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# Is micropollutant-loaded powdered activated carbon from a wastewater treatment plant toxic to the bivalve *Corbicula* sp.?

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## Abstract

**Background:** In order to reduce emissions of micropollutants (MPs) via effluents of wastewater treatment plants (WWTPs), additional treatment steps are suggested and already deployed in selected WWTPs. Next to advanced oxidation processes, the application of powdered activated carbon (PAC) is considered a promising and suitable option as MP removal rates of 80% and more can be achieved. However, this method might also hold a drawback as a complete retention of PAC applied within the WWTP cannot always be guaranteed. Hence, small amounts of MP-loaded PAC can enter receiving waters with potentially negative consequences for aquatic organisms. The present study investigated possible effects of MP-loaded PAC from a WWTP as compared to unloaded, native PAC on the bivalve *Corbicula* sp. in a 10-week exposure experiment. The PAC types were administered in concentrations of 1, 10 and 100 mg/L in a semi-static sediment–water system.

**Results:** Molecular biomarker responses for xenobiotic metabolism (i.e., glutathione-S-transferase (GST)) and oxidative stress (i.e., catalase (CAT) activity and lipid peroxidation) were analyzed and in none of the treatments, significant differences to the control could be detected, except for the CAT activity in the 1 mg/L PAC<sub>WWTP</sub> treatment. Moreover, the filtration rate of individual bivalves was measured after 5 and 10 weeks of exposure and compared to the initial filtration rate with the result that the presence of PAC did not affect the filtration rate of *Corbicula* sp. In summary, despite the selection of sensitive endpoints and a comparatively long exposure period, no significant effects were detected for unloaded and MP-loaded PAC even at the highest test concentration, which is far away from environmental relevance.

**Conclusions:** These results give an auspicious perspective for the application of PAC in WWTPs. Even when small PAC leakages from WWTPs occur, adverse effects for aquatic organisms appear to be neglectable based on our findings.

**Keywords:** Advanced wastewater treatment, Catalase, Glutathione-S-transferase, Lipid peroxidation, Oxidative stress, Filtration rate

## Background

Micropollutants (MPs), such as pharmaceuticals, detergents, pesticides and products used in the daily household, can be found ubiquitously in the aquatic environment [1–3]. Although they occur in very low

concentrations ranging from ng/L to µg/L [4, 5], they can have harmful effects on aquatic organisms [6–8]. Effects described so far range from molecular responses [9] and endocrine disruption [10] to behavioral changes [11] and population declines [12]. Effluents of conventional wastewater treatment plants (WWTPs) play an important role as a source of MPs because WWTPs are generally not equipped to remove these kinds of contaminants sufficiently from the wastewater [13]. In order to reduce further releases, conventional WWTPs are considered

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to be upgraded with advanced treatment technologies, which enable them to eliminate MPs to a satisfying extent [14]. Among different options, one promising method is the removal of MPs by adsorption onto powdered activated carbon (PAC) [15]. The PAC can be applied in the effluent from the biological treatment and can be kept in contact reactors to ensure well mixing with the wastewater and to make use of the adsorption capacity at the best possible rate [16–18]. Subsequently, the PAC needs to be separated from the effluent and may be used again during a recirculation process in the WWTP [19–21]. This concept has already been deployed in pilot and full scale in several WWTPs [16, 19, 22]. Various studies report successful elimination rates and demonstrate the feasibility of this method [16, 18, 21].

Despite these promising results, there might also be a drawback in the use of PAC, which lies in its separation and retention within the WWTP. As PAC can consist of very small particles (e.g., 10–150  $\mu\text{m}$ ), a complete retention of the PAC cannot be guaranteed and it is assumed that MP-loaded PAC is emitted into the receiving waters [18, 23, 24]. In a previous study, possible toxic effects from MP-loaded PAC toward the active filter feeder *Daphnia magna* were investigated [25]. No negative effects were induced by the presence of MP-loaded PAC, although the daphnids ingested PAC [25]. Despite this lack of negative effects, it should be noted that the exposure period was comparatively short (i.e., 48 h for the acute and 21 d for the chronic test) and the examined endpoints focused on immobilization and reproduction, rather than on molecular biomarker responses which are generally more sensitive.

In order to address the question of possible negative effects over longer exposure periods, we performed a long-term experiment over 10 weeks. Moreover, molecular biomarker responses, i.e., the activity of the enzymes glutathione-S-transferase (GST) and catalase (CAT) as well as the extent of lipid peroxidation, were investigated to allow for a more sensitive indication of possible adverse effects. These biomarkers were chosen as GST plays an important role in detoxifying xenobiotics, whereas catalase activity and lipid peroxidation are biomarkers for oxidative stress. These biomarkers are known to respond in *Corbicula* sp. in the presence of low concentrations of MPs, e.g., pharmaceuticals [26, 27].

The study was conducted with the invasive bivalve *Corbicula* sp. as a test organism. This bivalve lives in sandy sediments [28], is an active filter feeder and is therefore capable of filtering enormous amounts of water making it the ideal candidate for exposures with toxic compounds present in the water phase [29–32]. Moreover, it can also ingest sediment by pedal feeding which is another possible route of uptake [33, 34]. *Corbicula* sp. is already

known for its biomonitor and biofiltering abilities and shows sensitive biomarker responses when exposed to pollutants [27, 33, 35, 36]. Apart from this, the filtration rate has been shown to be a sensible endpoint as well [37, 38]. In the present study, we examined if the presence of PAC has negative effects on the filtration rate and induces biomarker responses in *Corbicula* sp. while being exposed to different concentrations of unloaded PAC or micropollutant-loaded PAC from a WWTP.

## Materials and methods

### Experimental design and test substances

The test organism *Corbicula* sp. was exposed in a semi-static test system for 10 weeks to micropollutant-loaded PAC from a WWTP (referred to as PAC<sub>WWTP</sub> in the following) and to unloaded PAC (referred to as PAC<sub>native</sub> in the following). Both types of PAC were applied in three concentrations (1 mg/L, 10 mg/L, and 100 mg/L) each. Additionally, one setup without addition of PAC served as control. The PAC that was used in the experiments was Norit<sup>®</sup> SAE Super (total surface area (B.E.T.) of 1150 m<sup>2</sup>/g; average particle size of 15  $\mu\text{m}$ ; Cabot Corporation, USA) and was provided by the WWTP Dülmen in North Rhine-Westphalia, Germany. This WWTP has a capacity of 55,000 population equivalents and is run by the Lippeverband (Germany). Within the frame of the project DSADS (“Den Spurenstoffen auf der Spur”), the plant was equipped with an advanced adsorption treatment step to eliminate MPs in May 2015 [39]. Native (unloaded) PAC was also obtained from the WWTP before use and served as PAC<sub>native</sub>. The PAC<sub>WWTP</sub> was collected over a period of 2 weeks in April 2017 from the sedimentation basin of the WWTP. Both types of PAC were frozen at  $-20\text{ }^{\circ}\text{C}$  followed by lyophilization (Heto PowerDry LL3000, Thermo Electron Corporation).

### Test animals

Individuals of *Corbicula* sp. were collected at the beginning of April 2019 from the river Lippe near Datteln, North Rhine-Westphalia, Germany and transported to the lab at the university campus in Essen, Germany. They were rinsed with deionised water before transferring them in a large storage tank containing a layer of washed quartz sand (size 0–1 mm, Baumit GmbH, Germany) as sediment and reconstituted water (containing 0.43 mg/L NaCl, 17.25 mg/L KCl, 98.42 mg/L NaHCO<sub>3</sub>, 187.43 mg/L MgSO<sub>4</sub>\*H<sub>2</sub>O and 446.8 mg/L CaCl<sub>2</sub>\*H<sub>2</sub>O in deionised water; see Osterauer et al. [40]) in a climate chamber with a 16:8 h light:dark cycle. The water was permanently aerated with high-porosity airstones (AS34, Tetra) to ensure constant oxygen supply. The mussels were fed once per week with a 3:1 (w:w) mixture of chlorella powder (*Chlorella pyrenoidosa*,

naturwaren-niederrhein GmbH, Germany) and ground TetraMin flakes (Tetra GmbH, Germany). The water was exchanged on a weekly base. Under these conditions starting at a temperature of 11 °C, the mussels were slowly acclimatized during several weeks to the exposure temperature of 16 °C.

**Exposure experiment**

The exposure experiment was conducted in 10-L plastic aquaria (Polystyrol, Savic, Belgium) containing quartz sand and reconstituted water. For the preparation of the exposure tanks, quartz sand was washed thoroughly with deionized water and dried at 70 °C. From a previous study, it is known that PAC in the water phase settles quickly to the bottom [25]. As it is likely that PAC would also settle and accumulate in lentic water stretches in the environment, PAC was spiked into the sediment of the test system. Therefore, the respective amounts of PAC<sub>native</sub> or PAC<sub>WWTP</sub> were homogeneously mixed with 1.4 kg dry quartz sand in order to achieve final test concentrations of 5 mg/kg, 50 mg/kg, and 500 mg/kg, which corresponds to a PAC concentration related to the water volume of 1 mg/L, 10 mg/L, and 100 mg/L, respectively. The lowest concentration was chosen to represent a vaguely estimated worst-case scenario. Although far above-expected environmental concentrations, the highest exposure concentration (100 mg/L PAC) was chosen as it was expected from former exposure studies with daphnids to cause effects [25]. Unspiked sand served as negative control resulting in a total of seven test aquaria (one for the control and three for PAC<sub>native</sub> and PAC<sub>WWTP</sub> each). The prepared sediment (corresponding to a layer of 2–3 cm) was transferred into clean tanks followed by the careful addition of 7 L pre-aerated reconstituted water. The tanks were constantly aerated and the test system was allowed to settle for 24 h before addition of test animals. This was done to ensure homogeneous conditions in each test setup. In each aquarium, 60 individuals with a size between 18 to 24 mm were exposed. For the determination of the filtration rate, five bivalves with a length of 20.5–22.0 mm of each exposure tank were marked with a red paint stick (Faber Castell). The animals were fed every week with the previously described feed mixture with an amount corresponding to 0.015 g per bivalve. Fresh exposure tanks were prepared weekly as described before and bivalves were transferred to these new tanks 24 h after feeding to avoid an increase of feed degradation products and feces in the tanks. The exposure took place in a climate chamber set to 16 °C and a light:dark cycle of 16:8 h. The parameters conductivity, pH, temperature, and oxygen saturation were checked weekly in the tanks immediately before replacement

of the bivalves and in the new tanks directly after adding the bivalves. Bivalve samples (eight individuals) were taken every 2 weeks for other purposes out of the scope of this study. The amount of food given to the remaining bivalves was adjusted accordingly. For the biomarker analyses, bivalve samples (eight individuals per tank: six for analysis and two as a backup in case of mortality) were taken at the end of the experiment after 10 weeks of exposure. The sampled animals were quickly rinsed in deionized water, shock frozen immediately in liquid nitrogen, and stored at – 80 °C until further use. In order to analyze stress levels prior to exposure, a sub-sample from the acclimatized bivalves was taken and frozen as well.

**Determination of the filtration rate**

The filtration rate was determined according to Martinez-Haro et al. [41] in order to gain knowledge if the filtration of bivalves was impaired by the presence of PAC. Prior to the start of the exposure as well as after 5 and 10 weeks of exposure, each marked bivalve was taken from the exposure tanks (24 h after feeding, simultaneously to aquaria change), rinsed with deionized water, and subsequently put for 2 h separately in a 50-mL glass beaker containing 50 mL of a 5 mg/L neutral red solution (prepared in pre-aerated reconstituted water). Before returning the animals back into the exposure tanks, they were put for 2 h in reconstituted freshwater to depurate. After removal of the bivalve from the beaker, the neutral red solution was acidified with 35% hydrochloric acid to a pH of 5. Subsequently, the extinction of aliquot of 1 mL was photometrically measured in duplicates at a wavelength of 530 nm (Specord 200, Analytic Jena, Germany). The concentrations of neutral red in the filtrates were determined by using a freshly prepared neutral red calibration line ranging from 0.5 to 10 mg/L. The filtration rate *m* was calculated as described by Quayle [42] and reviewed by Coughlan [43] as

$$m = \left( \frac{M}{n * t} \right) * \ln \frac{c_0}{c_t}$$

where *M* represents the volume of neutral red solution, *n* the number of bivalve individuals (i.e., one in the present study), *t* the time (filtration period), *c*<sub>0</sub> the initial neutral red concentration, and *c*<sub>*t*</sub> the final neutral red concentration after filtration period.

In order to compensate for individual variations of the bivalves, the filtration rate after 5 and 10 weeks, respectively, was normalized by division by the initial filtration rate of the same bivalve.

### Biomarker analyses

For the biomarker analyses, the frozen bivalves were measured (length and height) and allowed to thaw to a point at which the shell could be opened and surplus ice from the soft tissue could be removed. Each bivalve was prepared and analyzed separately ( $n=6$  pseudo-replicates per treatment). The soft tissue was transferred into snapcap vials (VWR, Germany) and 5 mL of lysis buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Igepal<sup>®</sup>-CA630 (Sigma-Aldrich, Germany), 5% glycerol, pH 7.4) containing 1% of protease inhibitor (P8340, Sigma-Aldrich) was added. Subsequently, the soft tissue was homogenized on ice with a dispersing tool (Ultra-Turrax T 25, Janke & Kunkel, Staufen, Germany). From this homogenate, an aliquot of 1.5 mL was centrifuged at 14,000g and 4 °C for 10 min. The supernatant was divided into aliquots for the different biomarker analyses and the determination of the total protein content. If required for the analysis, aliquots were diluted with dilution buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 5% glycerol, pH 7.4) accordingly. Aliquots were stored at - 80 °C until further processing.

For the total protein determination, the standard microwell Pierce BCA Protein assay kit (Thermo Scientific, USA) with serum bovine albumin in combination with a microplate reader (Tecan infinite M200) was used according to the instruction manual.

### Glutathion S-transferase

The GST activity was determined according to Boyland and Chasseaud [44] in which the absorbance increase of the glutathione-dinitrobenzene conjugate is detected via a photometer at a wavelength of 340 nm over time. The detection was performed as described by Brand et al. [45]. Briefly, 5  $\mu$ L of sample supernatant (which was diluted before 1:10) was mixed with 195  $\mu$ L 20 mM L-glutathione and 10 mM 1-chloro-2,4-dinitrobenzene in Dulbecco's phosphate buffer (2.7 mM KCl, 17.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 32.4 mM Na<sub>2</sub>HPO<sub>4</sub>, 137 mM NaCl, pH 6.5). The incubation of the reaction mixture was carried out in 96-well plates (Greiner Bio-one UV-star) for 10 min at 25 °C. The measurement of absorbance took place every 30 s for 5 min in triplicates in a microplate reader (Tecan infinite M200). The activity of GST was calculated and given as units (U) of GST per mg of total protein.

### Catalase

The catalase activity detection was conducted after Beers and Sizer [46] by measuring the absorbance decrease of hydrogen peroxide as described by Brand et al. [45]. For this approach, 210  $\mu$ L of diluted (1:100) sample supernatant was mixed with 90  $\mu$ L of 100 mM H<sub>2</sub>O<sub>2</sub> in a 96-well plate (Greiner Bio-one UV-star). The absorbance

of hydrogen peroxide was measured at 240 nm in triplicates at 25 °C every 15 s for 4 min. The catalase activity was calculated and given as U of catalase per mg of total protein.

### Lipid peroxidation

The amount of lipid peroxidation was determined via photometer by measuring malondialdehyde (MDA) in a thiobarbituric acid (TBA) solution as described in Brand et al. [45]. In summary, 110  $\mu$ L of the sample supernatant was mixed with 27.5  $\mu$ L sodium dodecyl sulfate (SDS, 8.1%) and 440  $\mu$ L of freshly prepared ice-cold staining reagent (0.5% TBA and 10% trichloric acid, pH 3.5) and incubated at 95 °C for 20 min. Subsequently, samples were cooled on ice for 5 min followed by centrifugation at 4000 g for 10 min at 4 °C. For the quantification, 150  $\mu$ L of the supernatant was measured in triplicates in 96-well plates (Brand, pure grade) at 532 nm and 25 °C in a microplate reader (Tecan infinite M200). The extent of lipid peroxidation was calculated as nmol MDA conjugate per mg of total protein.

### Data analysis

The statistical analysis of the obtained data was performed with the program GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA). The Kruskal-Wallis test followed by Dunn's multiple comparison test was performed to test for significant differences between the control and treated samples (significance level set to  $\alpha=0.05$ ).

## Results

### Test conditions

During the exposure, the test conditions did not differ between the tanks of the different groups (Table 1). The pH of the overlaying water was always between 7.0 and 7.6. The conductivity varied between 1000 and 1100  $\mu$ S/cm and the oxygen content was above 8 mg/L in all tanks and at all times. The temperature was constant at  $16 \pm 0.8$  °C throughout the exposure. Moreover, no dead bivalves were detected in the course of the experiment.

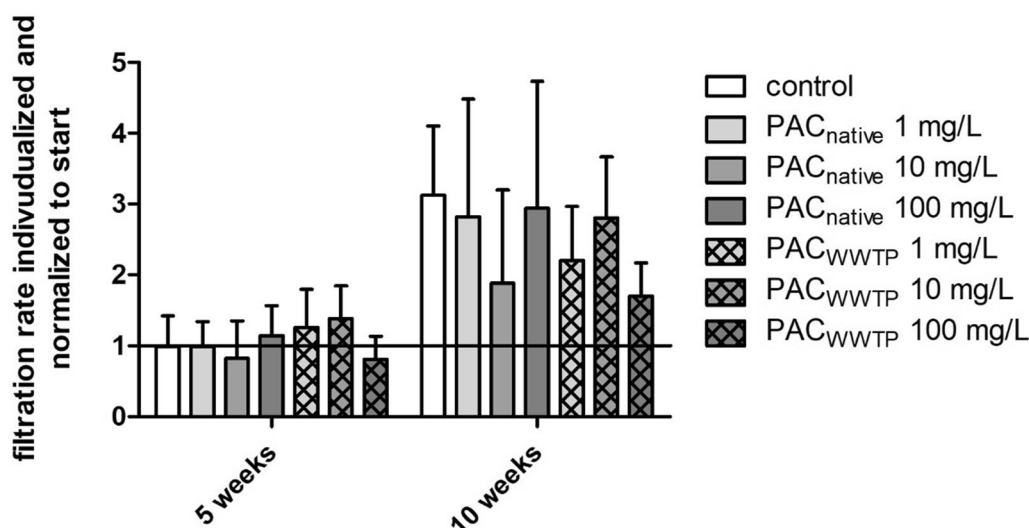
### Filtration rates

The filtration rate of each bivalve was calculated with respect to its initial filtration rate (Fig. 1). After 5 weeks of exposure, the normalized filtration rates of all exposure groups, including the control, were close to 1 (depicted as black line in Fig. 1) which means that the filtration rates (15–16 mL/h) had not changed with respect to the start of the exposure. After 10 weeks of exposure, bivalves from all treatments and control enhanced their filtration rates (approx. 34 mL/h) compared to the starting point. In general, a higher variation in filtration rates



**Table 1 Test conditions during the exposure experiment. Parameters are given as means with standard deviation**

Treatment	pH	O <sub>2</sub> [mg/L]	Temperature [°C]	Conductivity [µS/cm]
Control	7.5 ± 0.2	10.45 ± 1.25	16.06 ± 0.41	1061 ± 28
PAC <sub>native</sub> 1 mg/L	7.5 ± 0.2	10.50 ± 1.28	16.07 ± 0.45	1067 ± 33
PAC <sub>native</sub> 10 mg/L	7.5 ± 0.2	10.44 ± 1.28	16.11 ± 0.39	1058 ± 24
PAC <sub>native</sub> 100 mg/L	7.5 ± 0.2	10.33 ± 1.22	16.13 ± 0.49	1056 ± 21
PAC <sub>WWTP</sub> 1 mg/L	7.5 ± 0.2	10.58 ± 1.22	16.12 ± 0.45	1058 ± 23
PAC <sub>WWTP</sub> 10 mg/L	7.5 ± 0.2	10.58 ± 1.23	16.15 ± 0.46	1058 ± 24
PAC <sub>WWTP</sub> 100 mg/L	7.5 ± 0.1	10.22 ± 1.09	16.32 ± 0.46	1056 ± 21



**Fig. 1** The normalized filtration rates (dimensionless) of *Corbicula* sp. after 5 and 10 weeks of exposure with PAC<sub>native</sub> and PAC<sub>WWTP</sub>. Depicted bars are means (n = 5) with corresponding standard deviations. Bars below the line (= 1) indicate a lower filtration rate and bars above the line a higher filtration rate, respectively, as compared to the initial rate before exposure start. No significant differences (p < 0.05) between bivalves of the exposed groups compared to the controls were detected

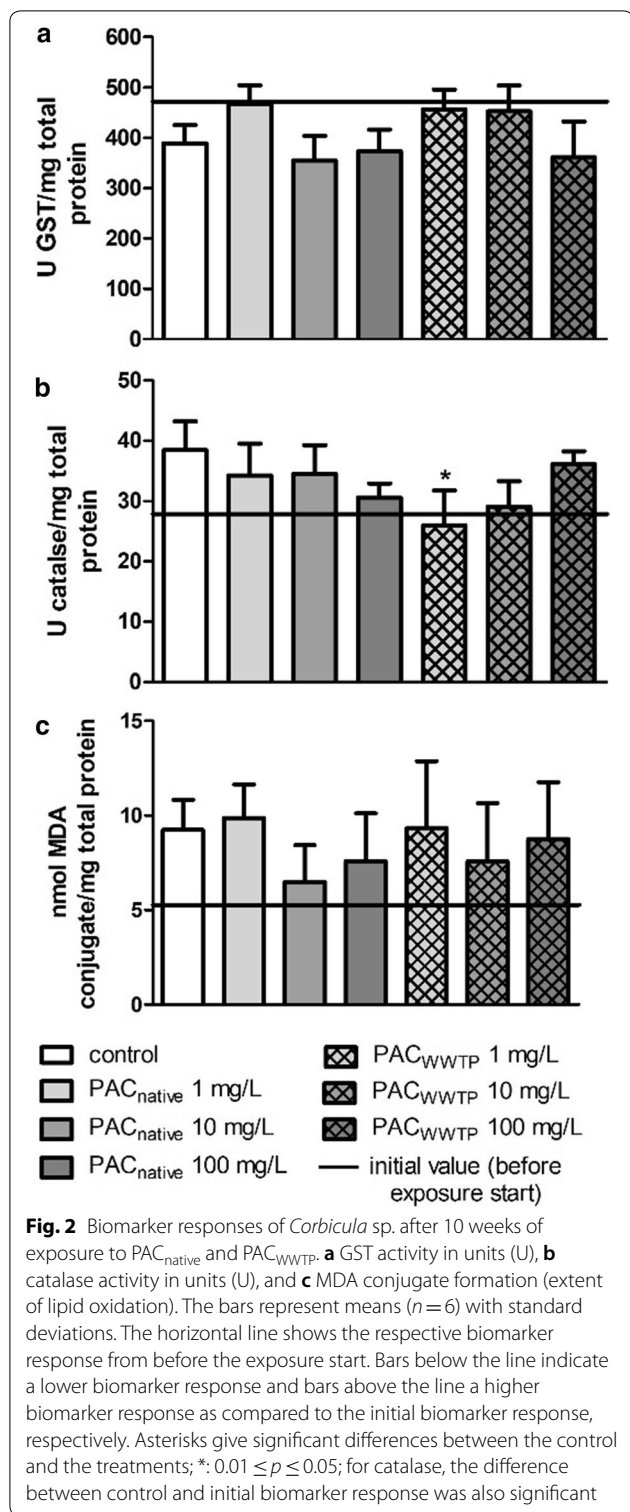
was observed after 10 weeks of exposure as compared to 5 weeks of exposure. At both exposure periods, no treatment showed a significant difference to the control (5 weeks:  $H=7.912$ ,  $p=0.2446$ ; 10 weeks:  $H=7.158$ ,  $p=0.3065$ ).

**Biomarker responses**

The results of the biomarker analyses are summarized in Fig. 2. Kruskal–Wallis test of the GST activity (Fig. 2a) showed significant differences ( $H=28.48$   $p=0.0002$ ). However, according to the post hoc test, no significant differences ( $p<0.05$ ) between the treatments to the control were detected. The bivalves exposed to 1 mg/L

PAC<sub>native</sub>, 1, and 10 mg/L PAC<sub>WWTP</sub> and the bivalves taken before the experiment showed the highest GST activity. The animals from the highest exposure concentrations from PAC<sub>WWTP</sub> and also 10 and 100 mg/L PAC<sub>native</sub> showed a lower GST activity which was on one level with the control.

For catalase, the highest activity was measured in the control (Fig. 2b). Significantly reduced activity to the control was found in bivalves taken before exposure and in the lowest PAC<sub>WWTP</sub> concentration (1 mg/L) ( $H=23.57$ ;  $p=0.0014$ ). No difference could be detected in the in the higher concentrated PAC<sub>WWTP</sub> treatments and in bivalves exposed to PAC<sub>native</sub>.



The other biomarker for oxidative stress, the extent of lipid peroxidation, showed a comparatively high variation between treatments with high standard deviations (Fig. 2c). The Kruskal–Wallis test indicated significant

differences ( $H=14.19, p=0.0480$ ), but the post hoc test could not detect any significant difference between the treatments.

### Discussion

The scope of the present study was to investigate possible effects of unloaded, native PAC and MP-loaded PAC from a WWTP on *Corbicula* sp. by examining different physiological biomarkers. Currently, research on toxic effects of activated carbon (AC) on benthic organisms is usually linked to remediation approaches of contaminated sites by application of high AC doses. For example, McLeod et al. [47] observed a slight decrease in growth and survival for *Corbicula fluminea* at concentrations above 0.7% AC in sediments which is approx. tenfold higher than the highest concentration tested in the present study. Kupryianchyk et al. [48] found a negative trend in species abundance of bivalves (Pisidiidae) after 15 months of exposure with up to 10% PAC. No negative effects (survival and growth) on bivalves after AC exposures of up to 3.4% were reported by McLeod et al. [49] and Tomaszewski et al. [50]. In general, negative effects only occur at very high concentrations of AC in sediments [51]. However, these studies analyzed relatively unobvious markers after applying AC not containing MPs. Interestingly, when performing exposure studies using MP-loaded PAC, also no negative effects on daphnids [25] could be detected. However, this was after shorter time periods and with less sensitive endpoints.

In the present study, we now demonstrated that again no effects were detectable using more sensitive toxicological endpoints, i.e., the filtration rate as well as molecular biomarker responses, in filter-feeding bivalves after 10 weeks exposure to native PAC and MP-loaded PAC from a WWTP. The filtration rate of bivalves is considered to be a very sensitive and reliable endpoint for the presence of many contaminants [52]. Several research articles have shown that bivalves including *Corbicula* sp. react with reduced filtration rates when exposed to pollutants [53] and also to particles, such as metal nanoparticles [37] and microplastic [38] at moderate concentrations and after relatively short exposure periods of 96 h. Reduced filtration rates may be caused by neurotoxicity [38], histopathological alteration in gills [54], or avoidance of pollutant uptake due to valve closing [53]. In the present study, no such effect was observed and no difference of the exposed bivalves to the controls could be detected despite comparatively high PAC concentrations and rather long exposure periods. It may be concluded that the bivalves in the present study did not perceive PAC<sub>native</sub> and PAC<sub>WWTP</sub> as pollutants, and hence, no valve closing was induced, which is known during pollutant contact [52, 53].

There was, however, a marked difference in the filtration rate between the five- and 10-week exposure although the bivalves were marked to compensate for individual variation. In the literature, high variations in the filtration rate commonly occur in experiments with *Corbicula* sp., e.g., ranging from 10 mL/h/bivalve [37] to 160 mL/h/bivalve [38]. Way et al. [29] described a large variation in the filtration rate within their study and also in the literature. Therefore, the difference between week five and week ten is presumably within the range of natural variations and not linked to the treatments.

Moreover, like other bivalves, *Corbicula* sp. is capable of selective particle intake and of avoiding ingestion by production of pseudofeces [29, 31]. The latter is a mechanism in which particles, which are filtered out by the ctenidia and not used as food, are bound to mucus and are excreted without passage through the digestive system [55]. This mechanism is one of the reasons why bivalves are capable to clear water at high rates [32] and are tolerant against high turbidities [29]. In a study conducted by Conway et al. [56] it was observed that bivalves used production of pseudofeces as an avoidance mechanism for nanoparticulate CeO<sub>2</sub>. Thus, in the present study, it is plausible that *Corbicula* sp. also removed PAC via this pathway so that no impairment of the neutral red filtration rates was detected.

In addition to the filtration rate, the three biomarkers GST and CAT activity as well as the level of lipid peroxidation were investigated. GSTs are a large group of enzymes, which exhibit a wide range of substrate specificity. They are important for the metabolism and detoxification of xenobiotic substances [57, 58]. GSTs occur ubiquitously in animals, including *Corbicula* sp., and changes in their activity are considered to be a sensitive biomarker to indicate the exposure of a wide range of pollutants. For example, in a study conducted by Aguirre-Martínez et al. [27], low concentrations (0.1–50 µg/L) of selected pharmaceuticals were sufficient to induce clear responses in GST activity in a 21-day exposure with *Corbicula fluminea*. Activities were increased by a factor of 2 to 3 in comparison to negative controls. Clear effects were also found after short (96 h) and long-term exposure (28 days) with paracetamol [26]. Further studies show that *Corbicula* sp. responds with amended GST activity when exposed to metals [59, 60], landfill leachate [61], and also to diamond nanoparticles [62]. In the latter, GST activity was increased significantly after already 7 days of exposure with a nanoparticle concentration of 0.01 mg/L. In the present study, after 10 weeks of exposure with PAC<sub>native</sub> and PAC<sub>WWTP</sub>, respectively, no significant differences to the control could be detected. Especially the animals from the higher concentrations

were expected to show effects, but the GST activities were on one level with the control.

The catalase is an enzyme involved in protection from oxidative stress by converting hydrogen peroxide to water and oxygen [63]. The alteration of the catalase activity is an ubiquitous biomarker which is induced in *Corbicula* sp. by several pollutants, such as pesticides [64] and metals [60] after likewise short exposure periods (96 h and 12 h, respectively). There are also several studies describing effects on catalase activity of *Corbicula* sp. after being exposed to particles, such as microplastics [38], diamond nanoparticles [62], and TiO<sub>2</sub> particles in combination with cadmium [65]. The catalase activity in *Corbicula* sp. appears to react sensitively toward the presence of these types of particles as a significant increase in activity was already detected after 3–14 days of exposure. However, in the present study, no increase in catalase activity could be detected in the treatments after 10 weeks of exposure. In one treatment, namely PAC<sub>WWTP</sub> 1 mg/L (lowest concentration), a significantly decreased activity was determined in comparison to the control indicating an inhibition of catalase. However, in the 10- and 100-fold higher concentrated treatments, the catalase activities did not differ from the control. Moreover, the decreased activity of this treatment is on one level with the initial catalase activity from before exposure start. Therefore, the obtained differences are probably due to individual variation and not induced by the presence of PAC and should not be overrated.

The last investigated biomarker, the extent of lipid peroxidation, is a measurement of damaged lipids of the cell membranes caused by oxidative stress [66]. Recent studies show that *Corbicula* sp. reacts sensitively with increased lipid peroxidation when exposed to pharmaceuticals [26, 27], pesticides [64], metals [59], and landfill leachate [61]. Furthermore, as for GST and catalase, there are also several studies describing distinct effects on lipid peroxidation after exposure to different types of particles, such as microplastics [38, 67], TiO<sub>2</sub> nanoparticles with cadmium [65], and also diamond nanoparticles at a low concentration of 0.01 mg/L [62]. Again, in the literature cited, the exposure periods were much shorter than in the present study. Still no effects on lipid peroxidation levels could be detected in our study.

In general, when regarding the investigated biomarkers, it can be summarized that neither PAC<sub>native</sub> nor PAC<sub>WWTP</sub> induced clear effects in the bivalves despite the comparatively long exposure period and the high PAC concentrations applied (100 mg/L). Unfortunately, no positive control was included in the experiment because the highest concentration (100 mg/L) of PAC<sub>native</sub> was strongly assumed to induce effects based on findings from previous experiments with daphnids

( $EC_{50, \text{reproduction}} = 5.1 \text{ mg/L}$  after 21 d [25]). Nevertheless, methodological issues can be ruled out as these analyses have been successfully established at the laboratory site with bivalves for similar research questions [45]. The lack of effects in the present study can be due to the fact that these substances are simply not toxic under the investigated conditions or maybe because PAC intake was possibly avoided by excretion via pseudofeces. A recent study confirmed the low toxicity of PAC from WWTPs also for daphnids [25].

Another important aspect is also the applied test design. As described in “Exposure experiment” section, the test system was allowed to settle for 24 h before adding the organisms. This was necessary to ensure homogeneous test conditions in each tank and reduce variability as much as possible. Moreover, this design was particularly chosen because it was known from preliminary testing that PAC settles quickly to the bottom [25]. Therefore, it is assumed that PAC also settles in lentic water stretches where it probably accumulates in the sediment. Naturally, due to this setup, less PAC was available in the water phase for the bivalves via filtration. However, in the present study, reproducibility and realistic conditions were favored in the test design.

Finally, the environmental relevance of the applied PAC concentrations has to be considered. As the PAC particles may accumulate over time at certain hot spots in aquatic ecosystems, the chosen lowest exposure concentration of 1 mg/L PAC in water (or 0.0005% PAC in sediment) is assumed to be a more or less realistic worst-case scenario. In WWTP full-scale applications, PAC is typically dosed in concentrations ranging from 5 to 30 mg/L [23], and retention rates are considered to be greater than 98% [20]. However, the exact amount of PAC that is emitted by WWTPs and reaches the environment is still unknown but is estimated to be rather low.

## Conclusion

In the present study, 10 weeks of exposure with  $PAC_{\text{native}}$  and  $PAC_{\text{WWTP}}$  did not lead to negative effects on the filtration rate of *Corbicula* sp. even at exposure concentrations, which are presumably far above predicted environmental concentrations. Moreover, no impact on biochemical responses (i.e., GST, catalase and lipid peroxidation) was observed. Thus, the results of the present study give strong evidence that the application of PAC in WWTPs can be considered to be safe for the aquatic environment as PAC leakages from WWTPs are assumed to be rather small, so that harmful effects to the aquatic wildlife are unlikely. Naturally, further research should be conducted to confirm these conclusions.

## Abbreviations

AC: Activated carbon; CAT: Catalase; GST: Glutathione S-transferase; MDA: Malondialdehyde; MPs: Micropollutants; PAC: Powdered activated carbon;  $PAC_{\text{native}}$ : Unloaded, native PAC;  $PAC_{\text{WWTP}}$ : MP-loaded PAC from a WWTP; SDS: Sodium dodecyl sulfate; TBA: Thiobarbituric acid; U: Units (enzyme activity); WWTPs: Wastewater treatment plants.

## Acknowledgements

The authors want to thank Daniel Grabner for his support during the biomarker analyses and Nadine Ruchter for advice in the bivalve handling.

## Authors' contributions

BS, SZ, and MW designed the experimental setup. The exposure experiment and biomarker analyses were carried out by MW. The data analysis was done by MW with the aid of SZ and BS. MW drafted the manuscript and SZ and BS reviewed the manuscript. Final reviewing and editing of the manuscript was done by BS. All the authors read and approved the final manuscript.

## Funding

Open Access funding enabled and organized by Projekt DEAL. This study was carried out in the course of the project “Den. Spurenstoffen auf der Spur” (DSADS 3). This project was funded by Bezirksregierung Münster.

## Availability of data and materials

Material and data are described in the main article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

Received: 24 August 2020 Accepted: 2 November 2020

Published online: 18 November 2020

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