Daphnia magna filtration, swimming and mortality under ammonium, nitrite, nitrate and phosphate

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Abstract

Biological methods are a promising approach to treating wastewater in order to produce water of an appropriate quality for sub-potable water purposes, thus reducing pressure on potable water sources. *Daphnia magna* are organisms that filter on small suspended particles and bacteria and so may be able to clarify and disinfect wastewater. However, *Daphnia magna* are sensitive to common chemicals and might be vulnerable to the quality of the wastewater. This study analyses the filtration, mobility and mortality rates of *Daphnia magna* exposed to seven days of changing concentrations of ammonium, nitrite, nitrate and phosphate. Inactivation increased with the time of exposure for both nitrite and ammonium, with a 50% inactivation in *Daphnia magna* filtrations after 7 days of exposure at nitrite concentrations above 6 ppm and ammonium concentrations above 40 ppm. The *Daphnia* filtration remained unaltered in the nitrate and phosphate concentrations. Mortality increased with nitrite and ammonium concentrations, but not with phosphate or nitrate. The swimming velocity of *Daphnia magna* individuals decreased when both nitrite and ammonium concentrations increased and also with phosphate concentrations above 30 ppm. However, *Daphnia magna* swimming velocities remained unaltered in the presence of nitrate concentrations below 100 ppm.

Introduction

Wastewater reuse is an alternative potential water source that might reduce pressure on drinking water resources (Bouzit et al., 2016). Treated wastewater needs to meet established water quality standards to be used for irrigation or urban cleaning, (among other applications (Ait-Mouheb et al., 2018), however, the high capital and operating costs of conventional tertiary treatments can make this unaffordable for some communities. Moreover, most conventional tertiary treatments rely on the use of chemicals, which might also produce some non-desired by-products that could be released into the environment (Jaramillo and Restrepo, 2017). Since water reuse is a promising alternative to using natural water resources in zones where water is scarce, it is important to continue researching alternative low-cost, environmentally-friendly tertiary treatments. One such alternative to explore is the use of natural depuration systems. For instance, filter-feeder organisms such as *Daphnia* could be used as an alternative method to remove particles found in secondary effluents.

Removing small suspended particles (with diameters below 30 μm) from wastewater poses a challenge because they can go through meshes, filters or settling tanks without being separated from the water phase. Furthermore, as these small particles make the water appear cloudy, this complicates disinfecting it with UV-based technologies. Therefore, it is crucial to find alternative ways to reduce the concentration of these small particles in wastewater. The genus *Daphnia* is a zooplanktonic population of Cladocera whose ability to disinfect wastewater (Burnet et al., 2017; Serra et al., 2014; Shiny et al., 2005), remove emerging contaminants (Matamoros et al., 2012) and prey on wastewater particles with diameters below 30 μm (Pau et al., 2013; Serra et al., 2018; Serra and Colomer, 2016) has already been demonstrated.

However, *Daphnia* might be sensitive to some compounds. For this reason they are sometimes used to test water quality (Heger et al., 2018; Van de Perre et al., 2018) because many compounds are toxic for these organisms (Sladkova et al., 2016). Nitrate (NO_3^-) concentrations above 56 mgN-NO_3^- L^{-1} (250 mgNO_3^- L^{-1}) together with a water temperature of 26 °C proved to have lethal effects on a *Daphnia* population (Maceda-Veiga et al., 2015). In addition, ammonia (NH_3) concentrations over 0.81 mgN-NH_3 L^{-1} were found to reduce the ingestion rate of *E.coli* by *Daphnia* (Norgaard and Roslev, 2016). However, in the same study, *Daphnia* filtration had already been reduced by 40% when the concentration level of ammonia was 0.1 mgN-NH_3 L^{-1}; in comparison with the control experiments which had no ammonia. Furthermore, high levels of nitrite in water has been found to have toxic effects on aquatic organisms, producing physiological disturbances (Jensen, 2003). *Daphnia obtusa* exposed to nitrite concentrations above 2 mgN-NO_2^- L^{-1} have a reduced survival time compared to the case without nitrite (Xiang et al., 2012). Similar results were obtained after *Daphnia similoides* were exposed to the same nitrite concentration (Xiang et al., 2011). The presence of clays and cadmium have also been found to reduce the heartbeat of *Daphnia* (Lari et al., 2017), which would be an indicator of adverse environmental conditions for *Daphnia*. Low food concentrations have also been found to reduce their heart rate (Lari et al., 2017).

Therefore, *Daphnia* vulnerability makes their application as a tertiary treatment challenging. Even though *Daphnia* remove small particles, reduce microbial loads and polish nutrients in secondary effluents, their applicability in wastewater treatment plants might be complicated under the presence of some chemical compounds.

It must be noticed that the most common nutrients in wastewater such as ammonium, nitrite, nitrate and phosphate have a great variability during the water treatment in the system, from the inlet to the outlet. For instance, in secondary treatments N-NH_4^+, N-NO_3^- and N-NO_2^- attain low values of 12 mg/L, 0.4 mg/L and 19 mg/L in settler effluents and somehow lower in membrane with non-detectable values of N-NO_2^- and N-NH_4^+ (Paredes et al., 2018). For the same wastewater treatment plant, the inlet presented higher nitrite concentrations with maximum values of 0.9 mg/L. Higher nutrient concentrations can be found at the primary effluent of a wastewater treatment plant with N-NH_4^+ of 98.2 mg/L, P-PO_4^{3-} of 17.3mg/L and N-NO_3^- of 23.5 mg/L (Praveen et al., 2018). Other authors state that the total nitrogen and phosphorous in untreated wastewater can range from 20-85 ppm and 4-15 ppm, respectively. However, the effect the most common compounds found in wastewater have on the *Daphnia* filtration efficiency has scarcely been studied, this study explores the effect the main wastewater compounds found in the tertiary treatment (N-NH_4^+, N-NO_2^-, N-NO_3^- and P-PO_4^{3-}) have on *Daphnia* activity. With this information, the applicability of *Daphnia magna* as an organism for wastewater treatment will be known and in addition, the level at which this system will be suitable (after primary or secondary treatments) will be determined for each type of wastewater and in terms of these contaminants.

The toxicity effects biotic and abiotic parameters have on *Daphnia magna* have been evaluated in terms of their behaviour, that is, filtering rate (Serra et al., 2018), heart beat (Lari et al., 2017), mortality and swimming velocity (Serra et al., 2018; Wickramarathna et al., 2014). Swimming activity has recently received special attention because it is a sensitive biomarker that can easily be affected by chemical substances (Bownik, 2017). Therefore, *Daphnia* mobility is widely used in toxicology tests (Bownik, 2017). In this study the filtering rate, mortality and swimming velocity will be considered in order to analyse the effect of ammonium, nitrite, nitrate and phosphate on *Daphnia* activity. Different concentrations of each chemical will be tested separately and will be compared to the experiments without the presence of the contaminants. One expects that if *Daphnia* filtration rate and swimming velocity does not differ from that without the contaminant, *Daphnia* can then be considered effective in removing small particles from wastewater.

Materials and methods

Daphnia magna characteristics

The *Daphnia magna* population was obtained from a laboratory culture kept in a 40 L container at 20.0±0.5°C and natural daylight photoperiod for one year at the University of Girona. A gentle supply of air ensured the water container was oxygenated and the *Daphnia* in the container were fed twice a week with a mixture of commercial spirulina powder and Baker's yeast (*Saccharomyces cerevisiae*). Thirty percent of the water from the container was renewed once a week.

For each experiment, *Daphnia* individuals were collected from the container using a 1.5 mm mesh in order to be able to discard individuals smaller than 1.5 mm long. Individuals retained in the mesh larger than 2 mm were also discarded and returned to the container. Therefore, only 1.5-2.0 mm-long *Daphnia* individuals were considered for the study. Using ImageJ software, the mean size of the *Daphnia* individuals was analysed from a video recording of 25 individuals and was found to be 1.6±0.3 mm.

Experimental method

Four chemicals were considered for the toxicology analysis: ammonium, nitrite, nitrate and phosphate. The effect each chemical had was tested using a range of concentration levels in align with real levels expected to be encountered in urban wastewater treatment plants (Metcalf&Eddy et al., 2002). Nitrogen dosages in the form of nitrate ($N\text{-}NO_3^-$) with concentrations of 0, 5, 10 25, 50, 75 and 100 ppm $N\text{-}NO_3^-$ were obtained from $NaNO_3$. Nitrogen dosages in the form of nitrite ($N\text{-}NO_2^-$) with concentrations of 0, 1, 2, 5, 10 and 20 ppm $N\text{-}NO_2^-$ were obtained from $NaNO_2$. Nitrogen dosages in the form of ammonium($N\text{-}NH_4^+$) with concentrations of o, 5, 20, 30, 35, 40, 50, 80 and 100 ppm $N\text{-}NH_4^+$ were obtained from NH_4Cl and the dosage of phosphorous in the form of phosphate ($P\text{-}PO_4^{3-}$) with concentrations of 0, 1, 5, 15, 20, 30, 40 and 50 ppm $P\text{-}PO_4^{3-}$ were obtained from NaH_2PO_4. The concentrations were obtained with a maximum uncertainty of 10% when considering the error made in the mass of each compound and the volume of water measured.

Therefore, the desired concentration of each specific chemical was introduced into a beaker which had been filled with 950 mL of mineral water (chemical composition: total dissolved solids=206 mg/L, bicarbonate (HCO_3^-)=165 mg/L, sulfates (SO_4^{2-})=3.7 mg/L, chloride (Cl^-)=18.8 mg/l, calcium (Ca^{2+})=78 mg/L, magnesium (Mg^{2+})=16.5 mg/L, sodium (Na^+)=8.3 mg/L and silica (SiO_2)=27.1 mg/L) and 50 mL of spirulina suspension. The spirulina suspension had been prepared by diluting 1 g of spirulina powder in 1 L of mineral water, which was then mixed for 30 s at 120 rpm and left for 1 h so that large spirulina particles would settle. The supernatant was used as the spirulina suspension for the experiments. After introducing the spirulina suspension into the beaker, 50 *Daphnia* individuals were collected from the laboratory culture and gently introduced into the experiments, thus obtaining a final *Daphnia* concentration of 50 ind L^{-1}. Three replicates for each chemical concentration were carried out. All the experiments were carried out employing the same laboratory light conditions and temperature (20 °C) as the initial laboratory *Daphnia* culture were used to, in order to avoid any external effects on their behaviour. Control experiments without *Daphnia* and control experiments without chemicals were also carried out to account for the removal of spirulina due to sedimentation.

Daphnia magna filtration capacity

The spirulina particle size distribution in each beaker was measured with the Lisst-100x particle size analyser (Sequoia Inc.). The Lisst-100x consists of a laser beam and an array of detector rings of progressive diameters which allow the light received at the scattering angles of the beam to be analysed. The device measures particle volume concentrations for 32 size-classes, (logarithmically distributed in the size range of 2.5-500 μm), using a procedure based on the diffraction theory of light. The Lisst-100x has been found to perform well when determining particle size distribution and concentration for both organic (Serra et al., 2001) and inorganic particles (Serra et al., 2002b, 2002a) in water suspension. Since *Daphnia* feed on particles less than 30 μm in diameter, the volume concentration of particles within the range of 2.5 to 30 μm was calculated and used as a proxy to evaluate particle removal. Cladocera are known to ingest organic particles when their size overlaps the sizes of the organic particles they feed on (Arruda et al., 1983; Gliwicz, 1990).

Since the decrease in particle concentration is expected to be exponential (Pau et al., 2013; Serra and Colomer, 2016), the characteristic time t at which c/c_0 decreased in $e^{-1}=0.37$ was considered as the characteristic time for all the experiments. This time was approximately 4 h of treatment. Therefore, c/c_0 was calculated in all the experiments. From this ratio, the filtration rate could be determined afterwards. The tests lasted for one week. *Daphnia* filtration measurements were carried out on four of the seven days (days 1, 2, 4 and 7) that the experiment lasted to estimate the evolution of *Daphnia* filtration under each chemical dosage. On each measurement day, the water was renewed with the same initial chemical dosage and spirulina concentration and filtration rates were determined. For this purpose, a new set of beakers with the same chemical dosages and the same initial spirulina concentrations were prepared. Daphnia individuals were collected with a mesh from the old beaker to the new one.

Daphnia mortality was also determined by counting the number of dead *Daphnia* on days 2, 4 and 7 for each chemical dosage. The results obtained for *Daphnia* mortality and *Daphnia* filtration rates are the mean of the results obtained from the three replicates carried out for each chemical dosage.

Daphnia magna trails and Daphnia magna speed

The velocity of *Daphnia* individuals was analysed by videotaping their movements. The camera recorded 25 frames per second and, for each case, the *Daphnia* trails were recorded for 1 minute, thus resulting in a total of 1,500 frames. These frames were analysed with ImageJ software using the mTrack plug-in following Maison et al. (2012)

and Pan et al. (2017). Ten *Daphnia* individuals were considered in each case and a mean value for the velocities was obtained with the software. For the analysis, only some of the chemical dosages were considered. The trails were recorded on days 2, 4 and 7.

Calculating Daphnia filtration without chemicals

The temporal evolution of the suspended particle concentration can be described through a first order equation with time t and a decay constant k from which the Daphnia magna filtration (F) can be determined (see supplementary material for a complete derivation of the equations).

The *Daphnia* filtration inactivation (INACT) at a certain chemical concentration c_x was estimated as,

$$INACT = log\left(\frac{F(0)}{F(c_x)}\right) \qquad (1)$$

Where F(0) is the Daphnia filtration without chemicals and $F(c_x)$ is the Daphnia filtration with the presence of a concentration c_x the chemical x studied ($x=NH_4^+$, NO_2^-, NO_3^- or PO_4^{3-}). Therefore, cases with lower *Daphnia* filtration rates under a certain concentration of a chemical compared to filtrations without chemical ($F(c_x)<F(0)$), would result in inactivation values (INACT) greater than 1. In such conditions, the presence of a chemical incapacitates *Daphnia* filtration. In contrast, cases with INACT=0 mean that the presence of the chemical does not produce any effect on *Daphnia* filtration and therefore $F(c_x)=F(0)$.

Results

The ratio between the suspended spirulina concentration measured at t=4h and the spirulina concentration obtained at t=0h was plotted versus the chemical dosage for each chemical concentration tested and for the measurement days i.e., days 1, 2, 4 and 7 (Figure 1). The ratio c/c_0 measured during the first four hours of exposure to the chemical on day 1 remains nearly constant with the chemical dosage for all the chemicals tested. However, c/c_0 increases with the chemical dosage for both NH_4^+ (Figure 1a) and NO_2^- (Figure 2b) on day 2 of exposure and also increases with the time of exposure from days 2 to 7 at each chemical dosage. At C_{NH4+}=40 mgN-NH_4^+ L^{-1} and after 7 days of exposure, c/c_0 reaches the c/c_0 obtained for the control experiment without *Daphnia* (Figure 1a). For the NO_2^- tests, at c_{NO2-}=10 mgN-NO_2^- L^{-1} and on day 7, c/c_0 attains the c/c_0 for the control experiment without *Daphnia* (Figure 1b). The ratio c/c_0 does not present any variation at all, i.e., neither with the chemical dosage nor with time of exposure to the chemicals NO_3^- and PO_4^{3-} (Figures 1c and 1d, respectively), and remains at the same ratio as the control experiment without chemicals.

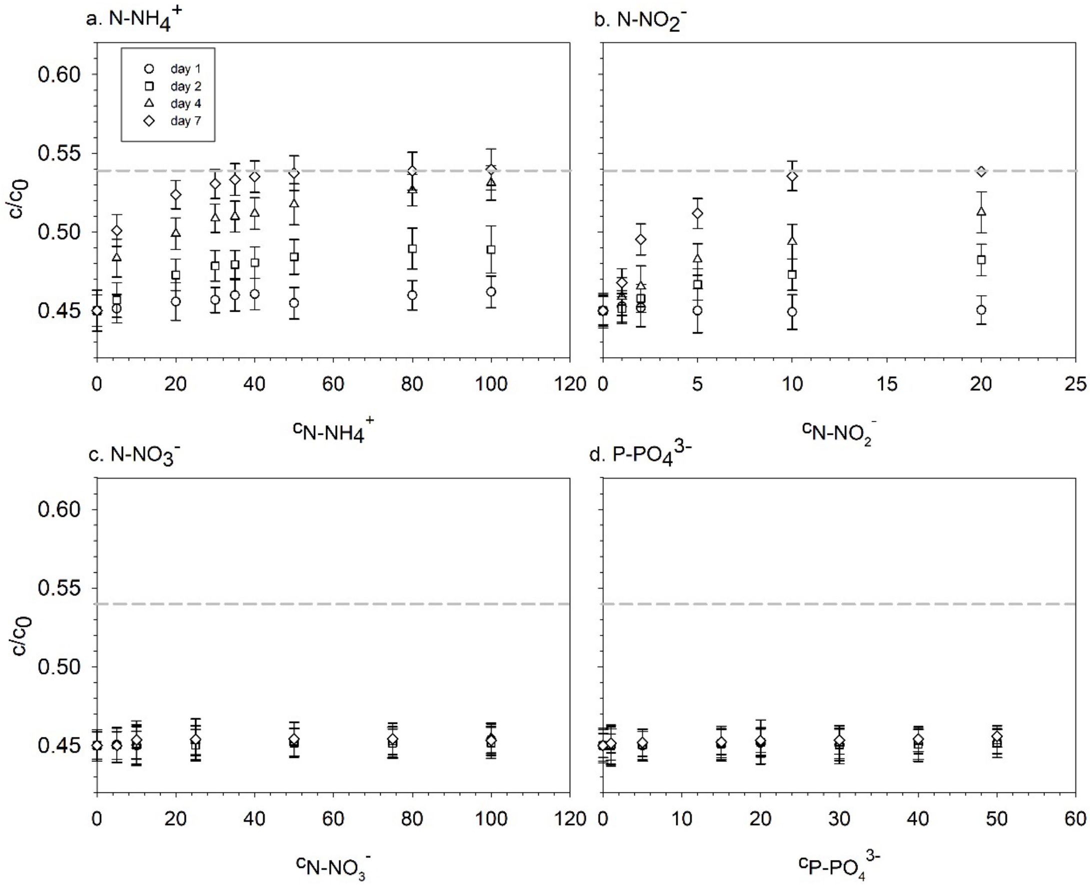

Figure 1. Ratio c/c_0 versus the chemical dosage (c in ppm) and with exposure time for the experiments with $N\text{-}NH_4^+$ (a), $N\text{-}NO_2^-$ (b), $N\text{-}NO_3^-$ (c) and $P\text{-}PO_4^{3-}$ (d). The horizontal dashed line represents the ratio c/c_0 for the control experiments without Daphnia.

The ratio between c/c_0 for the experiments with ammonium to that of c/c_0 in the absence of ammonium has been calculated and it is expected to follow a power function of the chemical concentration ($c_{N\text{-}NH4+}$, Figure 1) as,

$$\frac{\left(\frac{c}{c_0}\right)_{with\ chemical}}{\left(\frac{c}{c_0}\right)_{without\ chemical}} = \frac{e^{-k_{Chem}t}}{e^{-kt}} = e^{k't} = ac_{N-NH4+}^{b} \qquad (13),$$

where a=1 is a constantand the exponent b will vary with the exposure time T_{exp} through a power function of the time as

$$b = eT_{exp}^{d} \qquad (14).$$

From equations (13) and (14), k' can be solved as a function of both the chemical concentration $c_{N\text{-}NH4}$ and the exposure time T_{exp}, resulting in:

$$k' = \frac{eT_{exp}^{d}}{t}\ln(c_{N-NH4+}) \qquad (15),$$

where e and d are constants that will depend on the chemical. For the case of $N\text{-}NH_4^+$, e=0.003 and d=0.540. Therefore, it is possible to write

$$T_{exp} = \left(\frac{k\prime t}{e\ln c_{N-NH4+}}\right)^{1/d} \qquad (16)$$

Considering a case where the ratio $(c/c_0)_{\text{with chemical}}/(c/c_0)_{\text{without chemical}}$ =1.20, i.e., the $(c/c_0)_{\text{with chemicals}}$ is 20% greater than $(c/c_0)_{\text{without chemicals}}$, k'=ln(1.20)/t (from equation 13), where t=4h. This 20% increase in c/c_0 corresponds to c/c_0=0.54 of spirulina, which is equal to that obtained only by sedimentation, i.e., no spirulina particles were ingested by *Daphnia*. In such case, the values of c_x^{max}, T_{exp}^{max} that inhibit completely the Daphnia filtration will follow:

$$T_{exp}^{max} = \left(\frac{\ln 1.20}{e\ln c_{N-NH4+}}\right) \qquad (12).$$

Therefore, the conditions ($c_{N\text{-}NH4+}^{max}$, T_{exp}^{max}) that fall above the curved line in Figure 2 will inhibit Daphnia filtration. The same calculation was carried out for the experiments with nitrite, where e=0.008 and d=0.385. For the case of ammonium and nitrite T_{exp}^{max} versus c_x^{max} are presented in Figure 2. For c_x^{max} below 14 ppm of contaminant, the maximum exposure time for ammonium falls below that of nitrite, indicating that ammonium has a greater effect on *Daphnia* ingestion. However, for concentrations above 14 ppm, nitrite has a greater effect than ammonium on *Daphnia* ingestion. These results might indicate which of these contaminants limit the *Daphnia* filtration. However, more experiments should be done to exactly assess whether a combination of these chemicals would enhance the inhibition effect of Daphnia filtration compared with the experiments here presented.

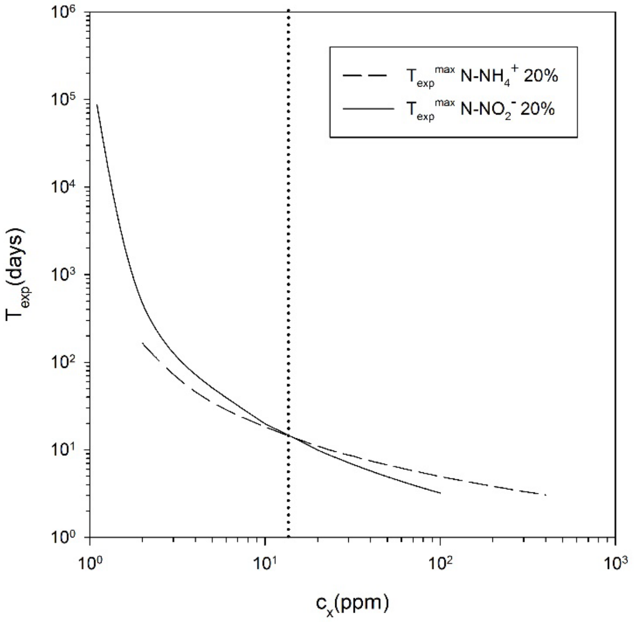

Figure 2. T_{exp}^{max} (in days) versus c_x^{max} (in ppm) for both ammonium (N-NH_4^+) and nitrite (N-NO_2^-).

The inactivation (INACT) for each concentration and with time was calculated from equation 12 and plotted in Figure 4. INACT increased with the chemical dosage and with the exposure time for both N-NH_4^+ and N-NO_2^- (Figures 3a and 3b). For these cases, INACT was above 0 during the first day of exposure and was above 1 in the second day. For the chemicals N-NO_3^- and P-PO_4^{3-}, the inactivation was 0, in accordance with the fact that c/c_0 remained unaltered with the presence of these chemicals and with the time of exposure (Figure 1c and 1d, respectively).

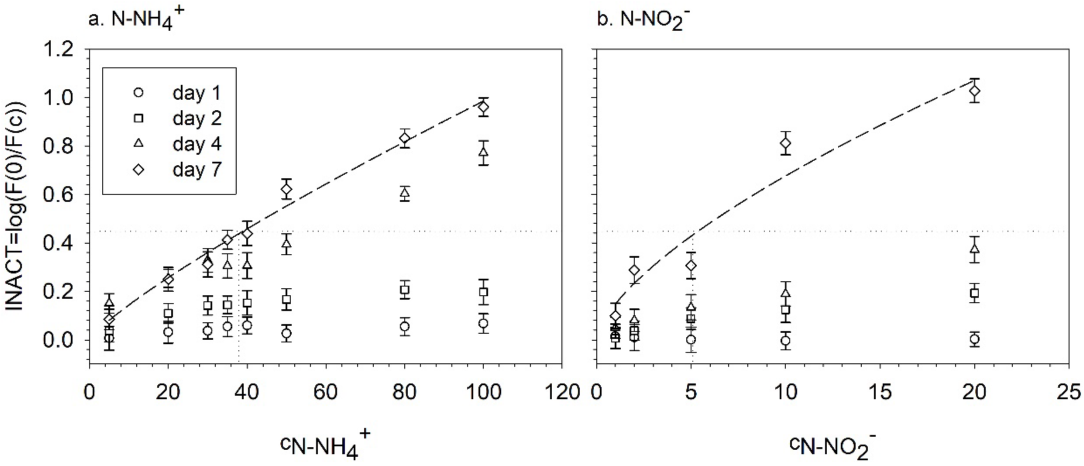

Figure 3. Inactivation INACT calculated from equation 1 versus the chemical dosage c (in ppm) and with exposure time (in days) for N-NH_4^+ (a) and N-NO_2^- (b). The dashed line corresponds to the evolution on INACT with c after 7 days of exposure to N-NH_4^+ and N-NO_2^-. The dotted horizontal lines show the position of a 50% of inactivation ($INACT_{50}$) after 7 days of exposure to N-NH_4^+ and N-NO_2^- and the vertical dotted lines the concentration of N-NH_4^+ and N-NO_2^- at $INACT_{50}$.

The ratio between *Daphnia* swimming velocity for each chemical dosage c_x (for x=NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}) and the swimming velocity for the control experiment without the presence of chemicals $v_{DPh}(c_x)/v_{Dph}(0)$ was calculated (see Figure 4 for each chemical). $v_{DPh}(c_x)/v_{Dph}(0)$ decreased with the dosage and the time of exposure for both NH_4^+ and NO_2^- (Figures 4a and b, respectively). $v_{DPh}(c_x)/v_{Dph}(0)$ remained constant with the chemical dosage and exposure time for the tests with NO_3^- (Figure 4c). For PO_4^{3-}, the ratio $v_{DPh}(c_x)/v_{Dph}(0)$ showed a slight decrease for $c_{PO4}{}^{3-}$=30 mgN-NO_3^- L^{-1}, whereas for $c_{PO4}{}^{3-}$>30 mgN-NO_3^- L^{-1} the decrease was greater, especially at exposure times after day 4 (Figure 4d).

Daphnia mortality increased for all the NH_4^+ dosages studied and with the exposure time (Figure 5a). For the experiments carried out with NO_2^-, no mortality was observed for the lowest NO_2^- dosage studied i.e., 1 mgN-NO_2^- L^{-1} (Figure 5b). For NO_2^- dosages above 2 mgN-NO_2^- L^{-1}, mortality increased gradually with the NO_2^- dosage and with the exposure time. Greater mortality rates were obtained for the highest dosages tested with NH_4^+ than for those tested with NO_2^- (Figures 5a and 5b, respectively). No mortality was observed for the case of the NO_3^- and PO_4^{3-} chemicals, neither in terms of chemical dosage nor exposure time (data not shown).

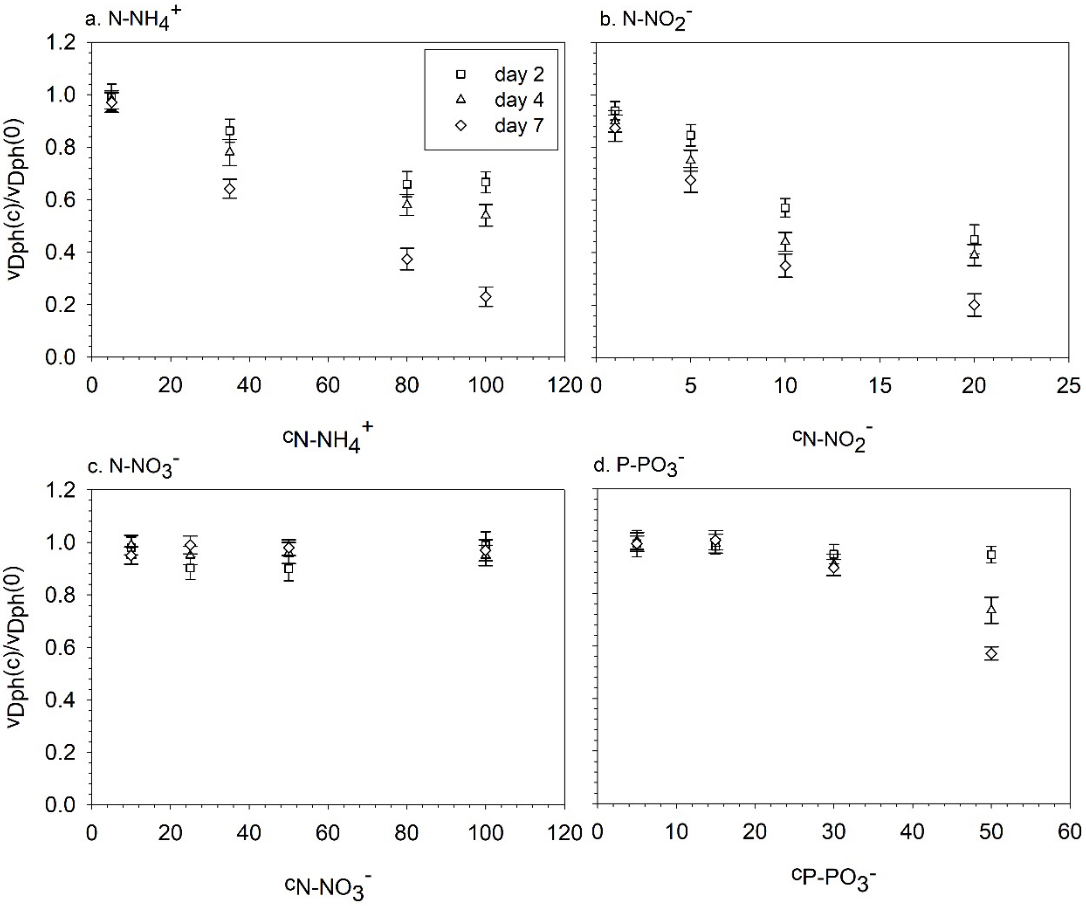

Figure 4. Ratio $v_{DPh}(c)/v_{DPh}(0)$ versus the chemical dosage c and with exposure time for N-NH_4^- (a) and N-NO_2^- (b), N-NO_3^- (c) and P-PO_4^{3-} (d).

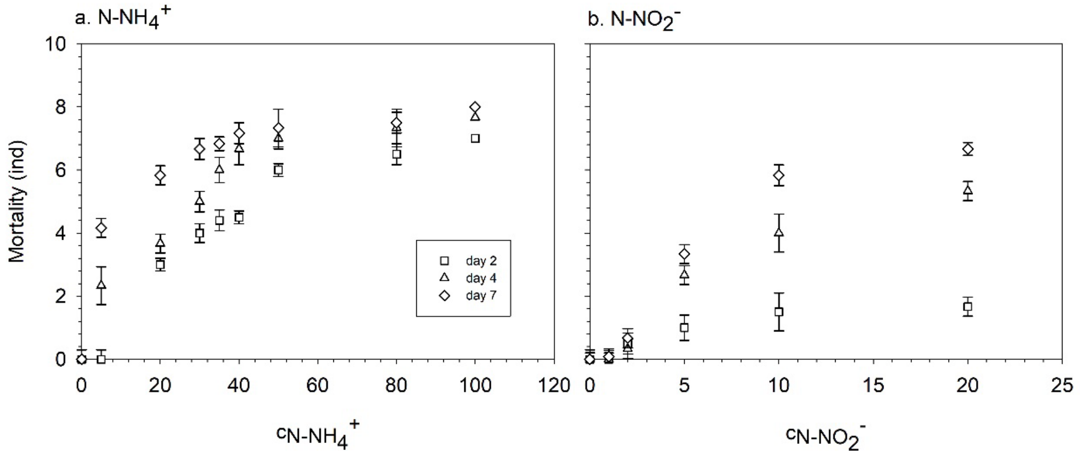

Figure 5. Mortality versus the chemical dosage c (in ppm) for different exposure times (in days) for N-NH_4^- (a) and N-NO_2^- (b).

Discussion

By exposing *Daphnia magna* to the presence of chemicals commonly found in wastewater treatment plants (NH_4^+, NO_2^-, NO_3^- and PO_4^{3-}), their filtering capacity, swimming velocity and their mortality rates have been found to be differentially affected.

The continued exposure of *Daphnia* to ammonium and nitrite increased the inactivation of *Daphnia* filtration. After one day of exposure the inactivation in *Daphnia* filtration was minimal, but this increased with the exposure time to ammonium and nitrite. That is, levels of ammonium above 35 mgN-NH_4^+ L^{-1} inhibited *Daphnia* filtration by 0.4 after 7 days of exposure, i.e., an 80% reduction in *Daphnia* filtration compared to the case without the presence of NH_4^+. Although there are no studies reporting the effect of *Daphnia* inhibition due to the presence of ammonium (NH_4^+), there are some that present *Daphnia* filtering inhibition due to the presence of ammonia (NH_3). The concentration of ammonia at a certain ammonium concentration was calculated following the methodology explained by Anthonisen et al. (1976). In our study, the concentration of 35 mgN-NH_4^+ L^{-1} at pH=7.5 and a temperature of 20 °C represents the presence of ammonia (N-NH_3) with a concentration of 0.3 mgN-NH_3 L^{-1}. In this case, the results for *Daphnia* inhibition are similar to those of Norgaard and Roslev (2016) who found that ammonia concentrations above 0.81 mgN-NH_3 L^{-1} had an inhibitory effect on the *E.coli* removal by *Daphnia*. From Norgard and Roslev (2016) work, such ammonia concentrations produced a 70% inhibitory effect on the *Daphnia* filtering rate, which is close to the 80% found in this present study. Furthermore, in the present study, *Daphnia* mortality for N-NH_4^+ above 35 mgN-NH_4^+ L^{-1} and after 7 days of exposure increased up to 14% and swimming velocities 50% below those for *Daphnia* individuals not exposed to N-NH_4^+. Whether the inhibition of *Daphnia* is caused by ammonium or ammonia ionic form would require further studies at different pHs and a molecular level. In addition, the crimson color of those *Daphnia* individuals under high ammonium and high nitrite concentrations faded with time and reaching a white/transparent color at the end of the experiment. The change in their color can be attributed to the impossibility to synthetize haemoglobin, suggesting the malfunctioning of important physiological functions (Seidl et al., 2005). Nevertheless, from the engineering point of view the concentration of 35 mgN-NH_4^+ L^{-1} at a neutral pH (7.5) would mean a warning signal for *Daphnia* reactor operation. The Daphnia magna inactivation above a 50% ($INACT_{50}$) after 7 days of exposure to NH_4^+ and NO_2^- can be calculated (Figures 4a and 4b). $INACT_{50}$ was reached after 7 days of exposure to concentrations of N-NH_4^+ above 40 ppm and N-NO_2^- above 5 ppm.

The presence of nitrite above 5 mgN-NO_2^- L^{-1} reduced the filtering rate of *Daphnia* after 7 days to c/c_0=0.51, representing a 13% reduction compared with the case without the presence of nitrite. In such conditions, mortality after 7 days of exposure was 6%. This mortality coincides with the 7% found by Xiang et al., (2011) for *Daphnia* after an exposure of N-NO_2^- for 21 days in the same nitrite concentrations. In addition, for *Daphnia* exposed to N-NO_2^- concentrations of 5 mgN-NO_2^- L^{-1} and with exposure times of 7 days, swimming velocities were reduced by 30% compared to those found for *Daphnia* non-exposed to the N-NO_2^- chemical. This percentage is lower than the percentage in Daphnia mortality for the same experiments (with a maximum of a 16% after 7 days of exposure). This result indicates that the presence of the chemical affects Daphnia and that longer exposure times above 7 days might produce greater mortalities.

Daphnia exposed to the range of nitrate concentrations (N-NO_3^-) tested were not found to present greater mortality or lower filtering rates than those for the non-exposed *Daphnia*. In addition, their swimming velocity also remained unaltered. *Daphnia* filtering and mortality rates were also unaltered when exposed to phosphate concentrations in the range from 0-50 mgP-PO_4^{3-} L^{-1} and compared with the non-exposed *Daphnia*. However, their swimming velocity for P-PO_4^{3-} concentrations of 50 mgP-PO_4^{3-} L^{-1} after 7 days of exposure, decreased by 40% compared to that of the non-exposed *Daphnia*. Currier and Elser (2017) exposed *Daphnia* to high PO_4^{3-} and found that after a period of one month the *Daphnia* exposed to high phosphate concentrations had lower growth and feeding rates than those found for *Daphnia* exposed to low PO_4^{3-}concentrations or those not exposed to PO_4^{3-}.

Therefore, in the present study, the swimming velocity seems to be the most sensitive parameter and clearly makes the effect high phosphate concentrations have on *Daphnia* evident. This result is in accordance with Bownik (2017) who stated that *Daphnia* mobility is a sensitive biomarker affected by various substances. Longer exposure times at these PO_4^{3-} concentrations are expected to deviate from those for non-exposed *Daphnia*.

Conclusions

Therefore, the use of a tertiary based technology based on *Daphnia magna* zooremediation might not produce satisfactory results for effluents rich in ammonium or nitrites (>35 mgN-NH_4^+ L^{-1} and >5 mgN-NO_2^- L^{-1}, respectively) when they are exposed to such contaminants for periods longer than 1 day. However, treated wastewater from a secondary wastewater treatment plant might not have such elevated concentrations of ammonium and nitrite and, in this case the performance of a treatment based on *Daphnia* would not be expected to alter. Therefore, the use of *Daphnia* as a tertiary treatment can be suitable provided the levels of the N-NH_4^+ and N-NO_2^- remain below 35 and 5 mg L^{-1}, respectively. In addition, short temporal exposures of one day to these contaminants might be overcome by the Daphnia population. Nitrate was not found to produce changes in *Daphnia* behaviour over the range of nitrate concentrations studied or during the exposure time considered. Phosphate did not produce any change in *Daphnia* mortality or their filtering rates either. However, the longest exposure time studied at the highest phosphate concentrations was found to produce a decrease in *Daphnia* swimming velocities. This indicates that longer exposure times to such conditions might cause changes in *Daphnia* behaviour. Therefore, a natural-based tertiary treatment based on *Daphnia* could be used based on their activity behaviour under the expected chemical composition range.

This study presents an evaluation of the *Daphnia* filtration, swimming and mortality under ammonium, nitrite, nitrate and phosphate separately. The effect of the combination of these chemicals and other compounds typical from wastewater treatment plants remains still unknown and can be the aim of a future study.

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Daphnia magna filtration. Theoretical model (Supplementary Material)

The temporal evolution of the spirulina concentration decreases through a first order equation with time and with a constant k as:

$$\frac{dc}{dt} = -kt \tag{1}$$

that has an exponential as a solution for the temporal evolution of the concentration as follows (Pau et al., 2013):

$$\frac{c}{c_0} = e^{-kt} \tag{2}$$

where k is the rate of particle removal by both sedimentation (k_s) and *Daphnia* filtration (k_{Dph}), i.e., $k=k_s+k_{Dph}$.

From Equation (2) k can be solved following

$$k = -\frac{1}{t} ln\left(\frac{c}{c_0}\right) \tag{3}$$

and k_s can be determined from those experiments without *Daphnia* (in which $k_{Dph}=0$). Therefore, k_{Dph} will be calculated for the rest of the experiments with *Daphnia*. The rate of decrease of c due to *Daphnia* filtration, is a function of the filtering rate of each *Daphnia* individual (F, in mL ind^{-1} L^{-1}) and the *Daphnia* concentration in such a way that (Pau et al., 2013),

$$k_{Dph} = F(c_x) \times C_{Dph} \qquad (4).$$

Calculating Daphnia magna filtration with chemicals

With chemicals, the decay in the suspended particle concentration is expected to be modified with respect to that found for the 'without chemicals', and follows,

$$\frac{dc}{dt} = -k_{Chem}t \tag{5}$$

where k_{Chem} is the total decay constant due to the presence of chemicals,

$$k_{Chem}=k_s+k'_{Dph} \tag{6},$$

and

$$k'_{Dph}=k_{Dph}-k' \tag{7},$$

where k' is the modification to the *Daphnia* filtration decay constant due to the presence of chemicals.

Equation (5) can be solved and results in,

$$\left(\frac{c}{c_0}\right)_{with\ chemical} = e^{-k_{Chem}t} \tag{8}$$

and therefore,

$$k_{Chem} = -\frac{1}{t}\ln\left(\frac{c}{c_0}\right)_{with\ chemical} \tag{9}$$

Using equations (6) and (9), k'_{Dph} can be obtained and then the filtration in the presence of chemicals ($F(c_x)$) can be calculated,

$$k'_{Dph}=F(c_x)\times C_{Dph} \tag{10}.$$

The ratio between equation (8) and equation (2) can be calculated following,

$$\frac{\left(\frac{c}{c_0}\right)_{with\ chemical}}{\left(\frac{c}{c_0}\right)_{without\ chemical}} = \frac{e^{-(k-k')t}}{e^{-kt}} = e^{k't} \tag{11}$$