**Do magnetic phosphorus adsorbents used for lake restoration impact on zooplankton community?**

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**Abstract**

Magnetic microparticles (MPs) have been recently proposed as innovative and promising dissolved inorganic phosphorus (DIP) adsorbents. However, before using them in a whole-lake restoration project, it is essential to assess their toxicological effects (direct and indirect) on aquatic biota. In the present study we hypothesized that zooplankton community is affected by MPs used for lake restoration. To test our hypothesis we designed a microcosms experiment (n=15) containing lake water and surface sediment from a hypertrophic lake. Temporal changes (70 days) on physico-chemical conditions and on zooplankton structure (rotifers, copepods and branchiopods) were monitored under different scenarios. In particular, three different treatments were considered: no addition of MPs (control) and MPs addition (1.4 g MPs L-1) on the surface water layer (T-W) and on the sediment (T-S). After 24 h of contact time, MPs were removed with a magnetic rake. A total of 15 zooplankton species (12 rotifers, 1 branchiopod and 2 copepods) were recorded and a high abundance of zooplankton was registered during the experiment for all treatments. No significant differences (RM-ANOVA test; p>0.05) in total abundance, species richness and species diversity among treatments were found. The absence of any effect of MPs on zooplankton can be explained because MPs did not significantly alter any of its physico-chemical (e.g. temperature, pH, O2) or biological (e.g. food quantity and quality) drivers. These results confirm the suitability of MPs as a promising tool for removing DIP in eutrophic aquatic ecosystems.

**Keywords:** magnetic particles, toxicity, zooplankton, lake restoration

1. **Introduction**

Eutrophication is one of the most striking problems affecting world water resources (Cooke et al., 2005; OECD, 1982; Sas,1989). Although the over enrichment by nutrients occurs naturally over centuries as lake age (Carpenter, 1981), human activities have intensified the rate and extent of eutrophication through both point-sources and non-point discharges. Overall, eutrophication is responsible for not only a drastic impairment of ecosystem structure and function (i.e. blooms of blue-green algae) but also for economic losses causing annual costs just in the U.S. of approximately $2.2 billion (Dodds et al., 2009).

Despite some large-scale efforts to reduce nutrient enrichment, cultural eutrophication continues to be the leading cause of water pollution for many freshwater and coastal marine ecosystems and is a rapidly growing problem in the developing world (Chislock et al., 2013; Smith and Schindler, 2009). Therefore, the ongoing deterioration of water quality together with the increasing demand for freshwater resources requires the implementation of imperative management and restoration measures. The essential and preliminary strategy for combating eutrophication by controlling algal biomass is the reduction of lake water phosphorus (P) concentration, which is the main limiting nutrient for the primary production (e.g. Hupfer and Hilt, 2008; Schindler et al., 2008). Among all strategies, external load control is the first and necessary step for achieving the success of any restoration program of eutrophicated ecosystems (Jeppesen et al., 2009; Smith, 2009). However, and because internal P loading frequently has a considerable impact on lake water P concentrations (Phillips et al., 1994), additional methods are also recommended such as P inactivation techniques. This approach is based on increasing sedimentary P binding capacity by adding iron (Fe), aluminium (Al) or Phoslock® (Boers et al., 1992; Egemose et al., 2011; Spears et al., 2013, 2015). As the effectiveness to remove P of most P-sorbing materials is dependent on the pH, potential redox, and/or presence of other dissolved ions (de Vicente et al., 2008; Lürling et al., 2014; Vohla et al.,2011;Westholm, 2006), more research is required.

In this context, Fe magnetic particles (MPs) have been proposed as a new and innovative restoration tool (de Vicente et al., 2010a, 2011; Merino-Martos et al., 2011). Briefly, first, MPs adsorb DIP from aqueous solutions and later, by applying a magnetic gradient, MPs and therefore, P adsorbed onto MPs, are recovered from the solution. Finally, P can be desorbed from MPs in an alkaline solution allowing both MPs recovery for subsequent adsorption processes and P reuse as a fertilizer. Among the most relevant advantages of using MPs for lake restoration, recorded in previous studies (Álvarez-Manzaneda et al., 2017; de Vicente et al., 2010a; Funes et al., 2016, 2017; Merino-Martos et al., 2011), we underline the next ones: (i) the high P:MPs molar ratio under both batch and flow conditions and oxic and anoxic conditions; (ii) the high P adsorption capacity in a wide pH range (5-9); (iii) the potential long-term decrease of P efflux from lake sediments as a consequence of the reduction of the sedimentary PMobile concentrations (even in anoxic conditions) and (iv) their low toxicological effects on both planktonic and benthic organisms. As a result of both P and MPs can be recovered from the polluted water, the use of MPs as a restoration tool for eutrophic systems could tackle two current and coupled problems: the eutrophication of aquatic ecosystemsand the worldwide depletion of P reserves for making fertilizers (e.g. Cordell et al., 2011; Mekonnen and Hoekstra, 2018).

Despite of the advantages of MPs, before using them in a whole-lake restoration project, it is essential to assess their toxicological effects on lake biota. Accordingly, basic acute and chronic experiments have been carried out in order to determine the risk assessment of MPs. Briefly, laboratory experiments to test the effects of MPs on immobilization and life-cycle of *Daphnia magna* and *Chironomus* sp. (Álvarez-Manzaneda et al., 2017); on the inhibition growth of *Chlorella* sp. and on mortality and cysts hatching of *Brachionus calyciflorus* (Álvarez-Manzaneda and de Vicente, 2017) have been already accomplished. In this context, it is important to consider that single-species toxicity tests achieved under laboratory conditions have several limitations: (i) they are usually focused in sensitive species being not representative of what occurs in a natural community (Cairns and Pratt, 1993; Rohr et al., 2016); (ii)the short duration of laboratory tests makes difficult to record population dynamics occurring at longer times and (iii) their simplicity hinder the detection of effects on structure and function of the ecosystems (Van den Brink et al., 2005). Hence, it is critical to develop outdoor microcosm experiments for evaluating the fate and effects (both direct and indirect) of chemicals at many different levels of organization through appropriate endpoints (Caquet, 2013).

In this context, in the present paper we evaluate, by using outdoor microcosms, the effects of MPs addition on one of the main components of the biological communities of aquatic systems, which is the zooplankton. Despite of the key role of this community in aquatic ecosystems (i.e. acting as a link between phytoplankton and secondary consumers and actively participating in nutrients recycling), there is a complete lack of studies focused on the assessment of the effect of P adsorbents used for lake restoration on zooplankton community by using microcosms. This is a relevant aspect as P adsorbents may direct (i.e. physical effects) or indirectly (i.e. by reducing phytoplankton biomass) affect to the zooplankton and the last effect of P adsorbents must be test by using more complex and realistic approaches than single-species toxicity tests. Therefore, in the present study we hypothesized that zooplankton community is affected by MPs used for lake restoration. To test our hypothesis we designed a microcosms experiment (n=15) containing lake water and surface sediment from a hypertrophic lake. This study is framed in a broader project focused on determining the consequences of MPs application on water quality and sediment P pools (Funes et al., 2017). In this experiment,MPs effects on physico-chemical conditions and on zooplankton compositionand structure were monitored under different scenarios.

1. **Material and methods**
   1. **Study site**

Honda lake is a well studied shallow (surface area=9 ha, Zmean=1.3 m; Zmax=3.2 m) and hypertrophic wetland (Carrillo et al., 1987; Cruz-Pizarro et al., 2003; de Vicente and Cruz-Pizarro, 2003; Funes et al., 2016). It is included in Albufera de Adra Natural Reserve, a Ramsar site that represents one of most important wetland in south Spain (Fig. 1). Its high trophic state is the result of both, the great extension devoted to intensive agricultural practices in its catchment area, which is responsible for the high external P load; and the relevance of P release from the lake sediment (internal P loading; de Vicente et al., 2006; 2010b).

* 1. **Experimental set-up**

Water and sediment samples were collected on July 2015. Lake water was collected using a peristaltic pump and sediment was sampled using an Ekman dredge at the deepest site of the lake. Once in the laboratory, homogenized surface sediment (6600 cm3; OECD, 2006) and lake water was distributed in 15 microcosms (PVC black containers; Ø=38 cm; h=58 cm), which were randomly placed in an outdoor roofed area. In addition, 25 L of lake water with concentrated zooplankton (which was obtained from vertical and horizontal hauls with a plankton net of 30 µm) were homogenized and distributed in the microcosms for getting a final volume of 40 L of lake water. The microcosms were kept oxygenated by using an aeration pump and they were covered with a mesh for avoiding the aerial colonization of flying insects or falling spores.

After a one-week stabilization period the experiment,which lasted for 70 days, started. Three treatments (five replicates per treatment) were considered: (i) no MPs addition (control, C); (ii) Treatment-Water (T-W), where MPs were added on the surface of the water and (iii) Treatment-Sediment (T-S), where MPs were added on the surface of the sediment.MPs were added to T-W and T-S in aqueous dispersion (120 g MPs L-1)by using a peristaltic pump in order to obtain a final concentration of 1.4 g MPs L-1 in each microcosm. As it is explained by Funes et al. (2017), this MPs concentration correspond to a MP:PMobile molar ratio of 85:1, three-fold higher than the ratio selected by de Vicente et al. (2010a) to test the P adsorption effectiveness of MPs in batch experiments. This ratio was increased in order to counteract possible chemical interferences (de Vicente et al., 2011). MPs supplied by BASF (Germany), have a composition of 97.5% Fe, 0.9% C, 0.5% O and 0.9% N and an average diameter of 800 nm (de Vicente et al., 2010a; Merino-Martos et al., 2011). In this study, Fe particles (MPs), have been selected due to their larger magnetization for a given external magnetic field if compared to Fe oxides. As a result of this, the removal of these particles using magnetophoresis is significantly facilitated because the magnetophoretic force depends on both the field gradient and particle magnetization. Moreover, the surface chemistry of MPs is expected to be very similar to that of Fe oxides as MPs become oxidized rather easily in water.

On the first day (day 0), baseline physicochemical and biological (zooplankton) data were obtained previously to the addition of the MPs. After 24 h of contact time (day 1), P loaded MPs were removed by fully immersing an especially designed magnetic rake in the microcosms down to the surface sediment. The contact time (24 h) was selected considering the fast adsorption kinetic of P on MPs reported by previous studies (de Vicente et al., 2010a; Funes et al., 2016). The efficiency of MPs removal by the magnetic rake was 91 and 32% for T-W and for T-S, respectively (Funes et al., 2017).

* 1. **Monitoring of the microcosms**

Temperature (T), pH, dissolved oxygen concentration (O2; mg L-1), conductivity (Cond; mS cm-1) and total dissolved solids (TDS; g L-1) were recorded at different times (days: 0, 2, 21, 35and 70) by using a multi-parameter probe (Hanna Instrument, HI 9829). In addition, total nitrogen (TN; APHA, 1995), total P (TP; APHA, 1995), total dissolved Fe (Tot-Fedis; Gibbs, 1979) and chlorophyll *a* (Chl*a*; Jeffrey and Humpfrey, 1975) concentrations were also measured. More details about chemical analysis can be found in Funes et al. (2017). Sampled and evaporated water in the containers was replaced, after each sampling, with filtered lake water.

Zooplankton community was studied by collecting integrated composite samples (3 L) with a tubular sampler (Ø=5.4 cm; h=25 cm) at the beginning, mid-time and end of the experiment (days: 0, 2, 21, 35 and 70). The sampled water volume was filtered through 30 µm-mesh size plankton net and preserved with 70% ethanol, until taxonomic identification. Organisms were counted and identified to species level (except for nauplii and copepodites which were merged in one category, hereafter N+C), according to Dussart (1969) and **Bledzki** and **Rybak (2016)** for copepods; Ruttner-Kolisko (1974) and Voight and Koste (1978) for rotifers and Alonso (1996) for branchiopods. At each sampling date, a minimum of 50 individuals of each species was counted and identified. Tests using the methodology proposed by Cain and Castro (1959) have shown that increasing the number of specimens did not imply an increase in the number of species.

Abundance (ind L-1), taxonomic groups (% abundance) and several ecological indexes were calculated for each sampling time. These indexes were focussed on evaluating: (i) diversity [Margalef index (Smg– Margalef, 1958); Shannon-Wiener index (H' (log2) – Shannon, 1948) and Pielou evenness index (J – Pielou, 1967)]; (ii) dominance [Simpson index (D – Simpson, 1949)] and (iii) similarity [Jaccard index (Jc – Jaccard, 1908)].

In addition, trophic state indexes (TSI) were calculated for TP and Chl*a* concentrations (Carlson, 1977), for TN concentration (Kratzer and Brezonik, 1981), for rotifer abundance (Ejsmont-Karabin, 2012), for total crustaceans abundance (TSICR1= 6.89 Ln(N, ind L-1) + 20.7 and cyclopoida biomass (TSICR2= 3.48 Ln(B, mg w.wt. L-1) + 60.2 (Ejsmont-Karabin and Karabin, 2013).

Finally, to verify that MPs effects on the zooplankton community are not derived from an indirect action through the phytoplankton community, food availability was calculated. The method of Huntley and Boyd (1984) was used to determine if food concentration was limited during the experiment. For these calculations, critical food concentration (Cc) was obtained from mean temperature data of water and mean adult body size of individuals. Species biomass was estimated from abundance data according to the formula proposed by Dumont et al. (1975) for branchiopods; Bottrell et al. (1976) for copepods and McCauley (1984) for rotifers. Food concentration (C) was calculated from Chl*a* concentration data, assuming a carbon Chl*a*-1 ratio of 70 (Guerrero and Rodríguez, 1997; Mullin and Brooks, 1970).

* 1. **Data analysis**

Statistical analysis was done by using Statistica 7.1 software (Stat Soft Inc.,1997). Differences in physico-chemical and biological variables as well asin the estimated indexes among treatments were tested by using one-way ANOVA, repeated measures ANOVA (RM-ANOVA) and Friedman ANOVA. In particular, one-way ANOVA test was performed to identify if there exist significant differences in the total abundance of each single species among treatments for any particular sampling date. In addition, RM ANOVA was used to test if there exist significant differences over time among treatments. Residuals normality (Shapiro-Wilk test), sphericity assumption (Mauchly’stest) and homogeneity of variances (Levene test) were checked before performing the analysis and Fisher´s Least Significance Difference (LSD) was used as post hoc test. Finally, Friedman ANOVA was used for testing significant differences in the species abundances over time for those species that only appeared in some sampling dates, using Wilcoxon Sign test as post hoc test.

At last, differences in species composition (in terms of total abundance of each species) among treatments were tested by using Principal Response Curves analysis (PRC; Van den Brink and Ter Braak, 1999) with CANOCO software. PRC is especially designed for micro and mesocosms experiments for evaluating changes generated over time in the structure of a community, being a very useful tool for ecotoxicological experiments (Moser et al., 2007; Pardal et al., 2004; Van den Brink et al., 2000) .

**3. Results**

**3.1. Effects of magnetic particles addition on environmental variables**

Table 1 shows the mean values of all environmental variables monitored during the experiment and Table 2 exhibits the associated RM ANOVA results. No significant differences among treatments were found (RM ANOVA; p>0.05) except for TP and TN concentrations. In this sense, significantly higher TP concentrations were measured in control than in T-W and T-S. Regarding to changes over time, significant differences were found for all parameters. A gradual reduction in T (from summer to autumn) was observed in all microcosms together with a reduction in Chl*a*, TP and TN concentrations. By contrast, pH, O2, Cond and TDS values depicted an increase. Tot-Fedis concentrations (data not shown) were on all occasions (treatments and time) below the limit of detection.

**3.2.Effects of magnetic particles addition on zooplankton community composition and structure**

A total of 15 zooplankton species (12 rotifers, 1 branchiopod and 2 copepods) were recorded (Table 3). A high abundance of zooplankton was registered during the experiment for all treatments. In general, rotifers were the most abundant group, being *Brachionus* the predominant genus and *Brachionusangularis* the most abundant species. This predominance is even more evident at the longer sampling times (days 35 and 70; Fig. 2). Copepods were the second majority group in abundance*,* being the developmental stages (N+C) the most abundant and *Acanthocyclops*sp*.* the predominant species. At last, branchiopods had the lowest total abundance in all treatments.

The results for total zooplankton abundances (Table 2) evidenced no significant differences neither among treatments (p=0.17) nor the interaction time x treatment (p=0.53), but significant differences were found over time (p<0.005) for zooplankton abundance. Even more, for all species no significant differences in their total abundances were found among treatments. When analyzing changes over time, most of species (*B. angularis*, *Brachionusplicatilis*, *Keratellaquadrata*, *Alonarectangula*,*Trichocerca*sp.,*Cephalodella*sp.*, Colurella*sp*., Lecane*sp.,*Hexarthraoxyuris*and N+C) evidenced significant changes but no interaction between treatment x time were found except for N+C.

Table 1 also shows mean values of the different ecological indexes. Species richness reflected by the Margalef index (Smg) was quite low, ranging from 0.26 to 0.66 for all treatments and times, which is likely to be a consequence of both the low species number and the high abundance values. The Shannon-Wiener diversity index (H´) ranged from 1.36 to 1.83 in C; from 1.40 to 1.97 in T-W and from 1.32 to 1.94 in T-S. Accordingly, intermediate values of evenness and dominance were found.Pielou evennessindex (J) was always lower than 0.8 while values of the Simpson dominance index (D) were lowerthan 0.6 in any treatment and time. For comparing community structure among treatments, Jaccard index (Jc) was estimated denoting that all treatments shared similar species at all sampling times (values close to 1). RM ANOVA evidenced that no significant differences in any of the ecological indexes was found among treatments (Table 2) reflecting that MPs addition did not cause any significant effect in the structure of zooplankton community. However, significant changes over time and treatment x time interaction were found just for Smg.

The results for the TSI drastically differ when considering different chemical and biological variables (Fig. 3). TSITP showed the highest value, with a mean value ranging from 88.7 for T-S to 94.7 for C, while TSIChl*a* and TSICR2 exhibited the lowest values (mean value 42.7 and 44.6 for 42.0 for TSIChl*a* and TSICR2, respectively). In general, all indexes evidenced a hypereutrohic state of the study lake except for TSIChl*a* and TSICR1 which denoted a meso-eutrophic condition. Again, no significant differences among treatments were found except for TSITP and TSITN (Table 2). Changes over time were significant for TSITP, TSITN, TSIChl*a* and TSIROT while treatment x time interaction was only significant for TSITP.

Next and considering that one of the most essential variables for zooplankton community growth is food availability, we explore if there was a lack of food resources for any species. The results evidenced that there were not food limitation (C>Cc) in any treatment at any sampling date.

Finally, PRC revealed no significant effects of treatment or its interaction with time according to Monte Carlo permutation test (Fig. 4; F=3.0, p=0.43). Most species (33%) have shown weights between -0.5 and +0.5 evidencing either a weak response or a response that is unrelated to that shown in Fig. 5. *A. rectangula* has the highest high positive weight and is thus inferred to decrease most strongly in abundance relative to the controls. In contrast, *B. Angularis* has a negative weight, indicating an increase in abundance relative to the controls.

1. **Discussion**

Although there exist a growing tendency for using microcosms experiments when testing chemical consequences of novel P adsorbents used for lake restoration (Funes et al., 2017; Lin et al., 2015; Wang et al., 2017;Yamada-Ferraz et al., 2015), up to date, there are scarce toxicological studies focused on using this methodological approach (Waajen et al., 2017). In this context, our results based on using microcosms have evidenced that MPs addition did not promote significant direct or indirect effect neither in composition nor structure of zooplankton community. The absence of any effect of MPs on zooplankton organisms can be explained because MPs did not significantly alter any of its physico-chemical(e.g. temperature, pH, O2) or biological (e.g. food quantity and quality) drivers.

Although zooplankton communities are known to be highly susceptible to a wide range of environmental factors, during the experiment temperature, pH and O2 concentrations were in their tolerance range. As a way of illustration, it has been found that for *B. calyciflorus*, one of the most abundant species in the study site, the mean number of offspring per female shows an optimum at 20 °C (Halbach, 1970), which is just the average temperature recorded along the study period. Additionally, pH played a secondary role in our study due to both the consistent pH values (pH ranges just from 8.7 to 9.3) and the absence of any significant pH change after MPs addition. In fact, pH values were very close to 9 in all experimental units and according to Mitchell (1992) the highest net reproductive rate of the *B. calyciflorus* occurs at pH 8.5 and 9.5.

Apart from abiotic factors, zooplankton drastically depends on food quantity and quality (DeMott, 1986; Gulati and DeMott, 1997; Persson et al., 2008). At this point is important to consider that although MPs addition caused a notably reduction (65%) in DIP concentrations in both T-W and T-S treatments (Funes et al., 2017), no significant effect on Chl*a* concentrations were found. The most likely explanation is that although DIP concentrations were much lower after MPs addition, they remained very high (TP >0.2 mg L−1) and previous studies found that significant and sustained changes in the biological community and the water transparency of shallow, temperate freshwater lakes may not appear unless TP concentrations are reduced below 0.05–0.1 mg L−1 (Jeppesen et al., 2000). Hence, as Funes et al. (2017) suggested, achievement of a significant reduction in Chl*a* concentrations may involve higher MP doses, either in the first application or by repeated additions, and requires long-term follow-up of changes in water quality. Accordingly, and as a result of the high food availability, zooplankton abundance did not change after MPs addition. In fact, available C (estimated from Chl*a* concentrations) for zooplankton was always, for all treatments, much higher than Cc evidencing the lack of any food limitation for zooplankton.

In relation to the food quality, it has been found that MPs did not change phytoplankton composition, being cyanobacteria (95.2%), the most dominant group in all treatments before and after adding MPs (del Arco et al., pers.com). Despite cyanobacteria are known for being inadequate as a food source for zooplankton, whether by their toxicity, large size, lack of essential compounds or due to feeding inhibitors (Lürling, 2003; Martin-Creuzburg and Von Elert, 2009); in eutrophic systems, higher abundance of rotifers is often observed with higher abundance of cyanobacteria (Bouvy et al., 2001; Leonard and Paerl, 2005; Starkweather, 1981) which indicates that not all cyanobacteria inhibit rotifer growth. However, contrasting results are reported in the literature. More recently, Soares et al. (2010) found, in 2-day life-cycle assays, that *B. calicyflorus* was capable of ingesting *Microcystis aeruginosa* although diets consisting only of *Microcystis* caused death of the animals. However, Starkweather and Kellar (1987) reported a similar median survivorship for *Brachionus* fed *Microcystis* and unfed animals, while in another study, *B. calyciflorus* showed the ability to utilize *M. aeruginosa* as food or to possess tolerance to the toxins (Fulton and Paerl, 1988). These results are specially striking if we consider that *M. aeruginosa* represents one of the most dominant phytoplankton species (1.94% of the total phytoplankton abundance; del Arco et al. pers.com.) in the study lake. In any case, it is important to take in account that natural waters are complex mixtures of different phytoplankton species and accordingly, rotifers will encounter a variety of food items of different quality. In this sense, a significant increase in population growth rate of *B. calicyflorus* was found when it was fed with a combination of *M. aeruginosa* and *Scenedesmus* obliquus compared to cyanobacteria based diet (Soares et al., 2010).

When analyzing the potential toxic effects of any P adsorbent used for lake restoration, both direct and indirect effects on lake biota may be taken into account. Here it is important to take in account that there exist a continuum of experimental approaches and tools which ranges from single-species toxicity tests to the intentional contamination of natural ecosystems through experimental laboratory food chains and indoor microcosms (Caquet,2013). In this continuum of experimental approaches, outdoor microcosms are characterized by a high complexity-ecological realism (including both direct and indirect effects) and by the possibility of replicability. In our study, no significant direct effects of MPs addition on zooplankton abundance and composition have been found. Contrarily to our results, del Arco et al. (2018) observed that, during MPs removal, *Daphnia magna* abundances drastically reduced regardless the concentration of MPs, competition or habitat structure. Discrepancies between our results and those obtained by del Arco et al. (2018) can be explained because of differences in both methodology and zooplankton species composition. Briefly, our study is based on microcosms (40 L) filled with a natural aquatic ecosystem while 1 L glass jars filled with 0.8 L of mineral water were used by del Arco et al. (2018) and accordingly, a much complex habitat is obtained in the present study. Even more, del Arco et al. (2018) used *D. magna* for evaluating MPs effects while the only branchiopod present in our study was *A. rectangula*. In this sense, previous studies have reported contrasting results when comparing the susceptibility to toxicants of *D. magna* and *Alona* sp. In particular, Bossuyt and Janssen (2005) found a major sensibility of *Alona* sp. to copper (immobilization test), while Sarma et al. (2007) did not observe consistent differences in the LC50 to methyl parathion (a pesticide) and mercury (HgCl2), between *Alona* sp. and *Daphnia*sp.

Indirect effects on zooplankton community were also negligible and only significant changes in *A.rectangula* were found at the end of the experiment. It is important to state that Álvarez-Manzaneda et al. (2017) found in chronic toxicity tests that Tot-Fedis (even at very low concentrations) had a negative effect on reproductive output in *D. magna* as it significantly reduced the number of female offspring but no effect on the number of male offspring was observed. Accordingly, it is likely that the late disappearance in *A. rectangula* population dynamics may be caused by an affection of its reproduction as similar MPs concentrations were used in both experiments. However, the absence of any general long-term effectof MPs addition on the others zooplankton groups may be explained by the high percentage MP recovery (Funes et al., 2017) and by the extremely low Tot-Fedis concentrations.

More in detail, rotifers are the dominant group in the zooplankton community in Honda lake as it was expected due to the extremely high trophic state (Gannon and Stemberger, 1978). The predominance of rotifers in the study site has been previously reported (Carrillo et al.,1987; Cruz-Pizarro et al., 2003; Martínez-Vidal and Castro, 1990). Among rotifers, *B. calyciflorus* was one of the most dominant species in the study lake. In relation to this species, when comparing our results with single-specie toxicological experiments reported in the literature for the same adsorbents (MPs), we found that the LC50 was higher (1.63 g L-1; Álvarez-Manzaneda and de Vicente, 2017) than MPs concentration used in this experiment (1.4 g L-1) and accordingly, our results confirmed the expected minor effect of MP addition on *B. calyciflorus.*

Finally, in the context of any successful restoration project for combating eutrophication, it is essential to keep in mind that the final goal is to reduce the lake trophic state. Changes in the trophic state of the study lake after adding MPs were assessed by estimating different trophic indexes. Although a wide outcome was observed depending of the variable, the general trend was that no significant differences were found among treatments except for the one based on TP concentrations (TSITP). The ultimate effect of MPs on TSITP (promoting a change from hypereutrophy to eutrophy) was observed as TP concentrations were drastically reduced after MPs addition. In view of TSIChl*a*, and TSICR1 lake trophic state was mesotrophic while a much higher state (hypereutrophic) was obtained when considering TP and TN concentration as well as rotifers (TSIROT) and crustacean abundance (TSICR2). The extremely low TSIChl*a* could be explained by the high inorganic turbidity which ultimately may cause a primary production limitation by light. In fact, de Vicente et al. (2010b) measured, by using a combination of field measurements, modelling and laboratory experiments, large resuspension fluxes in the study lake promoted by the lake morphometry and sediment grain size distribution.

All in all, the lack of any significant effects of MPs addition on zooplankton community is especially striking in the context of restoration programs for Mediterranean endorheic ponds where zooplankton is naturally the highest aquatic trophic level. In this sense, it is important to consider that Mediterranean endorheic ponds are the most abundant systems in the Iberian Peninsula (Dantín, 1929; 1940; 1942). More research before applying MPs in a whole-lake restoration project should be focused on the assessment of their effects on (i) other trophic levels by using microcosms (e.g. phytoplankton) and (ii) in artificial aquatic ecosystems (e.g. farm ponds).

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