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RESEARCH ARTICLE

Whole effluent assessment of industrial wastewater for determination of bat compliance

Part 1: paper manufacturing industry

Stefan Gartiser · Christoph Hafner · Christoph Hercher · Kerstin Kronenberger-Schäfer · Albrecht Paschke

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Abstract
Background, aim and scope The applicability of the Whole Effluent Assessment concept for the proof of compliance with the "best available techniques" has been analysed with paper mill wastewater from Germany by considering its persistency (P), potentially bio-accumulative substances (B) and toxicity (T).
Materials and methods Twenty wastewater samples from 13 paper mills using different types of cellulose fibres as raw materials have been tested in DIN or ISO standardised bioassays: the algae, daphnia, luminescent bacteria, duckweed (Lemna), fish-egg and umu tests with lowest ineffective dilution (LID) as test result. The potentially bio-accumulative substances (PBS) were determined by solid-phase micro-extraction and referred to the reference compound 2,3-dimethylnaphthalene. Usually, a primary chemical–physical treatment of the wastewater was followed by a single or multi-stage biological treatment. One indirectly discharged wastewater sample was pre-treated biologically in the Zahn–Wellens test before determining its ecotoxicity.
Results No toxicity or genotoxicity at all was detected in the acute daphnia and fish egg as well as the umu assay. In the luminescent bacteria test, moderate toxicity (up to $LID_{lb}=6$) was observed. Wastewater of four paper mills demonstrated elevated or high algae toxicity (up to $LID_A=128$), which was in line with the results of the Lemna test, which mostly was less sensitive than the algae test (up to $LID_{DW}=8$). One indirectly discharged wastewater sample was biodegraded in the Zahn–Wellens test by 96% and was not toxic after this treatment. Low levels of PBS have been detected (median 3.27 mmol L^{-1}). The colouration of the wastewater samples in the visible band did not correlate with algae toxicity and thus is not considered as its primary origin. Further analysis with a partial wastewater stream from thermomechanically produced groundwood pulp (TMP) revealed no algae or luminescent bacteria toxicity after pre-treatment of the sample in the Zahn–Wellens test (chemical oxygen demand elimination 85% in 7 days). Thus, the algae toxicity of the respective paper mill cannot be explained with the TMP partial stream; presumably other raw materials such as biocides might be the source of algae toxicity.
Discussion Comparative data from wastewater surveillance of authorities confirmed the range of ecotoxicity observed in the study. Wastewater from paper mills generally has no or a moderate ecotoxicity (median LID 1 and 2) while the maximum LID values, especially for the algae and daphnia tests, are considerably elevated (LID_A up to 128, LID_D up to 48).

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Preamble. A Whole Effluent Assessment (WEA) of two industrial sectors has been applied according to the OSPAR WEA Guidance document. Part 1 describes the testing strategy and methods used as well as the results obtained with wastewater from 13 paper mills in Germany and part 2 the results obtained with wastewater from two areas of the metal surface treatment industry (two printed circuit boards and eight electro-plating factories). Further investigations concerning the potential origin of elevated ecotoxicity observed in some samples are presented.

S. Gartiser (✉) · C. Hafner · C. Hercher · K. Kronenberger-Schäfer
Hydrotox GmbH,
Boetzinger Straße 29,
79111 Freiburg, Germany
e-mail: gartiser@hydrotox.de

A. Paschke
Department of Ecology,
Helmholtz Centre for Environmental Research,
Permoserstrasse 15,
04318 Leipzig, Germany

Conclusions Wastewater from paper mills generally is low to moderately ecotoxic to aquatic organisms in acute toxicity tests. Some samples show effects in the chronic algae growth inhibition test which cannot be explained exclusively with colouration of the samples. The origin of elevated algae ecotoxicity could not be determined. In the algae test, often flat dose–response relationships and growth promotion at higher dilution factors have been observed, indicating that several effects are overlapping.
Recommendations and perspectives At least one bioassay should be included in routine wastewater control of paper mills because the paper manufacturing industry is among the most water consuming. Although the algae test was the most sensitive test, it might not be the most appropriate test because of the complex relationship of colouration and inhibition and the smooth dose–effect relationship or even promotion of algae growth often observed. The Lemna test would be a suitable method which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration.

Keywords Wastewater ordinance · Paper manufacturing industry · Ecotoxicity · Genotoxicity · Algae test · *Vibrio fischeri* assay · Daphnia test · Umu assay · Fish-egg test · Lemna test · Zahn–Wellens test · Potential bio-accumulating substances · Whole effluent assessment · WEA · OSPAR

1 Background, aim and scope

Effect-based test methods detect combined toxic effects of all substances present in complex wastewater samples and are complementary to the "single substances approach". The aim of the study was to analyse the applicability of effect-based tests for the proof of compliance with the "best available techniques" using the examples of wastewater from the paper manufacturing and the metal surface treatment industries. For this, the Whole Effluent Assessment (WEA) concept of the OSPAR expert group has been applied (OSPAR Hazardous Substances Committee 2007). Here, the wastewater samples are assessed with regard to persistency (P), presence of potentially bio-accumulative substances (B) and toxicity (T). Within the Integrated Pollution Prevention and Control Directive (IPPC, 2008/1/EC), the WEA concept has been included as a suitable monitoring tool on effluent in several Best Available Techniques Reference Documents. One consequence of the IPPC Directive is that for direct dischargers as well as for indirect dischargers, the same best available techniques should be applied. Within the study, a systematic approach for determining persistent toxicity of indirectly discharged wastewater was applied.

2 Materials and methods

2.1 Paper mill wastewater samples

In total, 13 paper mills from several parts of Germany representing different types of raw materials used (groundwood pulp, cellulose, recovered paper with/without deinking, chemicals for special papers, etc.) have been analysed. Twelve paper mills directly discharge their wastewater after passing a biological treatment plant of their own and one paper mill indirectly discharges to a municipal treatment plant. All factories (except one indirectly discharging) use a primary chemical–physical treatment of the wastewater followed by a single or multi-stage biological treatment. Most paper mills use the activated sludge process, sometimes coupled with percolating filters upstream, which are also used for cooling purposes. In three factories, the first biological stage is anaerobic treatment (Table 1).

2.2 Testing strategy

The testing strategy and WEA principles have been described in the WEA Guidance document (OSPAR Hazardous Substances Committee, 2007). In principle, the same persistency, bio-accumulation and toxicity criteria used for identifying priority substances in water policy are applied with native wastewater samples. The overall test concept consists in coupling the effect-based tests from a "toolbox" with biodegradation tests. For indirectly discharged effluents, the Zahn–Wellens test (adopted from OECD 302 B) has been suggested as a suitable tool for determining the behaviour in wastewater treatment plants and for discriminating persistent toxicity from non-persistent toxicity caused e.g. by ammonium or readily biodegradable compounds. Therefore, in this study, all indirectly discharged wastewater samples have been biologically pre-treated in the Zahn–Wellens test with activated sludge (1 g dry solids per litre) from the respective municipal treatment plants which received the wastewater and afterwards tested concerning their ecotoxicity (Fig. 1, Table 2).

Toxicity of wastewater might be caused by salts. In the German Wastewater Ordinance (2004), this is considered by a correction factor, which takes into account that the salt concentration increases when the water cycles are closed, which is appreciated from an environmental point of view. For the salt correction factor, the sum of chloride and sulphate ion concentrations (in g L^{-1}) is divided by an organism-specific value (3 for fish eggs, 2 for daphnia, 0.7 for algae and 15 for luminescent bacteria) and subtracted from the lowest ineffective dilution (LID). Hereby as a first approximation, a sum of 1 g L^{-1} chloride and sulphate (in equal proportions) corresponds to a conductivity of 5,000

Table 1 Characteristics of the paper mills investigated

	Type of discharge	Production t/a	Raw material	Wastewater 1,000 m^3/a	WWTP
P1	D	140,000 woodfree and 140,000 special papers	90% cellulose 10% recycling paper	545	Sedimentation, bio-filter
P2	D	555,000 from recycling paper with deinking and 25,124 from recycling paper without deinking	2% cellulose 9% groundwood pulp 89% recycling paper	4,937	Sedimentation, activated sludge, final clarifier, ozonisation, bio-filter
P3	D	148,000 decor -, special -, and carbon paper	96% ECF cellulose 4% TCF cellulose	1,404	Sedimentation, moving bed reactor, activated sludge, final clarifier
P4	D	180,000 from recycling paper without deinking	100% A recycling paper	1,110	Precipitation, flotation, high load moving bed reactor, low load activated sludge, final clarifier
P5	D	378,000 woody coated paper	25% cellulose	5,200	Chemical-mechanical pre-treatment, flotation, cooling sprinkling filter, moving bed reactor, activated sludge, final clarifier, biofiltration
			75% groundwood pulp (TMP)		Thermomechanical pulp (TMP) treatment with sodium dithionite
P6	D	583,000 from recycling paper without deinking	100% recycling paper	1,580	Mechanical pre-treatment (disc filter), anaerobic treatment, cooling trickling filter, aerobic treatment (3 cascades), final clarifier
P7	D	125,000 from recycling paper with deinking	100% recycling paper	1,600	Sedimentation, moving bed reactor, activated sludge, final clarifier, flotation, sand filtration
P8	D	240,000 woody coated paper	33% cellulose 69% groundwood pulp	4,143	No mechanical pre-treatment, cooling trickling filter, activated sludge, final clarifier
P9	D	660,000 uncoated woodfree and woody paper	8% cellulose 32% groundwood pulp 60% recycling paper	7,069	Chemical–physical pre-treatment (multi-disc clarifier, flotation, turbocirculator), cooling trickling filter, moving bed reactor, activated sludge, final clarifier
P10	D	342,000 from recycling paper with deinking	100% recycling paper	3,820	Sedimentation, cooling trickling filter, anaerobic treatment, activated sludge, final clarifier
P11	D	1,192,000 woodfree coated paper	100% cellulose	3,772	Sedimentation, bio-trickling filter, activated sludge, final clarifier
P12	I	40,000 paper board	95% recycling paper without deinking 5% TMP cellulose	350	Sedimentation, indirectly discharged to municipal WWTP (20% of hydraulic load)
P13	D	295,000 from recycling paper with deinking	100% recycling paper	2,700	Flotation, anaerobic treatment, activated sludge, final clarifier, sand filtration

P paper mill, *D* direct discharger, *I* indirect discharger, *ECF* elemental-chlorine-free, *TCF* total-chlorine-free, *TMP* thermomechanical pulp, *WWTP* Wastewater treatment plant

µS L^{-1}. For example, if the waste water permit requires a LID_F of 2, a value of LID_F=3 is considered being acceptable if the wastewater contains more than 3 g/L chloride and sulphate (in this case LID_F= 3−3/3=2).

2.3 Biodegradability/Treatability

Two vessels with 4,000 mL each of all indirectly discharged wastewater samples have been biodegraded in the

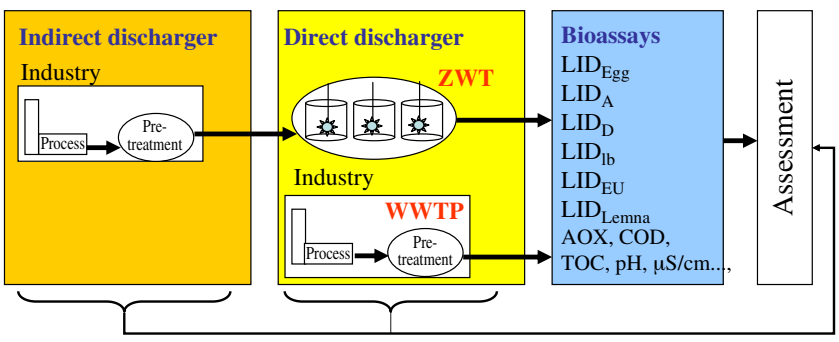

Fig. 1 Testing strategy for direct and indirect dischargers

Table 2 Test methods used from the wastewater ordinance

Wastewater ordinance	Method	Standard	Test organism	Test duration	Criteria
No. 401	Zebrafish embryo assay LID_{Egg} value	DIN 38415-6 ISO 15088: 2007	*Danio rerio*	48 h	Development of embryos (coagulated eggs, heartbeat, somites and tail differentiation) <10%
No. 402	Daphnia acute toxicity LID_D value	DIN 38412-30: 1989	*Daphnia magna*	24 and 48 h	90% of Daphnia maintain ability to swim
No. 403	Alga growth inhibition test LID_A value	DIN 38412-33: 1991	*Scenedesmus subspicatus*	72 h	Inhibition of biomass production <20%
No. 404	Fluorescent bacteria test LID_{lb} value	DIN 38412-34: 1997 EN ISO 11348-2: 1998	*Vibrio fischeri*	30 min	Inhibition of light emission <20%
	Duckweed growth inhibition test LID_{DW} value	ISO 2007: 2006	*Lemna minor*	7 days	Inhibition of growth based on fronds number and area <10%
No. 407 / 408	Zahn–Wellens test	DIN EN ISO 9888: 1999	Activated sludge	2–7 days	COD/DOC-elimination
No. 410	Genotoxic potential *umu* test LID_{EU} value	DIN 38415-3: 1996 ISO 13829: 2000	*Salmonella typhimurium* TA1535/pSK1002	2 h	Induction rate <1.5

Zahn–Wellens test (DIN EN ISO 9888) in order to provide sufficient material for subsequent ecotoxicity testing.

The wastewater samples were supplemented with an inorganic nutrient solution and continuously stirred and aerated with an aquarium pump. The pH was adjusted to pH7–8 each working day. Chemical oxygen demand (COD) determination was done using ready to use cuvette tests from Hach-Lange, Germany. The activated sludge used as inoculum was obtained from the municipal sewage treatment plants to which the respective wastewater is discharged. The bio-elimination extents were referred to the expected initial start concentration calculated from the COD of the original sample and the dilution by adding mineral medium and activated sludge (less the 20% of total volume). In parallel, an abiotic control without inoculum but with addition of copper sulphate (final copper concentration 20 mg L^{-1}) for reducing biological degradation is tested to determine non-biological elimination such as stripping or adsorption. Synthetic wastewater made up of peptone, yeast extract and urea, according to DIN 38412-26 (1994), has been used as reference substance for a functional control. After treatment for 7 days, the activated sludge was allowed to settle for about 1 h, and the supernatant was decanted, split in 100-ml polyethylene bottles, stored at −18°C and used for ecotoxicity testing with bioassays.

2.4 Ecotoxicity and genotoxicity testing

All tests have been carried out according to DIN or ISO standards (Table 2). As far as possible, the original wastewater samples have been tested after pH adjustment with hydrochloric acid or sodium hydroxide solution to 7.0± 0.2 without any further pre-treatment. Where suspended particles might have an influence on the test results by mechanically interfering with the test organisms (Daphnia) or by light absorbance (algae, luminescent bacteria test), the solids were allowed to settle for 1 to 2 h immediately before starting the incubation period. In parallel to the wastewater samples, one concentration of suitable reference compounds (ecotoxicity: 3,4-dichloroaniline or potassium dichromate; genotoxicity: 2-aminoanthracene, nitrofurantoine, 4-nitro-1,2-phenylenediamine, 4-nitroquinolineoxide) has been tested according to the Analytical Quality Assurance bulletin of the German Working Group of the Federal States on water issues (LAWA 2009).

In Germany, for wastewater evaluation, the acute fish toxicity test with *Leuciscus idus* was replaced in 2004 by the short-term fish-egg assay with zebrafish (*Danio rerio*, also called fish embryo assay) for animal protection considerations. The test is classified as a sub-organism test because the central nervous system of fish embryos is not fully developed (Oberemm 2000). The fish were cultivated at 26°C and 14:10 h light/dark cycle and were fed daily with TetraMIN® flakes and two times per week with newly hatched brine shrimps (*Artemia* sp.) The fertilised eggs were collected in a rectangular glass spawning box, covered by a stainless steel mesh and artificial plants and were separated manually from unfertilised eggs using an inverted microscope. The eggs were incubated over 48 h, which covers the time from the blastula to the stage with fully developed blood circulation. The test performance consists in exposing 10 fertilised eggs for each concentration in 24-well cell culture plates (2 ml each).

The Daphnia toxicity test was performed using the clone 5 of *Daphnia magna* STRAUS of the German Federal Environment Agency. Daphnia were held in Elend M4 medium and were fed daily with living algae cells (*Desmodesmus subspicatus* CHODAT, formerly called *Scenedesmus subspicatus*). Each concentration (dilution) was tested in two replicates with five daphnia each and incubated at 20°C in the dark. The test was evaluated after 24 h (DIN 38412-30).

For the algae growth inhibition test *D. subspicatus*, a planktonic fresh-water alga was used. After adding an algal nutrient solution, the vessels were inoculated with 10^4 algae per ml and incubated under defined light conditions (135 µE $m^{-2}s^{-1}$ photosynthetically active radiation) at 23± 1°C. Each concentration (dilution) was tested in two replicates, the control vessels in five replicates. At the beginning of the incubation period and after 72 h, the chlorophyll fluorescence (excitation wavelength 465 nm, emission wavelength 670 nm) has been measured for quantifying the biomass increase (TECAN Infinite 200F, Tecan, Switzerland).

The luminescent bacteria toxicity test with the marine bacteria *Vibrio fischeri* was performed using the LUMIStox system of the company Hach–Lange, Düsseldorf with liquid-dried bacteria of the strain *V. fischeri* NRRL-B-11177. The wastewater samples were tested after salinising with sufficient sodium chloride to obtain a 2% solution with two replicates at an incubation temperature of 15±1°C after 30-min contact time.

The duckweed *Lemna minor* represents freshwater aquatic plants. The growth inhibition was determined by both determining the frond numbers and the frond area after an incubation time of 7 days at defined light conditions (85–135 µE $m^{-2}s^{-1}$ photosynthetically active radiation) at 24±2°C with an imagine analysis system (Scanalyzer, LemnaTec, Germany). Each concentration (dilution) was tested in three replicates, the control vessels in six replicates. For the testing of dark-coloured test solutions compared to the algae growth inhibition test, the Lemna test has the advantage of light absorption and thereby resulting growth inhibition is irrelevant. As test result, the more sensitive of the two endpoints (frond numbers and frond area) is reported.

The umu test is a genotoxicity test with the biotechnologically modified bacterial strain *Salmonella typhimurium* TA1535/pSK1002. Gene toxins induce the umuC-gene, which belongs to the SOS-repair system of the cell. By coupling of the umuC-gene promoter with the lacZ-gene for ß-galactosidase, the activation of the umuC-gene can be indirectly measured spectrophotometrically at 420 nm through the formation of a coloured product from the ß-galactosidase substrate *o*-nitrophenyl-galactopyranoside (ONPG). The bacteria are exposed for 4 h to the wastewater with and without metabolic activation using microplates, and the genotoxin-dependent induction of the umuC-gene is compared to the spontaneous activation of the control culture. Each concentration has been tested three-fold in 96-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany). The induction rate corresponds to the increase of the extinction at 420 nm relative to the negative control. Bacterial growth and inhibition are determined turbidimetrically from the optical density at 600 nm. For growth factors below 0.5 (50% growth inhibition), the results are not evaluated. The result given is the smallest dilution step at which an induction rate <1.5 is measured. All samples have been tested in at least four concentrations. Subsequently, toxic or gentoxic samples have been further analysed until no growth inhibition or induction of genotoxicity was determined. Samples which are toxic at higher concentrations but non-genotoxic at growth factors >0.5 have been designated as "toxic".

All results of ecotoxicity and genotoxicity testing are given as the LID, which is defined as the reciprocal volume fraction of the wastewater sample at which only effects not exceeding the test-specific variability are observed (ISO 5667-16 1998, Annex A). This corresponds to the lowest dilution level (threshold level) where effects do not exceed the test-specific variability. The following thresholds effect levels are given in the respective standards: a mortality or inhibitory effect or an immobilisation of ≤10% (Fish-egg test, Daphnia test, Lemna test), an inhibitory effect ≤20% (luminescent bacteria test, algae test), an induction rate ≤1.5 (umu test).

2.5 Potentially bio-accumulative substances

The potentially bio-accumulating substances (PBS) were determined by solid-phase microextraction (SPME) according to a protocol adapted by Leslie and Leonards (2005) for the OSPAR WEA group. Briefly, a 1-cm long quartz glass fibre coated with 100 µm polydimethylsiloxan (PDMS) from Supelco (Bellafonte, CA, USA) was exposed at room temperature to 250 mL wastewater which was continuously stirred at 500 rpm over 24 h. The Erlenmeyer flask used was nearly headspace-freely filled with the sample and wrapped with aluminium foil during SPME. Gas chromatographic analysis was performed after thermodesorption of SPME fibre in the GC injector (in splitless mode) using a CP 9001 (Chrompack, Frankfurt a. M.) with flame-ionisation detector and a capillary column OPTIMA-1 (10 m long, 0.25 mm I.D., 0.1 µm film thickness) from Macherey–Nagel (Düren, Germany). The whole chromatogram was integrated between the retention times of nonane (n-C_9) and octa-triacontane (n-C_{38}). The obtained peak areas were normalised to the peak of the reference compound 2,3-dimethylnaphthalene (DMN; log Kow = 4.4) which was injected separately from a standard solution. The results (PBS concentrations) are expressed as mmol L^{-1} DMN equivalents. Note that this concentration

does not mean a PBS concentration in the water sample extracted but expresses per convention the PBS amount (as DMN equivalent) per volume of PDMS coating, the extracting phase. Additionally, two blank values from two PE bottles filled with distilled water (one new, one used before) were determined according to the same procedure.

2.6 Accompanying chemical analysis

Along with the biological tests, also physicochemical parameters of the Wastewater Ordinance such as pH, conductivity, COD, total organic carbon (TOC), total phosphate, ammonium and heavy metals have been determined, and here are only partly documented because of the limited space available.

3 Results with paper mill wastewater samples

3.1 Overview

Altogether, 20 wastewater samples from 13 different paper mills have been analysed in the research programme. One or two independent samples per paper mill have been taken. A repetition of sampling at the same site is designated as a "B" sample. The results are shown in Table 3. The COD of directly discharged paper mill effluents was between 24 and 498 mg L^{-1}; the respective TOC was between 7 and 136 mg L^{-1}. The inorganic nitrogen compounds (as sum of ammonium, nitrite and nitrate) with one exception (P6-B: N_{total}=14.8 mg L^{-1}) were below the requirements of the Wastewater Ordinance (N_{total}=10 mg L^{-1}). The limit values concerning total phosphate of 2 mg L^{-1} were slightly exceeded by two samples (maximum P6-B: P_{total}= 3.9 mg L^{-1}). The maximum conductivity of the samples was 3370 µS cm^{-1}, thus not indicating a toxicity caused by salts. AOX values were only available from five samples, and these were not elevated (maximum P11: AOX= 0.213 mg L^{-1}).

The results show no toxicity at all in the daphnia and fish-egg tests. No sample was genotoxic in the umu assay. However, the wastewater of four paper mills demonstrated an elevated or high algae toxicity while many others in contrast stimulated the growth of algae. This is in line with the result observed with the Lemna test, which mostly was less sensitive than the algae. With some wastewater

Table 3 Results with wastewater from the paper manufacturing industry

	COD	Duckweedassay	Algae assay	Daphnia test	Fish-egg test	Luminescent bacteria test	Umu assay	Conductivity	Potentially bio-accumulating substances
	mg L^{-1}	LID_{DW}	LID_A	LID_D	LID_{Egg}	LID_{lb}	LID_{EU}	µS cm^{-1}	mmol L^{-1}
P1	43	1	1 S	1	1	3	1.5	1,540	8.21
P2	210	2	3	1	1	≤2	1.5	3,040	7.24
P3	24	2	1 S	1	1	≤2	1.5	1,031	8.15
P3-B	32	2	1 S	1	1	≤2	1.5	1,281	2.92
P4	146	1	1 S	1	1	≤2	1.5	1,075	7.88
P4-B	125	4	1	1	1	≤2	1.5	1,779	2.03
P5	250	4	64	1	1	≤2	1.5	1,475	14.61
P5-B	244	3	128	1	1	4	1.5	1,278	1.40
P6	233	2	1 S	1	1	8	1.5	2,920	6.91
P6-B	237	3	1 S	1	1	3	1.5	3,370	1.12
P7	133	2	1 S	1	1	≤2	1.5	2,570	6.03
P7-B	102	1	1 S	1	1	3	1.5	2,080	0.89
P8	116	1	2	1	1	≤2	1.5	1,951	3.36
P9	446	3	16	1	1	6	1.5	1,693	4.28
P9-B	498	8	16	1	1	4	1.5	2,390	1.94
P10	247	8	6	1	1	≤2	1.5	2,472	7.50
P10-B	191	4	6	1	1	3	1.5	2,040	1.30
P11	43	2	1 S	1	1	3	1.5	1,283	3.18
P12 after ZW	88	3	1 S	1	1	6	1.5	1,293	0.78
P13	278	6	12	1	1	4	1.5	2,760	0.86

LID lowest ineffective dilution, *P* paper mill, *ZW* Zahn–Wellens test, *S* stimulation of algae growth

samples, the Lemna test revealed slight effects not detected with the algae test. In the luminescent bacteria test, half of the samples were inconspicuous while the other samples showed a moderate toxicity (up to $LID_{lb}=6$). The only indirectly discharged wastewater sample of paper mill P12 was biodegraded in the Zahn–Wellens test by 96% (see Fig. 2) and was not toxic after treatment.

Considering the sum parameter PBS, wastewater from the paper manufacturing industry exhibited low levels of PBS (0.78–14.61 mmol L^{-1}, median 3.27 mmol L^{-1}).

3.2 Origin of algae toxicity

Wastewater from paper mills often did not show a definite dose–response relationship in the algae test or even stimulated algae growth. Repeatability of algae tests has been studied on wastewater from paper mill P5. Figure 3 shows that all four independent tests consistently indicated considerable algae toxicity of P5. However, within the range of dilution factor 16 and 192, a flat decline of algae growth inhibition was observed, sometimes combined with an increase at higher dilution factors. As the LID_A is defined as the lowest dilution where for the first time the inhibition is below the threshold of 20%, the overall results fluctuate between LID_A 64 and LID_A 192. In the first trial, the threshold is even touched at a dilution factor of 32. The results demonstrate that obviously several effects like inhibition, light absorbance and growth promotion interact.

It is known that coloured samples might reduce photosynthetic efficiency and, therefore, inhibit algae growth. Paper mill wastewater often is considerably coloured because of the lignin fraction present in the water, which is not completely removable even not through bleaching processes. In order to determine the influence of colouration on testing results, all paper mill wastewater samples have been photometrically measured in the visible range (Fig. 4). The light absorption maxima of the chlorophyll from the algae used are 440 and 680 nm. In particular, both samples from paper mill P9 most strongly absorbed light in the whole visible range. However, these samples were only moderately toxic in the algae test. The outstanding samples with highest algae toxicity from factory P5 were not particularly notable with regard to their colouration. Also, the results with the Lemna test, which mostly were in the same direction as those with the algae test, gave an indication against the hypothesis that algae toxicity is mainly caused by colouration of the samples. As duck weeds swim at the water surface, their photosynthetic efficiency is not influenced by colouration. Therefore, attention was turned to other potential influencing factors. It was known that in factory P5 mainly TMP is used as raw material. Hereby, the external part of decorticated log wood rich in lignin, which is provided from a sawmill, is decomposed under heat and pressure. The resulting wastewater has a high COD of up to 5,000 mg L^{-1} and is biodegraded by around 90% in the wastewater treatment plant. For determining whether algae toxicity of P5 is caused by the TMP, a partial stream of the TMP wastewater was taken and at first degraded in the Zahn–Wellens test. The COD elimination reached 85% in 7 days (Fig. 5). Afterwards, algae and luminescent bacteria toxicity of the pre-treated wastewater was determined. The results demonstrate that the sample was rather unpolluted. The luminescent toxicity was $LID_{lb}=3$, the algae toxicity $LID_A \leq 4$. Therefore, the ecotoxicity found in wastewater from paper mill P5 cannot be explained with this partial stream. Presumably other raw materials such as biocides might be the source of algae toxicity. It is known that the successful closing of water circuits in paper mills which led to a reduction of the specific water consumption per tonnage produced from around 50 $m^3 year^{-1}$ in the 1970s to about 10 $m^3 year^{-1}$ increased microbiological problems

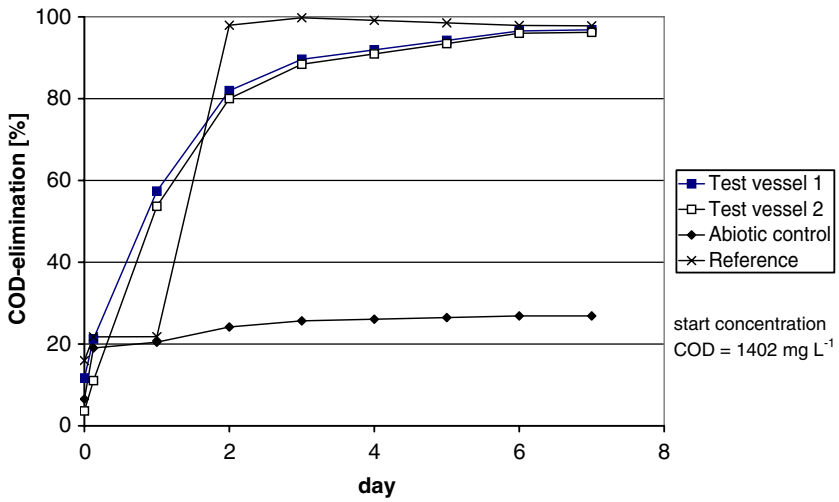

Fig. 2 COD elimination of paper mill wastewater P12 in the Zahn–Wellens test

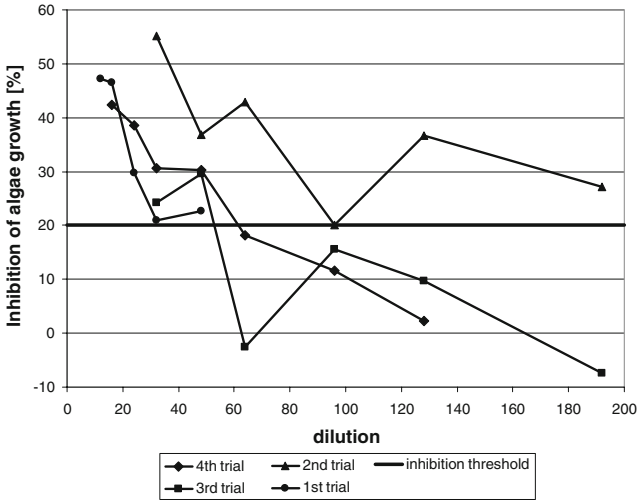

Fig. 3 Algae toxicity of P5 in four independent tests

in the circuits which subsequently were combated by biocides (European Commission 2001).

4 Discussion

In Germany, the application of bioassays in wastewater surveillance by local authorities has a long tradition. Several surveys on results with bioassays in different wastewater sectors have been elaborated, considering distinct timeframes (1993–1996, 1997–2000, 2001–2007). These comparative data of Diehl and Hagendorf (Diehl and Hagendorf 1998; Diehl et al. 2003) and Gartiser et al. (2008) presented in Table 4 confirm the range of ecotoxicity observed in the study in hand. Wastewaters from paper mills generally have no or a moderate ecotoxicity (median LID 1–2) while the maximum LID values especially for the algae and daphnia test are considerably elevated (LID_A up to 128, LID_D up to 48; it should be mentioned that all data of Table 4 from the period from 2001–2007 and most data of the previous periods refer to direct dischargers). The maximum value of the fish-egg test was not conspicuous (LID_{Egg} = 3) but is based on relatively few data. However, historic data with the acute fish test, which was replaced in 2004 by the fish-egg test, also demonstrated elevated fish toxicity (LID_F up to 48).

In a former WEA practical approach with another directly discharged paper mill wastewater sample, no toxicity or genotoxicity at all was observed in the algae, daphnia, fish-egg and luminescent bacteria tests, as well as in the umu assay and Ames test (Gartiser et al. 2009).

Literature data suggest that the discharge of pulp and paper mill effluents negatively affect light transmission through the content of lignosulphonates. However, the impact of colouration on phytoplankton development cannot be distinguished from inhibitory toxic effects on the phytoplankton (Karrasch et al. 2006). A survey of 12 pulp and paper effluents in Canada even found that effluent treatment using aerated stabilisation basins leads to average increases in colour of 20–40% (Milestone et al. 2004).

The application of bioassays for surveillance and wastewater permits in the pulp and paper sector is very common. An overview about national limit values is given by OECD (1999). For example, no acute toxicity to rainbow trout or *D. magna* is allowed in wastewater from kraft mills in Canada (LC50≥50 vol.%). Numerous studies on the effluent quality of the paper mill industry have been published which confirm that short-term and chronic effects on organisms may occur (OSPAR Hazardous Substances

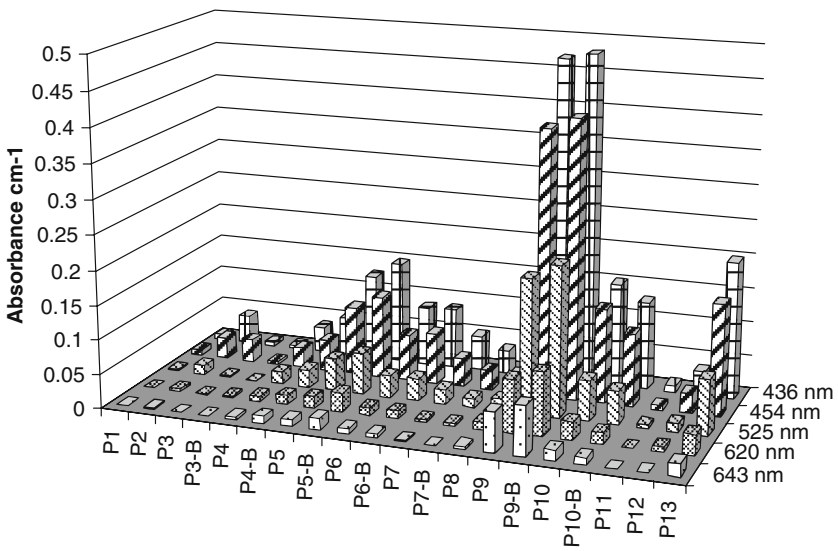

Fig. 4 Light absorbance of paper mill wastewater samples

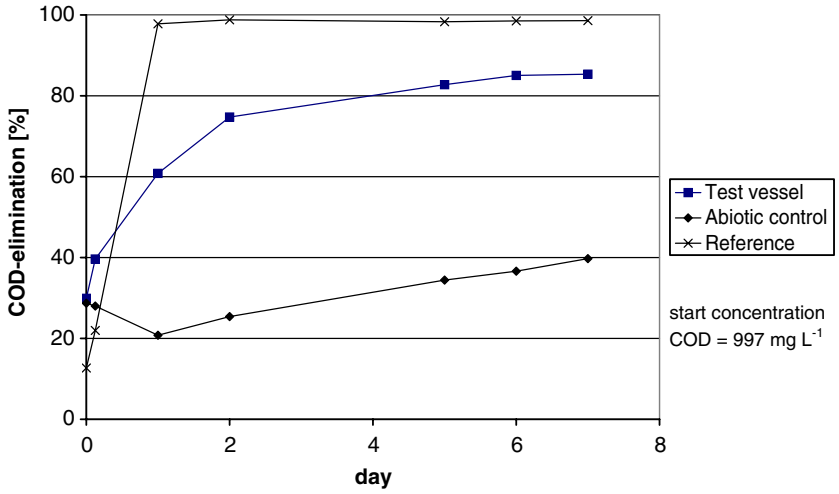

Fig. 5 COD elimination of TMP groundwood pulp P5-C in the Zahn–Wellens test

Committee HSC 2000; Kovacs and Ferguson 1990; Robinson et al. 1994; Hall et al. 2009). However, often, other test organisms have been applied or the effluents contained both pulp and paper partial streams so that the results are not directly comparable.

Most PBS values were in the same range as the blanks; thus, only minor potentially bio-accumulating substances are present. An interlaboratory study has proposed the following classification of water samples when using SPME as a screening method for PBS (Leslie 2006):

<5 mmol L^{-1} PBS very low level of PBS (clean) effluent
5–20 mmol L^{-1} PBS low level PBS effluent
>20 mmol L^{-1} PBS high level PBS effluent
<40 mmol L^{-1} PBS narcotic toxicity expected on this level

Thus, all wastewater samples analysed were in very low or low level respective pollution with PBS. It should be noted again that PBS concentrations refer to the volume of the extracting fibre and not to the water phase (because it is in contrast to the exhaustive solvent extraction a negligible-depletive, biomimetic extraction).

5 Conclusions

Wastewater from paper mills generally was low to moderate ecotoxic to aquatic organisms in acute toxicity tests. Some samples showed effects in the chronic algae growth inhibition test which cannot be explained exclusively with colouration of the samples. The origin of elevated algae ecotoxicity could not be determined. In the algae test, often, flat dose–response relationships and growth promotion at higher dilution factors have been observed, indicating that several effects are overlapping.

6 Recommendations and perspectives

It is recommended to include at least one bioassay in routine wastewater control of paper mills. Although the algae test was the most sensitive test, it might not be the most appropriate test because of the complex relationship of colouration and inhibition and the smooth dose–effect relationship or even promotion of algae growth often observed. The Lemna test would be a suitable method

Table 4 Effect-based data from paper mill wastewater surveillance by German authorities

Period	No. of tests (No. of paper mills)	Maximum					Median					90% Percentile				
		LID_A	LID_D	LID_{Egg}	LID_F	LID_{lb}	LID_A	LID_D	LID_{Egg}	LID_F	LID_{lb}	LID_A	LID_D	LID_{Egg}	LID_F	LID_{lb}
1993–1996	324 (20)	8	8		16	64	2	1		2	2	4	2		2	12
1997–2000	30 (3)	2	1		2	2	2	1		2	2					
2001–2007	380 (16)	128	48	3	48	8	2	1	2	2	2	6	2	2	6	3

Sources: 1993–1996: Diehl and Hagendorf (1998); 1997–2000: Diehl et al. (2003), 2001–2007: Gartiser et al. (2008). Data from 2001–2007 exclusively, data from 1993–2000 mainly belong to direct dischargers

$LID_{A,\ D,\ Egg,\ F,\ L}$ Lowest ineffective dilution for algae, daphnia, fish eggs, fish and luminescent bacteria

which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration. Because the paper manufacturing industry is among the most water-consuming industrial sectors, also the fish-egg test, which is used for the determination of wastewater charges, could be a useful parameter.

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