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Ecotoxicological evaluation of three tertiary wastewater treatment techniques via meta-analysis and feeding bioassays using *Gammarus fossarum*

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ABSTRACT

Advanced treatment techniques, like ozone, activated carbon and TiO_2 in combination with UV, are proposed to improve removal efficiency of micropollutants during wastewater treatment. In a meta-analysis of peer-reviewed literature, we found significantly reduced overall ecotoxicity of municipal wastewaters treated with either ozone ($n = 667$) or activated carbon (=113), while TiO_2 and UV was not yet assessed. As comparative investigations regarding the detoxification potential of these advanced treatment techniques in municipal wastewater are scarce, we assessed them in four separate *Gammarus*-feeding trials with 20 replicates per treatment. These bioassays indicate that ozone concentrations of approximately 0.8 mg ozone/mg DOC may produce toxic transformation products. However, referred effects are removed if higher ozone concentrations are used (1.3 mg ozone/mg DOC). Moreover, the application of 1 g TiO_2/l and ambient UV consistently reduced ecotoxicity. Although activated carbon may remove besides micropollutants also nutrients, which seemed to mask its detoxification potential, this treatment technique reduced the ecotoxicity of the wastewater following its amendment with nutrients. Hence, all three advanced treatment techniques are suitable to reduce the ecotoxicity of municipal wastewater mediated by micropollutants and may hence help to meet the requirements of the European Water Framework Directive.

1. Introduction

Wastewater treatment plants (WWTPs) equipped with secondary treatment, i.e. mechanical and biological methods, are not capable of degrading all contaminants present. Such contaminants, or micropollutants, are hence detected frequently at concentrations of up to a few microgram per liter in surface waters [1]. Thus, WWTP effluents are considered as one of the major pathways for micropollutants into aquatic ecosystems [2]. There, they may affect macroinvertebrate communities [3] as well as ecosystem functions, such as leaf litter breakdown [4,5].

To counteract the continuous release of such (in)organic micropollutants into surface waters – and the accompanied potential ecotoxicological implications – the European Commission, under the umbrella of the Water Framework Directive, requires a good status in terms of quantity and quality (=chemical and ecological) by implementing the best technique available to control their emission [6]. To achieve these requirements, end of pipe technologies may be useful in the medium term to reduce the release of micropollutants via point sources like WWTP effluents [7]. Ozonation, for instance, is an end of pipe technology that is economically feasible and technically realisable [8]. Moreover, it is capable of reducing the concentration of organic micropollutants in municipal wastewater [9,10]. Another option for chemical oxidation is photocatalysis, where reactive oxygen species are formed. TiO_2 is widely used as catalyst since it is photostable, non-toxic and insoluble [11]. Furthermore, the combined application of TiO_2 and ultraviolet (UV) irradiation is effectively degrading endocrine disrupting chemicals [11], organic chemicals in general [12], and was successfully applied in industrial wastewaters [13]. Hence, this technology may also be considered for implementation in municipal WWTPs. Besides these advanced oxidation technologies also the application of activated carbon – either granular or powdered – is currently under consideration as an additional treatment step to reduce concentrations of micropollutants [14]. In contrast to ozone or TiO_2 and UV, activated carbon adsorbs (in)organic chemicals from the water phase (i.e. wastewater) and hence, does not produce transformation products that may exhibit an even higher ecotoxicological potential than their parent compounds [15].

Especially this potential formation of transformation products makes it difficult to predict the ecotoxicological net effect of advanced treatment technologies in municipal WWTPs [16]. Thus, the main objective of the present study was to comparatively investigate the ecotoxicological consequences of the application

Abbreviations: COD, chemical oxygen demand; WWTP, wastewater treatment plant; TZW, water technology centre; SPE, solid phase extraction; DOC, dissolved organic carbon; TiO_2, titanium dioxide; UV, ultraviolet; PAC, powdered activated carbon; CI, confidence interval.

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of advanced treatment techniques – namely ozonation, TiO_2 and UV, and activated carbon – in municipal wastewaters. This issue was addressed (I) by conducting a meta-analysis of ecotoxicological data in literature dealing with these advanced treatment techniques and (II) by applying laboratory experiments. The feeding rate, a sublethal endpoint, of the leaf shredding amphipod *Gammarus fossarum* was selected as endpoint since former experiments suggest shifts in the organic matrix – potentially caused by ozone application – not to be the trigger of alterations in the feeding rate of *G. fossarum* [17]. In the same publication it was discussed that recolonization of leaf material by microorganisms (especially aquatic hyphomycetes), which may finally indirectly affect the investigated endpoint, are highly unlikely due to a lack of sources of such hyphomycetes and the short study duration [17]. Moreover, during a population level experiment it was displayed that levels of nutrients (e.g. NH_4^+) are not meaningfully affected by ozonation. Hence, this potential cause for effects is also considered to be of minor importance [18]. These insights were supplemented by an experiment, which used ten-fold enriched eluates of solid phase extraction (SPE) cartridges. The results suggested the fraction purified by the SPE-method applied, and hence not nutrients or heavy metals, are the trigger for the alterations in the feeding rate displayed by the test species *G. fossarum* [17]. Due to these explanations it can be assumed that shifts in the feeding rate of *G. fossarum* most likely display a reduction in the load of micropollutants. Therefore, *G. fossarum* was exposed to secondary treated wastewater from two different WWTPs, which were additionally treated with the above mentioned methods.

2. Material and methods

2.1. Meta-analysis

In order to locate studies assessing ecotoxicological properties of municipal wastewater treated with ozone, the combination of TiO_2 and UV irradiation (TiO_2 and UV), or activated carbon, a literature search was performed using the online database ISI Web of Science (Thomson Reuters; date 31st January 2011). The search strings used and the resulting number of paper hits are given in Table S1 of the supplementary data. In total more than 5000 articles were returned. However, only 16 dealt with ecotoxicological effects on various biomarkers and 25 used whole organism toxicity tests assessing the impact of ozone or activated carbon application, while in this context the employment of TiO_2 and UV was not yet investigated. The reference lists of the retained articles were inspected for pertinent additional publications [19]. However, only peer-reviewed publications were included from which information on treatment and control means, standard deviations and number of replicates could be deduced. All ecotoxicological effects were considered irrespective whether they assessed acute or chronic endpoints. Each comparison of the ecotoxicological mean effect, e.g. the proportion of dead organisms or any other measure of an adverse effect, caused by a given wastewater treatment (ozone or activated carbon treated or untreated) was considered as a separate observation (number of replicates = n). This approach resulted in a total of 780 comparisons used in the meta-analysis, 667 for ozonation and 113 for activated carbon.

Mean values and standard deviations were rescaled by dividing these original values by the largest value reported for each species, separately for each publication. Subsequently, Hedges' g, calculated from rescaled original values, was used as a standardised effect size, which is based on the difference between the mean effects caused by both treatments divided by the within groups standard deviations [20]. To be able to include all data in the analysis, for 21 cases from a range of biomarker and whole organism bioassays (e.g. bacteria, *Daphnia*, fish, yeast-based assays), where means and standard deviations of the original data were zero, rescaled values were set at zero and standard deviations were assumed to have an arbitrarily low value. Exclusion of these data pairs did not noticeably change results. Random-effects models were applied throughout because differences among observations in test species, experimental conditions and endpoints introduced substantial variation in addition to sampling error [20]. Large heterogeneity, which is defined as variation in the true effect size, suggested structure in the data set. Therefore, additional meta-analyses were performed that differentiated among biomarker, whole-organism tests, experiments conducted using eluates of SPE-cartridges loaded with the different types of wastewater, whole wastewater samples, and among groups of test organisms. Mean effect sizes are reported with 95% confidence intervals (CI).

Table 1
Quality parameter of secondary treated wastewater from WWTP Vidy (n = 3) and WWTP Wüeri (n = 3).

	Secondary treated wastewater from	
Parameter	WWTP Vidy (mean ± SD)	WWTP Wüeri (mean ± SD)
COD (mg/l)	29.75 (±6.13)	17.16 (±2.71)
NH_4-N (mg/l)	2.93 (±0.31)	0.05 (±0.02)
NO_2-N (mg/l)	0.43 (±0.16)	0.05 (±0.08)
NO_3-N (mg/l)	14.43 (±2.14)	9.75 (±2.19)
pH	7.56 (±0.12)	7.38 (±0.13)
DOC (mg/l)	7.5 (±2.12)	5.64 (±0.76)

COD = chemical oxygen demand; DOC = dissolved organic carbon.

2.2. Tertiary wastewater treatment techniques applied at pilot-scale

Wastewater composite samples (48 h) were taken from 11th to 13th January and 3rd to 5th May 2010 below the biological treatment (=secondary treated), below the sand filtration (=ozone treated; 0.84 and 0.72 mg O_3/mg dissolved organic carbon (DOC), respectively) and below the powdered activated carbon treatment (=PAC treated; 10 and 12 mg PAC/l, respectively; Norit SAE-Super) at WWTP Vidy (Fig. 1). This WWTP is located in Lausanne, Switzerland, and treats wastewater of a population equivalent of 200,000. Its average discharge is approximately 1300 l/s and the water quality parameters are provided in Table 1. The composite samples were taken time proportional and stored in stainless steel containers at 4 °C. Eight liters of the wastewater sampled below the biological treatment in May 2010 were subjected at the lab-scale to a treatment consisting of a combination of 1 g TiO_2/l (P25 Degussa, average particle size: 21 nm; average surface area: 51 m^2/g) and UV irradiation. The UV irradiation was realized with the laboratory weathering testing system Suntest XLS+ equipped with a daylight filter accompanied by the coupled air conditioning unit SunCool (ATLAS®, Linsengericht, Germany). The irradiation with a wavelength range of 300–400 nm took place at an intensity of 40 ± 5 W/m^2 for 60 min at 20 ± 3 °C. The intensity applied was slightly below values reported for southwestern Germany during summertime [21] and thus is considered an ambient UV irradiation. Subsequently, all wastewater samples were filtered (Whatman, GF/6, pore size <1 μm) to remove particulate organic matter, although this procedure may have removed some organic micropollutants, and TiO_2 present and were afterwards aerated for another 12 h. In both experiments, river water from the Hainbach (49°14′ N; 8°03′ E) – a near natural stream upstream of any settlement, WWTP effluent or agricultural activity – served as control water. Gammarids were exposed to river water (=control), ozone treated, PAC treated, TiO_2 and UV treated (only for samples from May 2010) and secondary treated (=biology) wastewater.

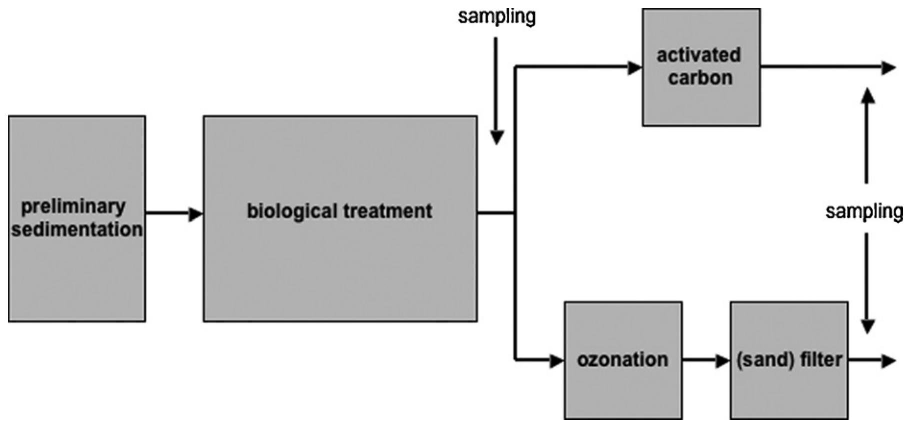

Fig. 1. Schematic diagram of the treatment processes at the WWTP Vidy. The 48-h composite samples were taken below the biological treatment (=secondary treated), below the sand filter (=ozone treated), or below the powdered activated carbon treatment (=PAC treated) as indicated by the arrows.

2.3. Tertiary wastewater treatment techniques applied at lab-scale

To assess potential alterations in the ecotoxicity of municipal wastewaters due to the application of three tertiary treatment methods – ozone, PAC and TiO_2 and UV – between two WWTPs, composite samples of secondary treated wastewater were sampled from WWTP Wüeri (details of this WWTP are given in Bundschuh et al. [5]) below the final sedimentation from the 2nd to 3rd (24 h) September 2010 and from WWTP Vidy below the biological treatment from 15th to 17th (48 h) of September 2010 (Fig. 1). The water quality parameters of both WWTPs are provided in Table 1. The composite samples were taken time proportional and stored in stainless steel containers at 4 °C. Eight liters of secondary treated wastewater from WWTP Wüeri and WWTP Vidy were treated at the lab-scale with an effective ozone concentration of 1.28 and 1.30 mg O_3/mg DOC, respectively, at the Water Technology Centre (TZW) in Karlsruhe, Germany. The ozone concentration was achieved by injecting air containing approximately 31 mg O_3/l for 5.8 and 12.0 min, respectively. Subsequently, the batches were purged for 10 min with a stream of nitrogen to remove any residual ozone and thus to stop ozone-mediated oxidation [16]. Success of this procedure was indicated by using the indigo–blue method [22]. Another 8 l of secondary treated wastewater were treated with 20 mg PAC/l with a contact time of 30 min. The last tertiary method, namely TiO_2 and UV, was applied as described in Section 2.2. Following the treatments, wastewaters were filtered (Whatman, GF/6, pore size <1 μm) to remove all particulate matter – PAC, TiO_2 and organic matter. Afterwards, wastewaters were aerated for another 12 h. Gammarids were exposed to river water from the Hainbach (=control), ozone treated, PAC treated, TiO_2 and UV treated and secondary treated (=biology) wastewater. For the experiment conducted with wastewater from WWTP Vidy respective treatments were supplemented by PAC treated wastewater amended with nutrients according to the concentrations described by Borgmann [23] for the SAM-S5 medium, as literature suggests that essential trace-elements may be removed by PAC [24,25].

2.4. Preparation of leaf discs

Leaf discs, used for the feeding trials, were prepared as described in detail in Bundschuh et al. [5]. Briefly, black alder leaves (*Alnus glutinosa* L. Gaertn.) were collected shortly before leaf fall in October 2007 from a group of trees near Landau, Germany (49°11′ N; 8°05′ E) and stored frozen at −20 °C until further use. After thawing, discs (2.0 cm diameter) were cut from each leaf with a cork borer. To establish a microbial community on the leaf discs, they were conditioned in a nutrient medium together with alder leaves previously exposed in the Rodenbach, Germany (49°33′ N; 8°02′E). Following a conditioning period of 10 days, the discs were dried at 60 °C to constant weight, and weighed to the nearest 0.01 mg. After being soaked in water from the Hainbach for 48 h, the leaf discs were assigned randomly to the vessels of the respective treatment.

2.5. Test organisms

The amphipod species *G. fossarum*, a species established at our laboratory for ecotoxicological bioassays [5], was chosen as test organism since it occurs at high densities in the headwater of the Furtbach, the receiving stream of the WWTP Wüeri. The test organisms, however, were obtained from another near natural stream (Hainbach) close to Landau one week prior to the start of the laboratory feeding trials since the individuals had to be prepared beforehand. Specimens infected with parasite were excluded from the experiment as parasites may affect gammarids' behaviour [26]. Afterwards, the remaining *G. fossarum* were divided into three size classes using a passive underwater separation technique [27]. Only adults with a cephalothorax length between 1.2 mm and 1.6 mm were used. Subsequently, the test organisms were kept in river water from the Hainbach at 20 ± 1 °C until the start of the experiment while preconditioned black alder leaves were provided *ad libitum*.

2.6. Feeding trial

One specimen of *G. fossarum* was placed together with two preconditioned leaf discs in a 250-ml-glass beaker [5] filled with 200 ml of river water, secondary, ozone, PAC or TiO_2 and UV treated wastewater. Beakers were aerated during the whole study duration at 20 ± 1 °C. For each treatment 20 replicates were set up. Five additional beakers per treatment containing only two leaf discs accounted for microbial decomposition and abiotic losses in leaf mass during the feeding trials. Amphipods, remaining leaf discs and any leaf tissue shredded off were removed after seven days of exposure, dried and weighed as described above. The feeding rate was calculated in milligram per milligram dry weight of *Gammarus* per day [28].

2.7. Data analysis

Due to shortcomings of null hypothesis significance testing [29,30], the statistical analysis was based on unpaired 95% CIs using methods described by Altman et al. [31]. If CIs of differences did not include zero, the test outcome was judged as significantly different. Adjustments for multiple comparisons were applied when necessary and reported as adjusted CIs (CI_a) [31]. To obtain approximate p-values of 0.05, 0.01 and 0.001, we calculated 95%, 99% and 99.9% CI_as, respectively. The freeware R version 2.11.2 was used for all statistical analysis and figures [32,33]. Due to seasonal variability in the feeding rate of *G. fossarum*, only the relative deviations among simultaneously conducted treatments should be compared with relative – and not absolute – deviations of other experiments.

3. Results and discussion

3.1. Meta-analysis

The present meta-analysis displayed a significant decrease in the overall ecotoxicity of municipal wastewater following ozone or activated carbon treatment, respectively (Fig. 2A), which was not apparent in our earlier meta-analysis due to lower number of observations then available [16]. This significantly reduced ecotoxicity was obviously irrespective whether whole organisms or biomarkers were used for the ecotoxicological assessment (Fig. 2A). But the cumulative effect sizes for biomarkers were always higher than for whole organisms. This supports the assumption that both types of bioassays differ in their way to assess ecotoxicity. Biomarkers were developed to monitor specific modes of toxic action like endocrine disrupting potential [14]. Hence, these tests hold limited power to detect any ecotoxicity of transformation products or residual compounds with modes of action different from those of the parent compounds [16]. Whole organism bioassays, in contrast, are affected by the chemical composition of the (waste)water in multiple ways. This suggests that these bioassays are capable of detecting effects independent of a particular mode of action, as long as the produced substances or alterations in the chemical constitution of the (waste)water [25] exert any measurable ecotoxicological effect.

However, if the cumulative effect size of whole organisms that assessed alterations in the ecotoxicological potential of municipal wastewater treated with ozone was subdivided in two groups, namely bioassays conducted with whole effluent samples (=no SPE) or with eluates of SPE-cartridges loaded with differently treated wastewaters, huge differences became obvious (Fig. 2B). First, the statistically significantly decreased overall ecotoxicity for whole organisms, exposed to whole effluent samples, masked the difference in cumulative effect sizes if subdivided in three groups of organisms (bacteria, fish, and invertebrates; Fig. 2B). While both fish and bacteria indicated a significantly increased ecotoxicity, the 150 observations summarised by invertebrates showed a significantly reduced ecotoxicity of whole effluent wastewater samples following ozonation [34]. These deviations in ecotoxicity identified by the meta-analysis, however, were not obvious in two individual publications, which assessed the alteration in ecotoxicity by using invertebrates, bacteria and/or fish [35,36]. These adverse effects for bacteria and fish seemed to be caused by toxic transformation products formed during ozonation as hypothesised by other authors [15,37]. Moreover, the overall decrease in ecotoxicity was even more pronounced for experiments using SPE-eluates than for whole effluent samples. This became particularly obvious if bacterial bioassays were considered (Fig. 2B). They displayed a significantly increased ecotoxicity of whole effluent municipal wastewater samples following ozonation but at the same time a significantly reduced ecotoxicity if bacteria were exposed to the respective SPE-eluates. This discrepancy in ecotoxicity may be driven by the ozone mediated formation of transformation products that may exhibit higher polarity than their parent compounds as recently noticed for propranolol [38]. Hence, these transformation products were not purified by SPE-methods and were subsequently not tested by the respective bioassays [39].

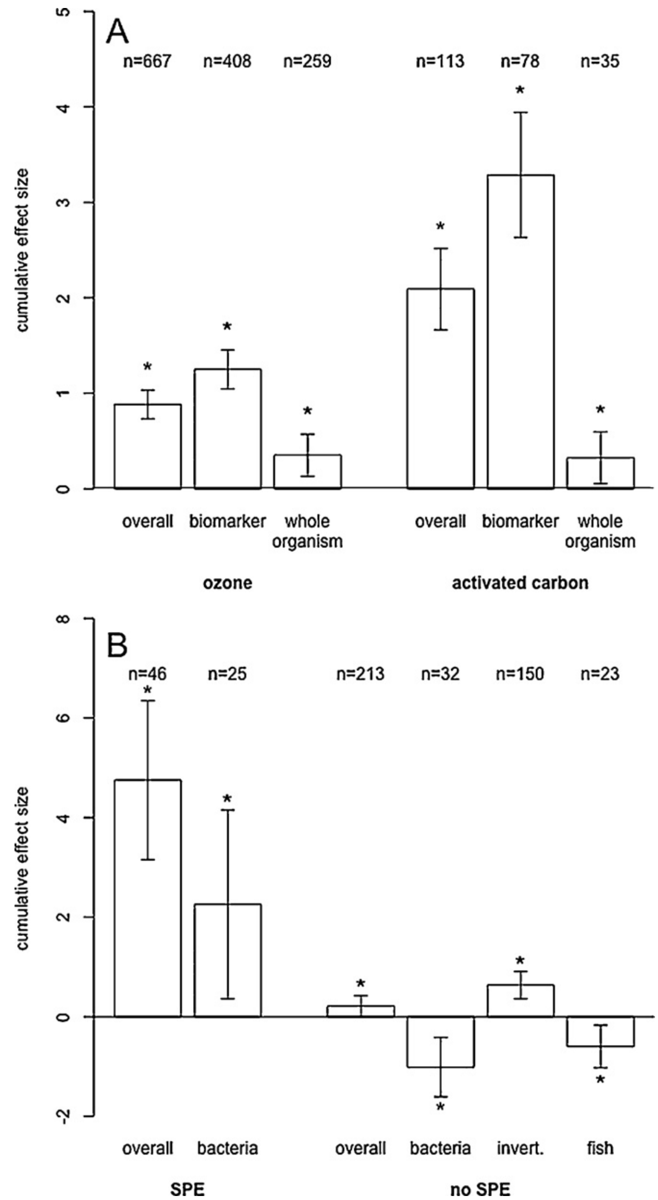

Fig. 2. Cumulative effect sizes (±95% CI) were calculated from rescaled original effect values that indicate any ecotoxicological response in the endpoint assessed, while each observation was considered as a separate replicate (=n). The original effect values were derived from published studies on ecotoxicological effects of municipal wastewater subjected either to ozone or activated carbon. (A) Cumulative effect sizes calculated based on biomarkers, whole organisms, and both together (=overall), however, subdivided in two groups addressing either the effect of ozone or activated carbon application in municipal wastewater. (B) Cumulative effect sizes of experiments focusing on effects of ozone application on whole organisms subdivided in experiments using whole wastewater samples (=no SPE) or SPE-eluates. For both groups the overall cumulative effect size is displayed, which was further subdivided in three groups (bacteria, invertebrates, fish). For the SPE-eluates, however, only the cumulative effect size for bacteria is reported as the number of replicates was too low to calculate reliable values for the other groups. A cumulative effect is considered significant when the zero value is not included in the 95% CI, which is highlighted by asterisks (*). Positive effect sizes indicate decreased toxicity.

3.2. Tertiary wastewater treatment techniques

Only two studies identified by our literature search compared the ecotoxicity of whole effluent municipal wastewaters following the application of ozone and activated carbon, respectively [14,36]. This complicates a direct comparison of both treatment techniques regarding their detoxification potential. Hence, the present study investigated the efficiency of ozone, activated carbon and TiO_2

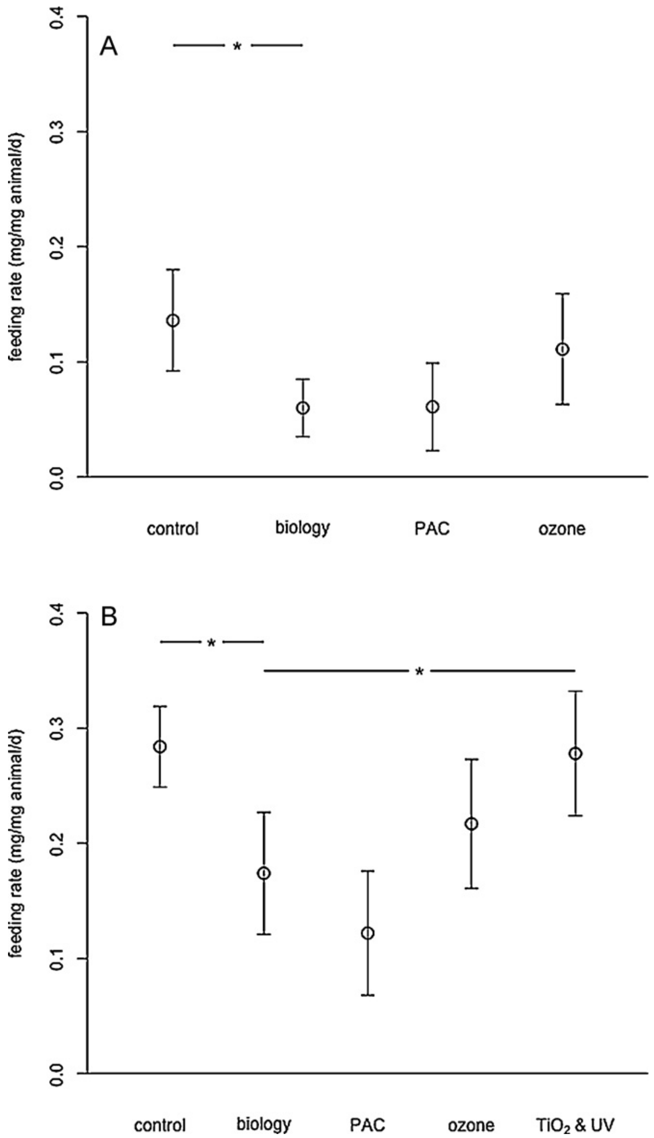

Fig. 3. Mean (±95% CI) feeding rate of *G. fossarum* exposed to (A) control (=river water), secondary treated (=biology), PAC treated and ozone treated wastewater directly sampled from the treatment process at WWTP Vidy in January 2010. (B) Using May 2010 samples, gammarids were additionally exposed to wastewater treated with TiO_2 and UV at the lab-scale. Asterisks denote statistically significant differences between two treatments.

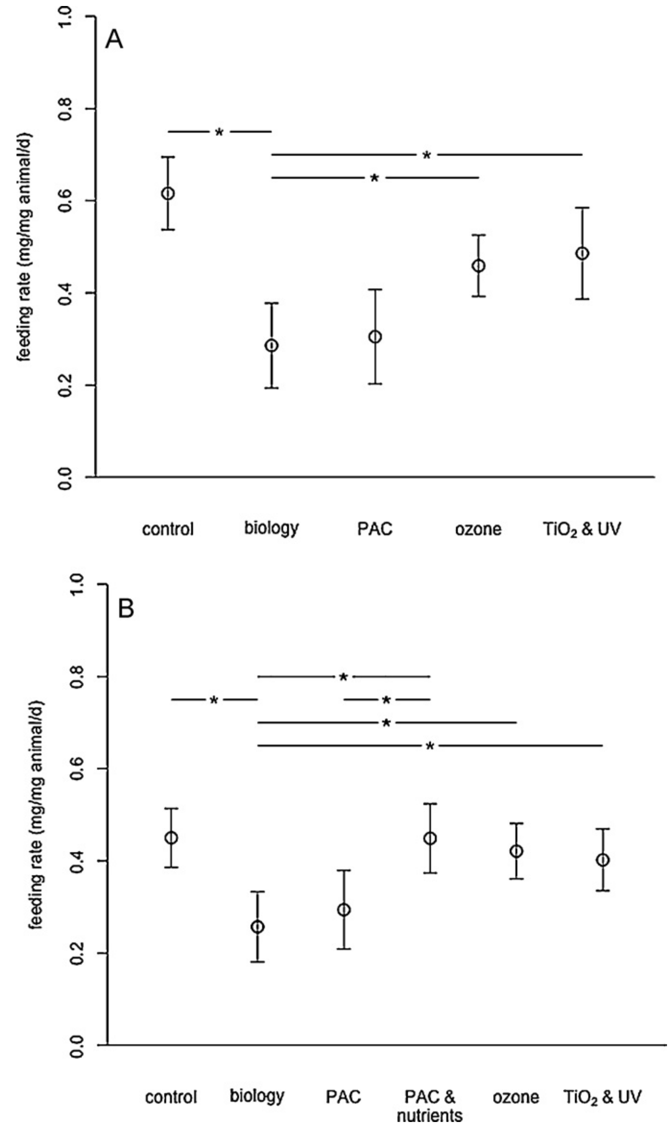

Fig. 4. Mean (±95% CI) feeding rate of *G. fossarum* exposed to control (=river water), secondary treated (=biology) wastewater from (A) WWTP Wüeri and (B) WWTP Vidy subjected at the lab-scale to PAC, ozone, or TiO_2 and UV. PAC treated wastewater from WWTP Vidy was additional assessed following the amendment of nutrients. Asterisks denote statistically significant differences between two treatments.

in combination with UV, while the latter was not yet assessed regarding municipal wastewaters. This assessment was based on four feeding trials using, the leaf shredding amphipod *G. fossarum* [5,17], applied to wastewaters from two WWTPs, which were additionally treated with one of the three advanced treatment techniques. All these experiments displayed a statistically significantly reduced feeding rate and hence a significantly higher ecotoxicity of secondary treated wastewater (=biology) if compared to the control (=river water) (WWTP Vidy January 2010: difference of means: 0.08 mg/mg/d; unpaired 95% CI 0.03 to 0.13; $n = 20$; $p < 0.01$; Fig. 3A; WWTP Vidy May 2010: difference of means: 0.11 mg/mg/d; unpaired 95% CI 0.05–0.17; $n = 20$; $p < 0.001$; Fig. 3B; WWTP Wüeri September 2010: difference of means: 0.33 mg/mg/d; unpaired 95% CI 0.21 to 0.45; $n = 20$; $p < 0.001$; Fig. 4A; WWTP Vidy September 2010: difference of means: 0.19 mg/mg/d; unpaired 95% CI 0.10 to 0.29; $n = 20$; $p < 0.01$; Fig. 4B).

In contrast to our earlier publications [4,17], the feeding trials of the present study did not reveal a significantly reduced ecotoxicity due to ozonation at the pilot-scale at WWTP Vidy, although a tendency towards toxicity reduction is obvious (January 2010: difference of means: 0.05 mg/mg/d; unpaired 95% CI_a −0.01 to 0.11; $n = 20$; $p > 0.05$; Fig. 3A; and May 2010: difference of means: 0.05 mg/mg/d; unpaired 95% CI_a −0.05 to 0.14; $n = 20$; $p > 0.05$; Fig. 3B). This may be explained, at least for the feeding trials conducted in January, by a high variability of the data and hence an increased probability for a type II error. However, the combined application of TiO_2 and UV at the lab-scale during May 2010 resulted in a statistically significant increase in feeding rate, and hence reduction in ecotoxicity, compared to the secondary treated wastewater (difference of means: 0.10 mg/mg/d; unpaired 95% CI_a 0.01 to 0.20; $n = 20$; $p < 0.05$; Fig. 3B). This may indicate that ozone treatment at the pilot-scale (0.84 and 0.72 mg O_3/mg DOC) was not sufficient to cause a sufficiently strong alteration in the loads of micropollutants. But the chemical analysis showed comparable removal efficiencies for the investigated analytes among treatments (Table S2). Hence, it may be suggested that the ozone concentration applied at the pilot-scale resulted in toxic transformation products that jeopardized the positive effects caused by the oxidation of parent compounds [37]. However, 40% higher ozone concentrations applied at the lab-scale (1.28 and 1.30 mg O_3/mg DOC) to wastewater sampled from WWTP Vidy and Wüeri, respectively, revealed significantly higher feeding rates of *G. fossarum* if compared to the corresponding secondary treated wastewater (WWTP Wüeri: difference of means: 0.17 mg/mg/d; unpaired 95% CI_a 0.04 to 0.30; $n = 20$; $p < 0.05$; Fig. 4A; and WWTP Vidy: difference of means: 0.16 mg/mg/d; unpaired 95% CI_a 0.05 to 0.28; $n = 20$; $p < 0.05$; Fig. 4B), although the removal efficiencies of analytes were comparable to the other experiments of the present study (Table S2). Again, TiO_2 and UV resulted in significantly increased feeding rates compared to secondary treated wastewater from both WWTPs (WWTP Wüeri: difference of means: 0.20 mg/mg/d; unpaired 95% CI_a 0.04 to 0.36; $n = 20$; $p < 0.05$; Fig. 4A; and WWTP Vidy: difference of means: 0.14 mg/mg/d; unpaired 95% CI_a 0.02 to 0.27; $n = 20$; $p < 0.05$; Fig. 4B). These results suggest that the combined application of the photocatalyst TiO_2 and ambient UV-irradiation may be a promising tool to detoxify besides industrial [13] also municipal wastewaters. This is also supported by the removal efficiencies of the investigated analytes, which were consistent among the three experiments regarding this treatment technique and comparable to those of ozone and PAC (Table S2).

In all four experiments, the application of PAC to municipal wastewaters resulted in no alteration in ecotoxicity compared to secondary treated wastewater (WWTP Vidy January 2010: difference of means: 0.00 mg/mg/d; unpaired 95% CI_a −0.05 to 0.05; $n = 20$; $p > 0.05$; Fig. 3A; WWTP Vidy May 2010: difference of means: 0.06 mg/mg/d; unpaired 95% CI_a −0.04 to 0.15; $n = 20$; $p > 0.05$; Fig. 3B; WWTP Wüeri September 2010: difference of means: 0.02 mg/mg/d; unpaired 95% CI_a −0.15 to 0.19; $n = 20$; $p > 0.05$; Fig. 4A; and WWTP Vidy September 2010: difference of means: 0.04 mg/mg/d; unpaired 95% CI_a −0.10 to 0.18; $n = 20$; $p > 0.05$; Fig. 4B), although the removal efficiency of the analysed substances was at the same level as for wastewater treated with ozone or TiO_2 and UV (Table S2). This suggests that the PAC treatment may alter the chemical constitution of the wastewater as it is capable to adsorb e.g. calcium ions from water [40]. As calcium ions are important for development and moulting of crustaceans [41], a calcium deficiency may have overridden the positive effects of PAC regarding the removal of micropollutants as hypothesised by Lundström et al. [36]. This assumption is further supported by a significant negative cumulative effect size, and hence increased ecotoxicity observed in the published literature, for invertebrates ($n = 8$) and macrophytes ($n = 2$) exposed to municipal wastewater following activated carbon treatment (cumulative effect size: −0.48; 95% CI_a −0.72 to −0.25; $n = 10$; data not shown). Also, Filby et al. [24] suggested a reduced level of trace elements to be the reason for a reduced reproduction of fish exposed to wastewater treated with activated carbon. These assumptions are supported by the present study as, PAC treated wastewater from WWTP Vidy, which was amended by nutrients (*inter alia* $CaCl_2$), displayed a significantly increased feeding rate compared to the respective PAC treated wastewater without nutrient amendment (difference of means: 0.15 mg/mg/d; unpaired 95% CI_a 0.03 to 0.28; $n = 20$; $p < 0.05$; Fig. 4B) and compared to secondary treated wastewater (difference of means: 0.19 mg/mg/d; unpaired 95% CI_a 0.07 to 0.31; $n = 20$; $p < 0.01$; Fig. 4B). Another experiment suggested that a general positive effect of the nutrient amendment could be excluded, as *G. fossarum* exposed to river water, which was amended with nutrients, displayed identical mean feeding rates as gammarids exposed to river water without nutrient addition (Fig. S1). Thus, the hypothesis of PAC mediated nutrient removal, which compensates any potentially positive effect due to the reduced load of micropollutants, is further facilitated.

4. Conclusion

Our meta-analysis as well as the bioassays conducted, suggest the advanced wastewater treatment methods tested here, namely ozone, activated carbon and TiO_2 in combination with ambient UV, to be suitable to reduce the load of micropollutants and the associated ecotoxicity of municipal wastewaters. However, the present study also displays that these techniques should be applied carefully. Bioassays and meta-analysis reveal that the application of ozone may result in toxic transformation products that may conceal the positive effects caused by the oxidation of parent compounds. Moreover, the application of activated carbon in municipal wastewater reduces besides the load of micropollutants also the bioavailability of nutrients. This issue needs to be considered when evaluating the detoxification potential of these treatment methods based on whole effluent samples. As wastewater is, after its release, mixed with surface water – that usually carries a broad range of nutrients – in the receiving water body the deficiency in such elements is presumably not of concern for aquatic communities. Thus, the three treatment techniques assessed in the present study may help to meet the requirements of the European Water Framework Directive claiming a good status of surface waters in terms of quality (=biological and chemical).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.05.079.

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