxicity of the textile wastewater.	
	Artemia franciscana	24-h mortality effect test ARTOXKIT M in agreement with Ecotoxicological method 8060 of APAT-IRSA (2003).	Immobilization percentage	Immobilization percentage varied from 0% to 40%. *A. franciscana* was not recommended for testing toxicity of textile wastewater.	
	Vibrio fischeri	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* according to UNI EN ISO 11348-3 (2007).	EC50; LOEC; NOEC; TU	One effluent was toxic: EC50=3%, LOEC=0.9, TU=33.1, while the second one did not cause any toxicity. Bacteria *V. fischeri* were relatively good bioindicator for testing toxicity of the textile wastewater.	
Samples from five sampling points (incl. influent and effluents) from the pilot plant and the full-scale WWTP in Koblenz (Germany).	*Vibrio fischeri*	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* according to UNI EN ISO 11348-3 (2007).	EC50	EC50 for influent was 1.49±0.41 REF (Relative Factor of Enrichment). Reduction of toxicity by 86.1% in the full-scale WWTP. Baseline toxicity was effectively removed in the activated sludge systems.	[14]
	Desmodesmus subspicatus	72-h growth inhibition test according to ISO 8692 (2012).	Algae cell number	Increase in algae growth in all treatments was found. The use of the classic growth inhibition test to determine phytotoxic effects of wastewater should be considered.	
	Daphnia magna	48 h acute immobilization test according to ISO 6341	Percentage of immobilizatio	No adverse effect of wastewater on *D.magna*	

		(2012).	n	Tests with *D. magna* occurred to be of limited relevance in evaluation of toxicity of wastewater.	
	Potamopyrgus antipodarum	28 d reproduction test according to OECD method no. 242 (2016).	Mortality; Number of embryos.	Mortality of snails did not exceed 10%. The mean reproductive output 17.9 ± 5.6 embryos in the control test. Exposure to wastewater effluents increased the reproduction by 10.4-31.1%. No reproductive toxicity after direct exposure to conventionally treated wastewater.	
	Lumbriculus variegatus	28 d reproduction test according to OECD method no. 225 (2007).	Number of worms; Biomass of worms	No significant effect on reproduction. Biomass exposed to the wastewater decreased compared to the control from 25.5 to 34.2%. Decrease of biomass of earthworms but no effect on reproduction.	
Influent and effluent from three full-scale WWTP in Tuscany (Italy).	*Raphidocelis subcapitata*	72-h growth inhibition test according to UNI EN ISO 692:2012 (2012).	Inhibition percentage	Inhibition percentage varied from 10.1 to 98.2%. Reduction of toxicity after treatment. One of two the most sensitive bioindicators used in this work. 90% of samples induced a significantly algal inhibition.	[15]
	Sorghum commune, Lepidium sativum, Cucumis sativus	72-h germination and early growth test according to UNI 11357:2010 (2010).	Inhibition percentage; Germination Index (GI)	A large variation of results. Both toxic and stimulatory effects were found. Inhibition phenomenon was observed in 37% of samples.	
	Daphnia magna	48 h acute immobilization test according to UNI EN ISO 6341:2013 (2013).	Inhibition percentage	Inhibition percentage was 0% except one sample, when it was 100%. *D. magna* almost never response to the samples tested.	
	Vibrio fischeri	Measurement of luminescence of the naturally bioluminescent marine bacteria *Vibrio fischeri* according to UNI EN ISO 11348-3 (2007).	Inhibition percentage	Inhibition percentage varied widely from -40.8% to 95.4%. Reduction of toxicity after treatment processes was usually found.	

				One of two the most sensitive bioindicators used in this work. 90% of samples induced a significantly bacterial inhibition.	
Samples from six sampling points (incl. influent and effluents) from the full-scale WWTP treated pigment contained wastewater (China).	*Photobacterium phosphoreum*	Acute toxicity tests of luminous bacteria according to National Environmental Protection Administration, China (NEPA 1995).	EC50; TU	TU varied from 0 to 5.5. The highest toxicity was found at anoxic tank effluent (TU=5.5). Reduction of toxicity after treatment (to TU=0) for the final effluent was noticed.	[16]
	Daphnia magna	48-h acute immobilization test according to OECD method no. 202.	EC50; TU	TU varied from 1.1 to 13.6. The acute toxicity to *D. magna* was reduced by 91.8%, to which the anaerobic and aerobic biological treatment units contributed 65.3% and 12.5%, respectively.	
	Danio rerio	96-h static acute toxicity test according to ISO 7346-3:1996 (1996).	EC50; TU	TU varied from 2.0 to 3.7. Only 20% of the acute TU was removed.	
Samples from eight sampling points (incl. influent and effluents) from the full-scale WWTP treated acrylonitrile contained wastewater (China) were tested.	*Daphnia magna*	48-h acute immobilization test according to OECD method no. 202 (2004).	LC50; TU	TU varied from below 0.4 to 125. Systems anaerobic oxic (A/O) and anaerobic oxic-aerobic biological fluidized tank (A/O-ABFT) was efficient in removal of toxicity to *D. magna*. Effluent was not toxic *to D. magna.*	[17]
	Danio rerio	96-h static acute toxicity test according to OECD method no. 203 (1992)	LC50; TU	TU varied from below 0.4 to 29.6. After going through the A/O and ABFT process systems, the final effluent showed no acute toxicity to *D. rerio*.	
Samples from each stage of treatment (incl. influent and effluents) from three full-scale WWTP of different treatment systems (SBR, conventional	*Scenedesmus obliquus*	72-h growth inhibition test according to OECD method no. 201 (2006).	Inhibition percentage (it refers to cell density, chlorophyll-A synthesis, superoxidedismutase (SOD) activity); Percentage of cell viability (it	The increase of cell growth was observed in all WWTPs studied. Only the effluent from NaClO disinfection units inhibited the cell growth by 131.8%. Analogous results were found for activity of SOD and chlorophyll-A synthesis. Percentage of cell viability decreased in the	[18]

activated sludge and Linpur) were tested.			refers to cell membrane integrity)	range from 0.33% to 17.5%. The acute toxicity of municipal wastewater on chlorophyll-A synthesis in, *S. obliquus*, was significantly correlated to phosphorus and organic carbon concentrations. SOD activity and chlorophyll-A synthesis were found to be sensitive endpoints for the municipal wastewater studied.	
Samples from five sampling points (incl. influent and effluents) from three full-scale WWTP treated municipal wastewater using A/O system (China) were tested.	*Danio rerio*	96-h acute static test according to OECD method no. 203 (1992).	Mortality rate (%)	Mortality rate varied from 0% to 50%±10%. Acute toxicity was reduced along with the A/O process of treatment. Acute toxicity on zebrafish decreased in accordance with the COD removal.	[19]
Effluents from 17 municipal WWTPs of different size, i.e. different PE, were tested.	*Pseudokirchneriella subcapitata*	72-h chronic growth inhibition biotest according to Algaltoxkit procedure (1996).	Inhibition percentage	The mean growth inhibition percentage varied from 11 to 100%. High acute hazard was noted for four WWTPs tested. *P. subcapitata* was a sensitive bioindicator for treated wastewater.	[20]
	Thamnocephalus platyurus	24-h mortality acute biotest according to Thamnotoxkit procedure (1995).	Mortality rate (%)	The mean mortality rate varied from 3 to 100%. *T. platyurus* mortality demonstrated a very strong positive correlation with NH_4^+ and a strong with total N.	
	Tetrahymena thermophila	24-h chronic growth inhibition biotest according to Protoxkit procedure (1998).	Inhibition percentage	The mean growth inhibition percentage did not exceed 40%. The mean toxicity did not exceed acute hazard for all samples from WWTPs. Stimulation effect was obsrved.	

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