Beyond microplastics: Water soluble synthetic polymers exert sublethal adverse effects in the freshwater cladoceran *Daphnia magna*

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HIGHLIGHTS

- Including water soluble synthetic polymers to hazard assessment is urgently needed.
- Toxicity of water soluble synthetic polymers is assessed in *D. magna*.
- 21-d exposure to water soluble synthetic polymers impacted *D. magna*'s life history.
- Water soluble synthetic polymers did not exert acute effects on *D. magna*.

GRAPHICAL ABSTRACT
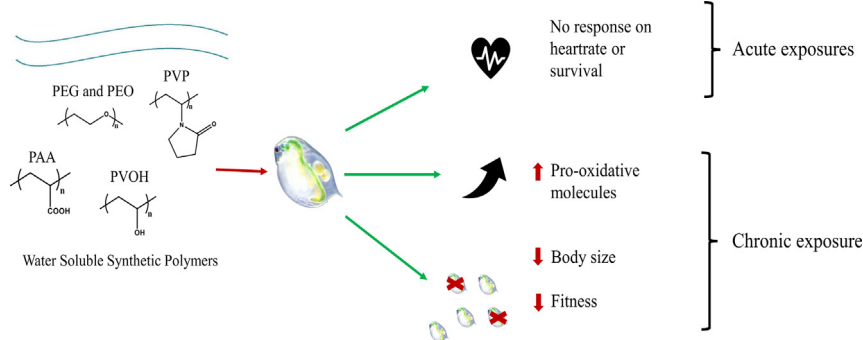
ARTICLE INFO

ABSTRACT

Plastic pollution is considered one of the causes of global change. However, water soluble synthetic polymers (WSSPs) have been neglected so far, although they are used in several industrial, dietary, domestic and biomedical products. Moreover, they are applied in wastewater treatment plants (WWTPs) as flocculants and coagulant agents. Hence, their presence in the aquatic environment as well as their uptake by aquatic organisms is probable, whereas no data are available regarding their potential adverse effects. Here we show in the freshwater key species *D. magna* exposed to five different WSSPs life history changes along with an altered level of reactive oxygen species, although acute mortality was not observed. Since daphnids act as keystone species in lake ecosystems by controlling phytoplankton biomass, even sublethal effects such as WSSPs induced changes in life history may result in cascading effects, from lower to higher trophic levels, which in turn could affect the whole food web.

1. Introduction

Water soluble synthetic polymers (WSSPs) account for ca. 6 % of the global polymer market (Wang et al., 2021) and find application in a variety of different fields and industries like food, cosmetics, pharmaceuticals, paint, textile, paper, construction, adhesive, coatings and water treatment (Kadajji and Betageri, 2011). Unlike the majority of the chemicals that are employed in Europe, WSSPs are not registered under REACH

(Regulation, Evaluation, Authorization and Restriction of Chemicals) (Huppertsberg et al., 2020). This is because, according to the Regulation (EC) No 1907/2006 (Regulation EC No 1907/2006 of the European Parliament and of the Council of 18 December 2006 Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemical Agency, Amending Directive 1999/4 2006), polymers should be exempted from registration and evaluation until they pose any risk to human health or the environment. However, according to Huppertsberg et al. (2020), an estimation of the annual production volume of WSSPs in Europe can be made if the educts of polymer synthesis that are REACH registered are taken into account, leading to a production volume in the range of millions of tons (e.g., poly(ethylene glycol (PEG) > 10^6 t/a, poly(acrylic acid) (PAA) 10^5–10^6 t/a).

Although WSSPs are massively used, information regarding their concentrations and prevalence in the natural environment is still scarce, so their fate and impact remains mostly unclear.

Nevertheless, some studies confirm their presence in the freshwater compartment, for example poly(vinyl pyrrolidone) (PVP) which is present in a variety of medicines, food products and supplements (Julinová et al., 2018) was detected in a concentration range comprised between 0.9 and 7.1 mg/L in the effluents of wastewater treatment plants (WWTPs) in Düren (Germany) (Antić et al., 2011). Also recently, Wang et al. (2021) revealed the presence of PEG (widely used in pharmaceuticals and personal care products, for blood and organs storage and in the automotive industry (Jang et al., 2015; Kadajji and Betageri, 2011; Wang et al., 2021)) in fresh falling snow with a concentration of 2.19 µg/L in the city of Montreal (Canada), confirming air-born dispersion. Once they enter our water bodies, they are free to interact with the biota on different levels of the biological hierarchy. Moreover, their ability to bind heavy metals or organic pollutants gives these toxins the potential to increase their mobility and thus increase their bioavailability (Julinová et al., 2018).

Despite the fact that these polymers cannot be seen washing up on shores like microplastics (MP) do, they occur in our rivers, lakes and other water depositories. (Gross and Kalra, 2002). Once they reach the environment they can possibly exert - unnoticed - a plethora of different adverse effects (possibly affecting different levels of the biological hierarchy, from molecular to individual, up to population or community), rendering them potentially hazardous for the biota and the entire ecosystem (Gross and Kalra, 2002). Given the paucity of reports regarding possible repercussions of WSSPs, we investigated the effects of five synthetic commonly used water soluble polymers on the freshwater model organism *Daphnia magna*. In detail we focused our research on the effects of poly(vinyl alcohol) (PVOH), PVP, PAA, PEG and poly(ethylene oxide) (PEO), (Fig. SI 1). All polymers chosen were of low molecular weights (M_w (weight average molecular weight) < 10,000). To additionally study the effect of molecular weight on *Daphnia magna*, the polymers with ethylene oxide repeat units (PEO and PEG) were used in low and high molecular weights (M_w 1000 and 900,000).

We conducted acute toxicity tests on *D. magna* and a 21-day reproduction test to assess any life-history alteration on the exposed cladocerans. At the end of the chronic test we also measured the levels of reactive oxygen species (ROS). Moreover, we performed a heartrate acute assay on 48-hour old daphnids, as it was proven to be a reliable and quick test for rapid screening on possible adverse effects of toxic compounds (Park et al., 2019). With this work, we aim to investigate and compare for the first time the effects of five widely employed WSSPs on different endpoints in *D. magna* since it is not known if these frequently used compounds evoke adverse effects on aquatic organisms.

2. Material and methods

2.1. Daphnia magna cultivation

A single clone of *D. magna* BL 2.2 was chosen for the experiments from the large and well-established laboratory collection. This *Daphnia* clone originated in Belgium and is maintained in husbandry since 1997 (Imhof et al., 2017).

The organisms used originated from aged-synchronized mothers held in 1,5 L glass beakers (Weck GmbH u. Co. KG, Wehr-Öflingen, Germany).

All the animals were maintained in M4 artificial medium (Elendt and Bias, 1990) with the addition of 4.48 µg/L of selenium dioxide (SeO_2) and kept at 20 ± 0.5 °C with a photoperiod of 14 h light: 9 h dark with 30 min of dusk and dawn. The *Daphnia* culture was fed ad libitum with the green algae *Acutodesmus obliquus* to achieve 2 mgC/L. The mothers were separated from the offspring (<24 h) so that no more hatching occurs and to ensure the use of similar-aged daphnids for the experimental procedure.

All the specimens that were used during these experiments were born from the same mothers and randomly selected for the treatments.

2.2. Chemicals and reagents

The WSSPs that were used during this work (PVOH (75 % hydrolyzed, $M_w \sim 2000$, Acros Organics), PVP (Kollidon 17PF, $M_w \sim 7000$–10,000, BASF), PAA (Mw ~ 1800, Sigma Aldrich), PEG ($M_w \sim 1000$, Sigma Aldrich) and PEO ($M_w \sim 900,000$, Sigma Aldrich)) are shown in Fig. SI 1. The stock solutions were prepared by dissolving 0.5 g of each WSSP in 99.5 g of MilliQ water, in order to obtain five stock solutions at 0.5 w% containing one polymer each. The so prepared stock solutions where diluted in ultra-pure water (0.055µS) (PURELAB Flex, ELGA LabWater).

For the protein quantification Bradford protein assay solution ROTI-Quant ($5\times$ concentrated, Carl Roth, Karlsruhe, Germany) was used (Bradford, 1976; Song et al., 2021). 2′-7′ Diclorofluorescin–Diacetate (2,7-DCFH-DA) (Sigma-Aldrich, St. Louis, USA) was used for the determination of the reactive oxygen species content (Deng et al., 2009).

2.3. Acute tests

2.3.1. Acute exposure experiment

A 48-hour acute exposure experiment was performed following the OECD 202 guidelines (Test No. 202: Daphnia Sp. Acute Immobilisation Test, 2004). Four concentrations (1 mg/L, 5 mg/L, 10 mg/L and 50 mg/L) of the selected WSSPs were used for this test. The procedure was performed on four replicates with 5 neonates each per treatment, exposed in beakers containing 100 mL of the modified M4 medium. During this test mortality and motility within 48-hour were considered as the only endpoints.

2.3.2. Heartrate assay

A heartrate acute assay was conducted on 48-hour old daphnids. Ten animals per concentrations plus ten for control were randomly selected for the exposure to each polymer (forty animals for each WSSP treatment, two hundred animals in total). For the first 48-hour the animals were maintained in the local husbandry and fed with 1 mgC/L of *A. obliquus*. After this period, they were mounted on sterile syringe needles (Ø 0.8 × 22 mm 21G × 7/8", B. Braun Melsungen AG, D-34209 Melsungen, Malaysia) using a drop of Vaseline (MOLYDUVAL, Ratingen, Germany) as adhesive and inserted in 2.5 mL cuvettes (disposable cuvettes, PS 12.5 × 12.5 × 45 mm, BRAND GMBH, Wertheim, Germany) filled with the modified M4 medium and algae as a food source (see more details in the extended methods in the SI). After a 45-minute acclimatization time the animals were recorded with a high-speed camera (i-Speed 3 Olympus Shinjuku, Japan at 60 fps to avoid aliasing), focusing on their hearts, for at least 300 frames (5 s) of which only 300frames were used for further analysis. The organisms were then moved to new cuvettes containing three different concentrations of each of the WSSPs (1 mg/L, 5 mg/L and 10 mg/L) and a blank that consisted of culture medium with the addition of 0.2 % ultra-pure water. This addition corresponded to the same volume of polymer solution that was added in the highest concentration. Ultra-pure water was chosen as control because it was used as solvent for the preparation of the WSSPs' stock solutions. After another 45 min of acclimatization in the treatments the animals were recorded again as described above.

2.4. Chronic toxicity test and ROS quantification

A 21-day chronic exposure experiment was carried out according to OECD 211 (Test No. 211: Daphnia magna Reproduction Test, 2008) using three different concentrations (1 mg/L, 5 mg/L and 10 mg/L) of the selected WSSPs. Due to handling the experiment was set up on two consecutive days. On the first day the exposure started with PVOH, PEO and PEG, on the consecutive day started the procedures with PAA and PVP. For each exposure group an individual control was set up, resulting in two control groups.

Each treatment and the two controls had fifteen replicates, each containing one individual of *D. magna* (with an age < 24-hour). The experiments were performed in 160 mL beakers (Weck GmbH u. Co. KG, Wehr-Öflingen, Germany) containing 50 mL of the modified M4 medium. In order to guarantee constant exposure conditions, the exposure medium was renewed every second day. *Daphnia* were fed with 0.5 mgC/L of *Acutodesmus obliquus* every second day. As life history parameters we recorded different morphological parameters at primiparity and at the end of the exposure, the number of reproductive events, the amount of daphnids hatched daily (neonates) and the levels of ROS.

The measurements of the specimens at primiparity and at the end of the chronic exposure was carried out using a dissecting microscope equipped with a digital camera (Leica M50, Wetzlar, Germany; camera: OLYMPUS DP26, Hamburg, Germany; Light: Leica KL 300 LED, Wetzlar, Germany). The following morphological parameters were considered: body length (measured from the anterior edge of the compounded eye to the base of the tail-spine) and body width (defined as the maximal length between the dorsal and ventral edge of the carapace at the region of the heart) (Fig. SI 2). These morphological parameters were recorded using the software cellSens Dimension (v1.11, OLYMPUS, Hamburg, Germany). For the measurements at primiparity the animals were caught and then released immediately after the picture for the measurements was taken. At the end of the exposure the specimens were photographed and then sacrificed in liquid nitrogen.

At the end of the chronic exposure the animals of each treatment were pooled and homogenized with a pestle in potassium phosphate buffer (100 mM, pH = 7.4, with 100 mM KCl, 1 mM EDTA and protease inhibitors 1/100 v/v) directly in a 1.5 mL Eppendorf tube (Eppendorf Group, Hamburg, Germany). The homogenate was then centrifugated at 15000g for 30 min at 4 °C. The supernatant was used to perform biochemical analyses with two technical replicates. Before quantifying the ROS levels, we assessed the sample's protein content according to the Bradford's method (Bradford, 1976). To be able to evaluate the amount of protein we had to use all the exposed animals, hence for this procedure we relied on the use of technical replicates. The protein quantification was performed using a microplate reader (Synergy HT, BioTek Instruments, Friedrichshall, Germany) (λ_{ab} = 595 nm). The data obtained via this assay were then used for normalization. Then we quantified the amount of ROS using the fluorimetric method described by Deng and coauthors (Deng et al., 2009). This method relies on the fluorescence change (λ_{ex} = 585 nm, λ_{em} = 530 nm)) determined in the 2'7' DCFH-DA (Sigma-Aldrich, St. Louis, Missouri, USA) by the presence of pro-oxidative molecules. ROS concentration was normalized to the protein content and expressed in arbitrary units AU DCF/mg proteins.

2.5. Statistical analyses

The statistical analyses were performed using R (R version 4.0.2, © 2020 The R Foundation for Statistical Computing). To test for normal distribution and homogeneity of the residuals we used the Shapiro Wilk and Levene Test together with a visual inspection using Q-Qplot (quantile-quantile plot) for estimating normal distribution. Since the conditions of normality and homogeneity were met, we used a linear regression model (ANOVA) followed by Tukey HSD post-hoc test with Holm's correction method for multiple comparison by using the package *emmeans* (Lenth et al., 2020).

3. Results

3.1. Acute toxicity tests

3.1.1. 48-hour acute toxicity test

After the 48-hour acute toxicity test neither immobilization nor mortality were registered in the test organisms for the control and any of the four concentrations tested (1 mg/L, 5 mg/L, 10 mg/L and 50 mg/L) of all used WSSPs.

3.1.2. Heartrate acute assay

The heartrate acute assay did not reveal any significant alteration on the heart beat rate of the exposed daphnids for any of the used treatments as shown in Fig. 1. The only treatment where there could be a trend is the one with PVP, for which we could see a decrease in the heartrate with an increase of the concentration of exposure which however is not significant (Table SI 1 and Fig. SI 3 for additional statistical information).

3.2. 21-day chronic toxicity test

3.2.1. Effects on reproduction

At the end of the chronic exposure experiment significant alterations in the number of neonates were registered (Fig. 2). In particular we observed a decrease in the number of offspring in the animals exposed to PVOH 5 mg/L (t = −4.451, p = 0.0001) and 10 mg/L (t = −4.585, p = 0.0001), PEO 10 mg/L (t = −2.827, p = 0.027), PEG 5 mg/L (t = −3.580, p = 0.002) and PEG 10 mg/L (t = −5.103, p < 0.0001) when compared to the corresponding control group 1 (Table SI 2).

Alterations were also detected in the total number of reproductive cycles (Fig. 3). In detail we observed a reduction in the number of reproductive events in the organisms exposed to PVOH 10 mg/L (t = −3.120, p = 0.018) and to PEG 10 mg/L (t = −3.629, p = 0.003) when compared to the corresponding control group, and in the animals exposed to PAA 10 mg/L (t = −2.862, p = 0.026) and to PVP 5 mg/L (t = −3.103, p = 0.015) when compared to the corresponding control group 2 (Table SI 3).

No significant alteration was recorded in the number of days to primiparity.

During the exposure we registered one dead individual in the 15 individuals in "control group 1", in PEO 1 mg/L, in PEO 5 mg/L and in PAA 5 mg/L.

3.2.2. Effects of WSSPs on morphological parameters

Regarding morphological parameters no alterations were detected at primiparity (defined as the age at first reproduction) neither in terms of body length nor body width (Table SI 4 and 5).

Alterations were however recorded at the end of the 21-day exposure.

In detail a significant reduction in body-length (Fig. 4) was registered for the cladocerans exposed to PVOH 10 mg/L (t = −3.495, p = 0.005) compared to the corresponding control group, while an increase in length was observed in the organisms exposed to PVP 10 mg/L (t = 3.284, p = 0.008)) compared to the corresponding control group (Table SI 6). A reduction in body-width was observed just for PVOH 5 mg/L (t = −3.073, p = 0.023) compared to the corresponding control group (Fig. 5) (Table SI 7).

3.2.3. ROS production

After the 21-day chronic exposure the surviving *Daphnia* of each treatment were pooled together and homogenized to perform an evaluation on the levels of ROS (Fig. 6). The levels of pro-oxidative molecules appear to increase along with the concentration of exposure, for every treatment except for the one with PVOH, where it seems the lowest concentration determined a major increase of ROS when compared to the two higher ones.
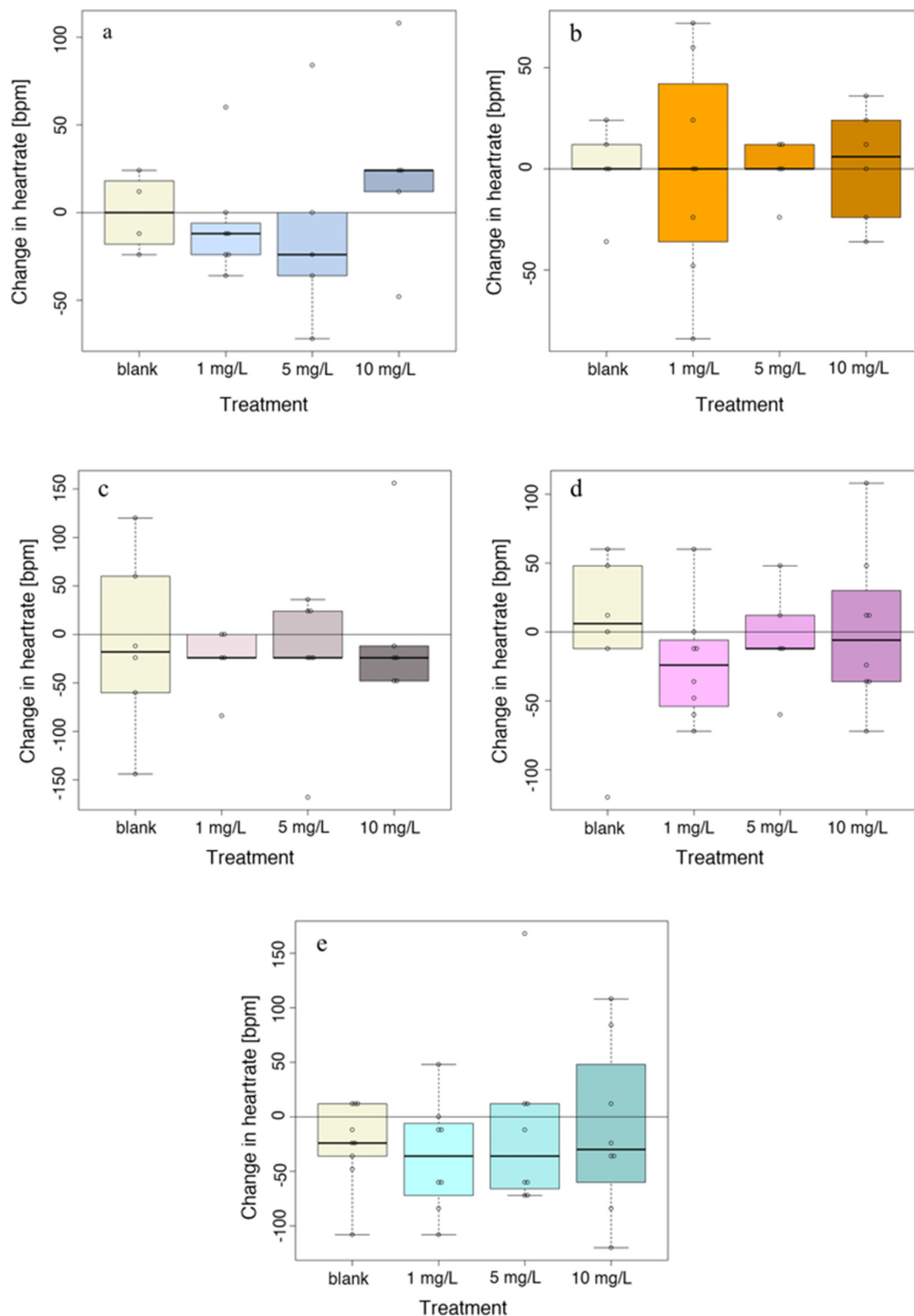
Fig. 1. Change in the heartrate (bpm) as a result of the heartrate acute assay. The boxplots represent the delta in the heartrate before and after the exposure. (a) Treatment with PVOH. (b) Treatment with PEO. (c) Treatment with PEG. (d) Treatment with PAA. (e) Treatment with PVP. Labels on the x-axis indicate treatment, points indicate delta data of one animal. Additional statistical information can be found in Table SI 1 and Fig. SI 3.

4. Discussion

Nowadays plastics in the environment are one of the most discussed topics, both by researchers and the public. However, the discussion so far has been focused mainly on the potential threat represented by particulate plastics, neglecting the hidden risk that WSSPs might pose.

Given their extensive use they also occur in the environment (Antić et al., 2011; Wang et al., 2021). In contrast to particulate microplastics, WSSPs do homogeneously dissolve in water (Kvale et al., 2020). Thus, aquatic organisms are expected to be uninformedly exposed to these polymers and not predominantly at the surface or the sediments of the water body as observed for microplastics.
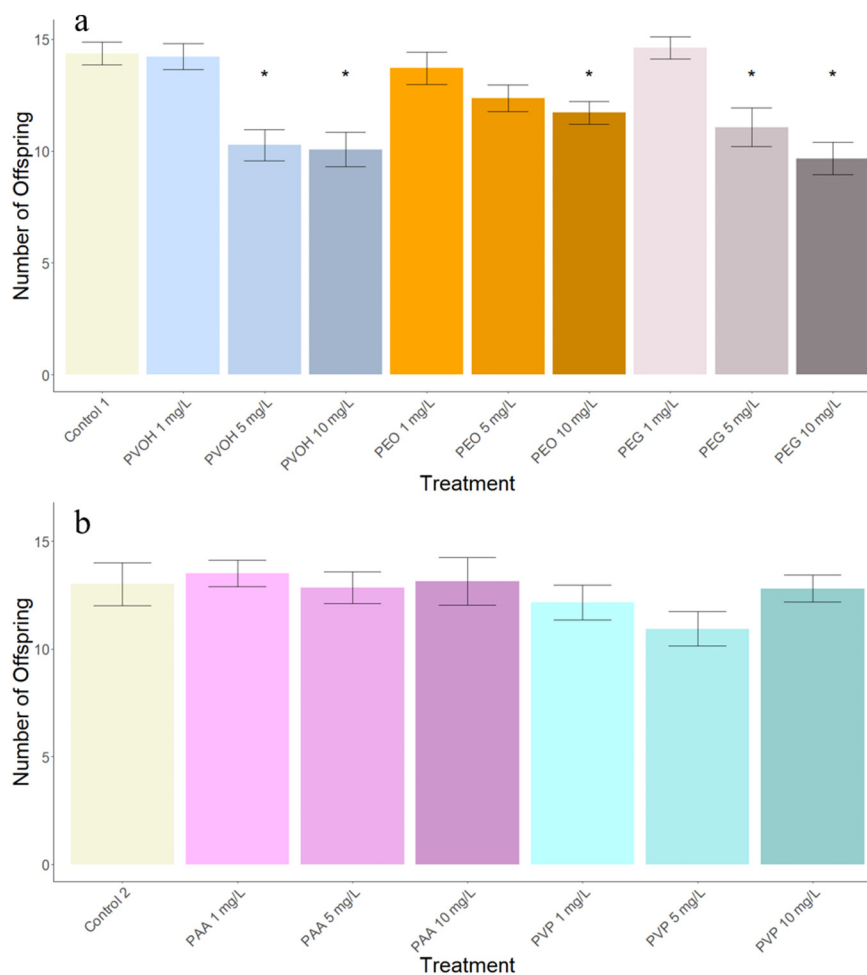
Fig. 2. Number of offspring during the chronic exposure. (a) number of offspring of the animals treated with PVOH, PEO and PEG compared to Control 1. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. (b) number of offspring of the animals treated with PAA and PVP compared to Control 2. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. Additional statistical information can be found in Table SI 2.

Acute toxicity testing with model organisms like *D. magna* is a standardized procedure for the assessment of the ecological risk for non-target organisms resulting from different chemicals. The scientific literature has plenty of examples of these kind of standardized test being used to analyze the effects of a plethora of water-soluble compounds, like pharmaceuticals, pesticides etc. (Daniel et al., 2019; Horton et al., 2018; Haap et al., 2008; Pretti et al., 2009; Okamoto et al., 2015; Cappelli et al., 2020; Han et al., 2006). Hence, we employed these tests first also for WSSPs. However, the investigated WSSPs do not exert any acute effect up to 50 mg/L on *D. magna*. Since the highest concentration of a WSSP (PVP) measured at the outflow of a sewage treatment plant was reported as 7.1 mg/l (Antić et al., 2011) it is unlikely that the tested WSSPs show any acute toxicity in nature. However, even if not acute toxic, any chemical compound can interfere with the physiology of an organism and therefore might affect its fitness. Therefore, the heartrate assay was included in our investigations since the cardiac rhythm could represent a reliable measure to assess the physiological effects of the exposed (Fekete-Kertész et al., 2018; Xu et al., 2017; Duong et al., 2021). In our research, however, the results we obtained do not show any significant alteration to the heartrate of *Daphnia* for the concentrations of WSSPs under investigation.

Despite the fact that this test suggests that WSSPs did not induce any acute physiological alterations we did observe a chronic impairment in the life history of the exposed organisms for most of the employed polymers, both in terms of number of living offspring and reproductive events. In particular, a lower amount of produced neonates was observed for

the animals that were exposed to PVOH (5 mg/L and 10 mg/L), PEO (10 mg/L) and PEG (5 mg/L and 10 mg/L). A reduced amount of reproductive cycles was recorded in the treatments with PVOH (10 mg/L), PEG (10 mg/L), PAA (10 mg/L) and PVP (5 mg/L). Further, a reduction in body size was recorded for the animals exposed to PVOH (5 mg/L and 10 mg/L), while the organisms exposed to PVP (10 mg/L) were observed to grow bigger compared to the control.

Differences between PEO and PEG, that might be due to the different molecular weight of these polymers composed of ethylene oxide repeated units (M_w respectively 1000 and 900,000), can be observed just in terms of reproductive outcome. In fact, while just the cladocerans exposed to the highest concentration of PEO show a significant decrease in the number of produced offspring, for the animals treated with PEG we registered a decrease in the amount of neonates at both concentrations 5 mg/L and 10 mg/L, together with a reduction in the number of reproductive events at 10 mg/L. These results suggest that the toxicity increases with the increase of the molecular weight. No significant differences were however recorded on the investigated morphological parameters.

The reproductive success and *Daphnia* body measurements are important parameters to consider, as they are frequently assessed as endpoints in ecotoxicological studies and as their impairment might ultimately translate on multiple levels of the biological hierarchy. Numerous studies have in fact showed the toxicity of different compounds assessing these variables. For example Sancho et al. (2018) demonstrated that the exposure to the pyrethroid etofenprox impaired both the reproductive outcome and
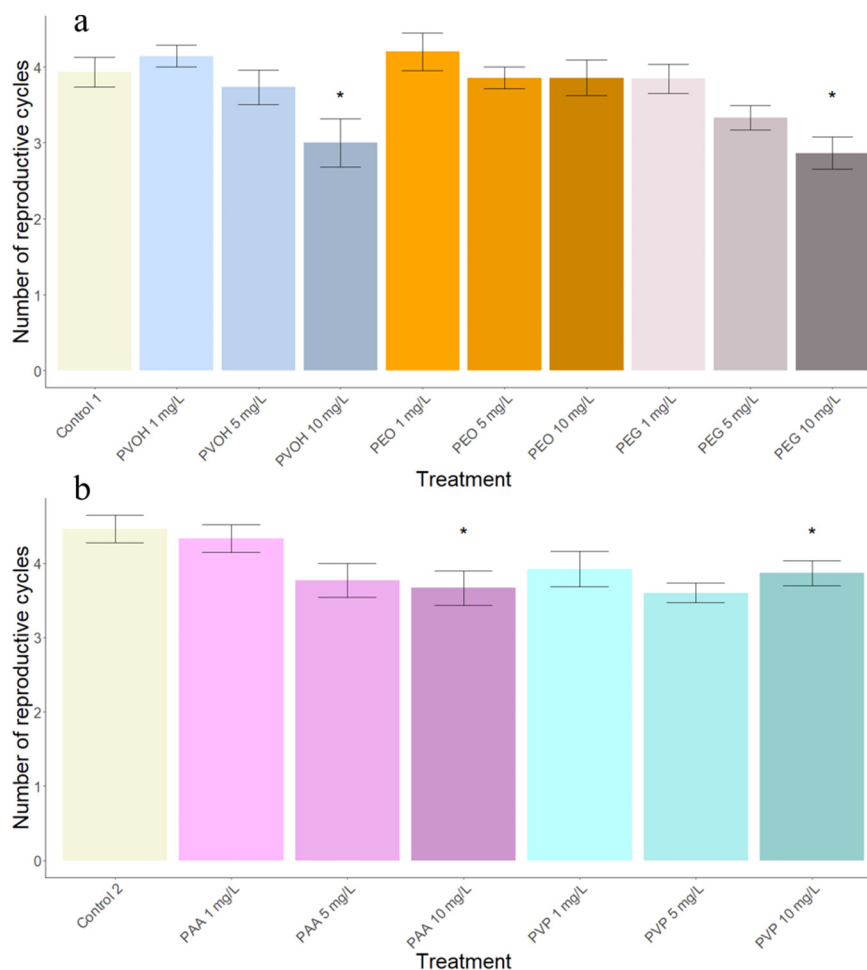
Fig. 3. Number of reproductive cycles during the 21-day chronic toxicity test. (a) number of the reproductive cycles of the animals treated with PVOH, PEO and PEG compared to Control. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. (b) number of reproductive cycles of the animals treated with PAA and PVP compared to Control 2. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. Additional statistical information can be found in Table SI 3.

the growth in *D. magna* at environmentally relevant concentrations. The same was recorded by Rocha et al. (2014) on *Daphnia* exposed to anticholinesterasic drugs. Further, also microplastics exposure showed to have detrimental effects over growth and reproduction (Zimmermann et al., 2020; Jaikumar et al., 2019; Bosker et al., 2019; Schwarzer et al., 2022). Detrimental effects on the reproductive success might manifest at first on the population level, with a decline of *Daphnia* biomass. This could then translate over the biological community and the ecosystem as an essential link of the freshwater food web would be diminished (Bosker et al., 2019).

Alterations in morphological parameters might also have relevant environmental repercussions, both in case of a size reduction and increase. A decrease in body size like we observed in the organisms exposed to PVOH might in fact make the daphnids more susceptible to gape-limited predators like *Mesocyclops* (Chang and Hanazato, 2003; Krylov, 1992) contributing so to the reduction of their biomass(Schwarzer et al., 2022). By contrast, an increased in size, as was recorded for the animals exposed to PVP, might render them more vulnerable to visual predators e.g. the small sunfish *Lepomis macrochirus* or *Notonecta undulata,* for which a smaller body size was observed as an inducible defense in different exposed *Daphnia* species (Dodson, 1989). However, if size alterations as a consequence of PVOH or PVP exposition would overweigh an inducible defense in nature must be investigated in future.

A potential reason for the effects we observed on life history may be oxygen depletion since water soluble polymers may cover the gill

surface or interact with oxygen transfer. A study conducted by Goodrich et al. (1991) on *Oncorhynchus mykiss* (rainbow trout), proved that cationic WSSPs are able to bind via ionic interactions onto the fishes' gills, resulting in a toxic effect on the organisms due to an impaired oxygen transfer through damaged gill surfaces. However, since the compounds employed in this work are not charged and *Daphnia*'s gills are physically separated on the thoracopoda it is unlikely that they collapse and stick together as it was previously observed on *O. mykiss* and hence exert negative effect on the cladocerans. Further, the fact that the heartrate of *D. magna* was not increased during exposure renders an impaired oxygen transfer unlikely.

A further potential reason why we observed an impairment in the reproductive success and a reduction in size when it comes to the PVOH treatment might be the onset of an altered oxidative status, as suggested by the increased amount of ROS within the organisms. Several studies (e.g. Trestrail et al., 2020) have shown how exposure to different contaminants can enhance the intracellular ROS production, leading to the activation of different energy expensive physiological defenses (De Felice et al., 2020; Xia et al., 2020; Parolini et al., 2018). Under normal conditions the organisms use most of their energy reserves for growth, reproduction and for their basal metabolism (Arzate-Cárdenas and Martínez-Jerónimo, 2012). However, when they have to face the adverse effects induced by the presence of one or more toxic compounds, such as WSSPs, the energy required for the basal metabolism is much higher. Ultimately this results in an energy depletion and a
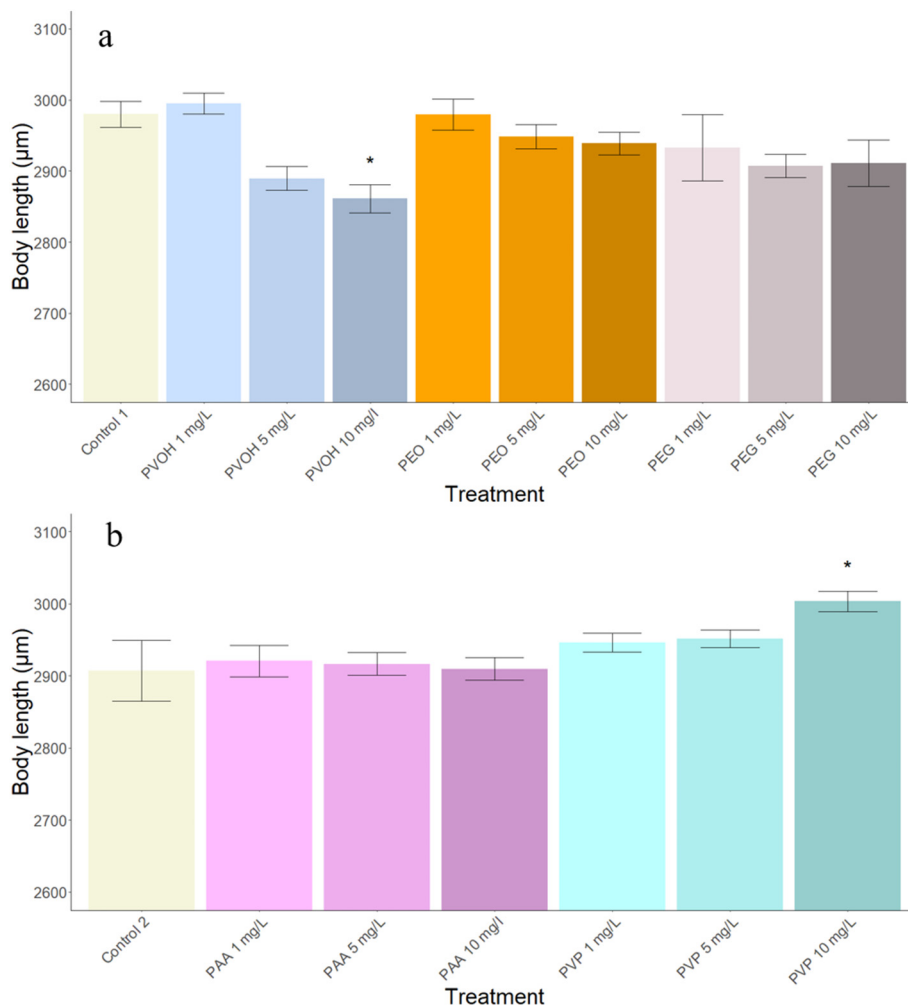
Fig. 4. Body length of the exposed animals at day 21. The measures are expressed in μm. (a) Body length of the animals treated with PVOH, PEO and PEG compared to Control 1. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. (b) Body length of the animals treated with PAA and PVP compared to Control 2. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. Additional statistical information can be found in Table SI 6.

different allocation of the resources to detoxification and antioxidant enzymes (Pieters et al., 2006; Knops et al., 2001; Nogueira et al., 2004). The preferential allocation of resources in favor of the biochemical defenses of the organisms might so explain the impairment of the reproductive success we observed in our study. This phenomenon was previously described for different substances, by e.g. Parolini et al. (2018) after the exposure of *D. magna* to benzoilegonine or by Liu et al. (2022)after the exposure of the same model organism to poly (vinyl chloride) (PVC) microplastics.

However, an altered oxidative status does not explain the findings concerning *Daphnia* exposed to PVP, for which we observed an increase in body length at the higher concentration over the 21-day exposure, together with the highest concentration of ROS among the tested animals. The increased growth rate of the organisms exposed to PVP might be due to a phenomenon called hormesis (Calabrese and Baldwin, 1998; Gems and Partridge, 2008; Mattson, 2008). This term refers to the biphasic response that can be observed after the exposure to some chemicals: lower doses might show a beneficial effect in terms of growth rate, fitness and stress resistance, while higher concentrations of the same compound could exert a toxic effect.

Nevertheless, a difference in resource allocation in favor of the antioxidant defenses is consistent with a lower reproductive outcome. Even though there was no induced mortality or any alteration in days to

primiparity among all exposed daphnids, the reduction in the number of offspring and reproductive events could still have an impact over population and ecosystem dynamics. *D. magna* is relevant for ecotoxicological studies, as it represents a key species in lentic pelagic ecosystems. They are primary consumers for phytoplankton and an essential food source for secondary consumers (Miner et al., 2012). They therefore represent a crucial link for carbon and energy transfer between primary producers and higher trophic levels (Diel et al., 2020). A decrease in the number of daphnids might impair the top-down control of the phytoplankton, possibly leading to algal blooms and eutrophication (Ekvall et al., 2014). Moreover, a reduction in daphnids biomass could alter the bottom-up control of higher trophic levels, disrupting so the entire ecosystem function (Bosker et al., 2019).

5. Conclusion

Our study showed that, even though no acute effect was detected in terms of mortality, immobilization or alterations on the heartrate, the chronic exposure to five commercially available and extensively used WSSPs exerts sublethal effects on *D. magna*. We did record impairments on life history traits and morphological parameters of the exposed organisms. This may be concerning as the WSSPs mediated impairment of *Daphnia*'s fitness would lead to negative effects on the population level, which,
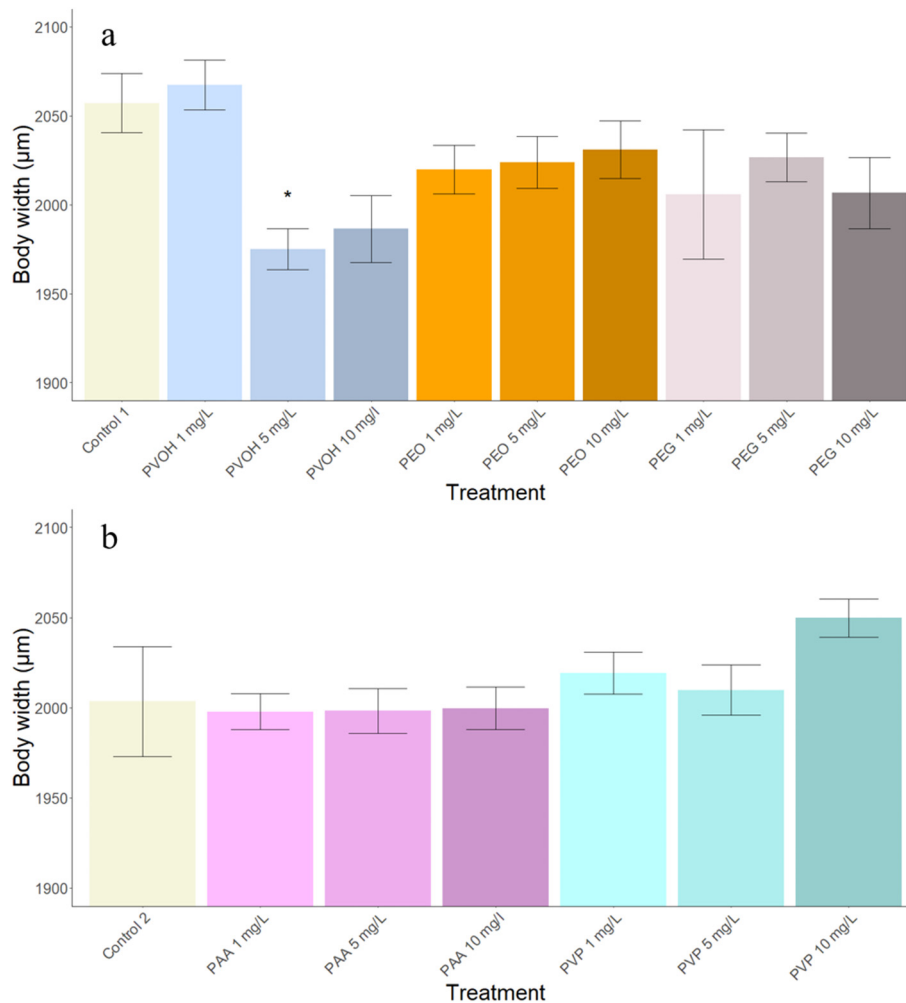
Fig. 5. Body width of the exposed animals at day 21. The measures are expressed in μm. (a) Body width of the animals treated with PVOH, PEO and PEG compared to Control 1. Asterisks represents significances ($p < 0.05$) compared to the control. Error bar represents standard error. (b) Body width of the animals treated with PAA and PVP compared to Control 2. Asterisks represents significance ($p < 0.05$) compared to the control. Error bar represents standard error. Additional statistical information can be found in Table SI 7.

over the long run, might potentially cascade over higher trophic levels, hence impairing the entire community and ecosystem. Nevertheless, a huge gap of knowledge still exists what impact the WSSPs could have and what are their mode of action, since our results suggest that the molecular weight of WSSPs may alter their toxicity. Hence, it would be highly desirable to have further investigations on the potential threat represented by these and other WSSPs, using not only *D. magna,* but also including other organisms.

CRediT authorship contribution statement

Simona Mondellini: Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing, Formal analysis, Visualization, Project administration, Validation, **Matthias Schott:** Conceptualization, Methodology, Investigation, Writing - Review & Editing, Formal analysis, Validation, **Martin G. J. Löder:** Conceptualization, Resources, Writing - Review & Editing, Validation, Project administration, Supervision, Funding acquisition, **Seema Agarwal:** Project administration, Funding acquisition, Resources, Development of Reference Material, **Andreas Greiner:** Project administration, Funding acquisition, Resources, Development of Reference Material, **Christian Laforsch:** Conceptualization, Resources, Writing - Review & Editing, Validation, Project administration, Supervision, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Christian Laforsch reports financial support was provided by Horizon 2020. Christian Laforsch reports financial support was provided by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157608.
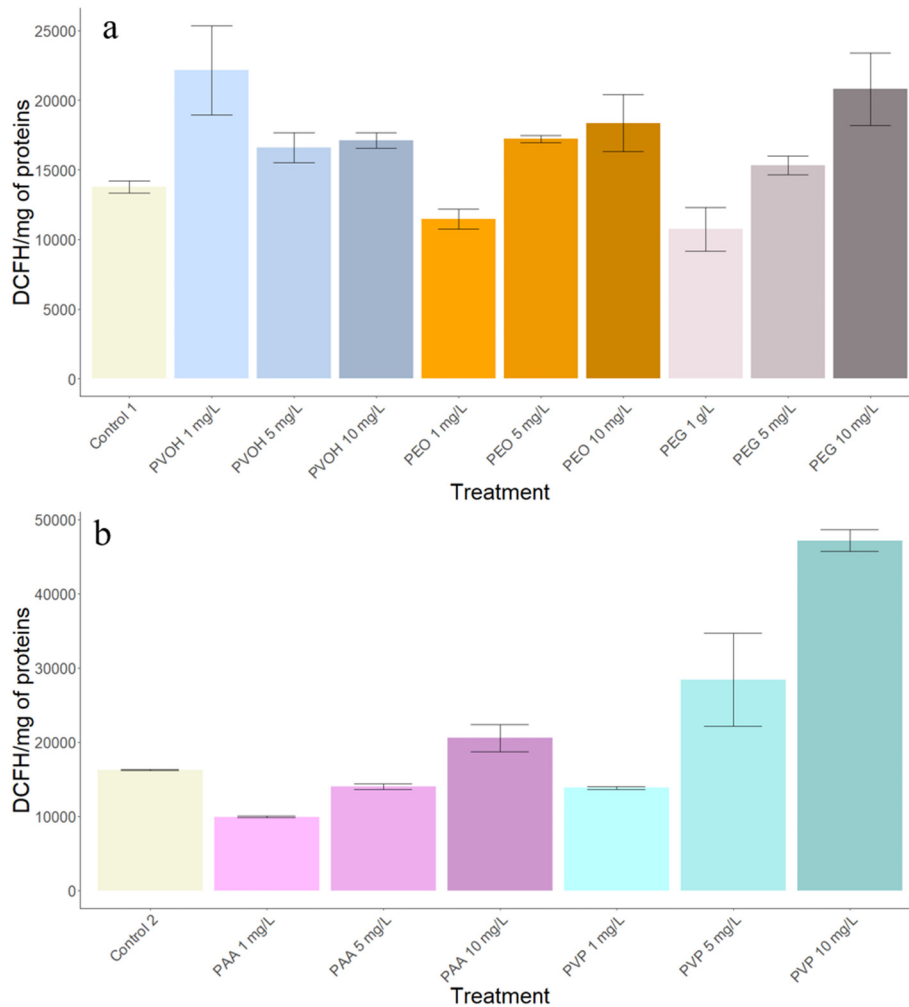
Fig. 6. Levels of ROS expressed as arbitrary fluorescence units and normalized to de protein content of each sample. (a) Levels of ROS of the animals treated with PVOH, PEO and PEG compared to Control 1. Error bar represents standard error. (b) Levels of ROS of the animals treated with PAA and PVP compared to Control 2. Error bar represents standard error.

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