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Assessment of toxic effects of magnetic particles used for lake restoration on *Chlorella* sp. and on *Brachionus calyciflorus*

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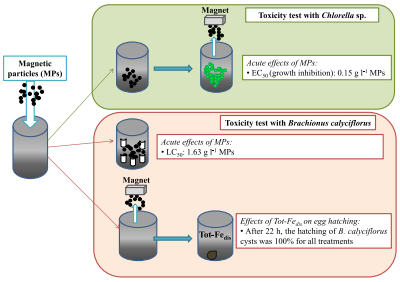
HIGHLIGHTS

- EC_{50} (algal growth) for *Chlorella* sp. was 0.15 g MPs l^{-1}.
- High turbidity and Tot-Fe_{dis} cause negative effects on algal growth.
- For *B. calyciflorus*, LC_{50} was 1.63 g MPs l^{-1} (corresponding to 30.7 mg P l^{-1}).
- Hatching rate of *B. calyciflorus* cysts was 100% for all treatments (Tot-Fe_{dis}).
- MPs in a real whole-lake application may cause minor lethal and sublethal effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Laboratory tests, by following standardized Organization for Economic Co-operation and Development (OECD) protocols, were run for evaluating the acute effects of iron magnetic microparticles (MPs), recently proposed for lake restoration, on *Chlorella* sp. (algal growth) and on the rotifer *B. calyciflorus* (mortality). In addition, the MPs potential indirect effects on rotifer egg bank were assessed by performing hatching rate test with *B. calyciflorus* cysts in contact with dissolved iron (Tot-Fe_{dis}). In the algal growth test, no inhibition occurred at the two lowest MPs concentrations (0.01 and 0.05 g l^{-1}) which would correspond, considering the adsorption efficiency ratio (Phosphorus: MPs), to P concentrations lower than 0.94 mg P l^{-1}, much higher than typical concentrations found in natural waters. For higher MPs dose (EC_{50} for *Chlorella* sp. was 0.15 g l^{-1}), no nutrient limitations but high turbidity and Tot-Fe_{dis} values cause negative effects on algal growth. For the case of *B. calyciflorus*, LC_{50} was 1.63 g MPs l^{-1} (corresponding to 30.7 mg P l^{-1}). When analyzing Tot-Fe_{dis} effect, the hatching rate of *B. calyciflorus* cysts was 100% for all treatments. To sum up our results for *B. calyciflorus* acute and chronic toxicity tests, it is extremely unlikely the mortality of adult organisms in contact with MPs as well as an affectation of the rotifer egg bank. In conclusion, it is expected that MPs addition in a real whole-lake application cause minor lethal and sublethal effects on both *Chlorella* sp. and *B. calyciflorus*.

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1. Introduction

Phosphorus (P) translocation from its land reserves to the aquatic environment is a direct consequence of the impact of human action on the environment which lastly drastically affect to the

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biogeochemical P cycle (Cordell et al., 2011). Hence, we are facing two coupled and worldwide increasing problems: (i) the global food resources depletion link to the reduction of P reserves essential for making fertilizers P (Gilbert, 2009) and (ii) the eutrophication, nutrient enrichment, of aquatic ecosystems (OECD, 1982; Sas, 1989; Cooke et al., 2005).

Eutrophication is the leading cause of water pollution for many freshwater and coastal marine ecosystems and is a rapidly growing problem in the developing world (Harper, 1992; Schindler, 2006; Smith and Schindler, 2009). Because of the main limiting nutrient for the aquatic primary production is P, it is essential to reduce P concentration when restoring eutrophicated systems. It is well accepted that P availability in lake water can be reduced by three ways (Hupfer and Hilt, 2009): (i) Reducing P external load, (ii) Increasing P retention by the sediment and (iii) Increasing P export from the system. Among these techniques, the control of P external load is an essential and preliminary step before implementing any other management strategy (Smith, 2009; Jeppesen et al., 2009). In fact, controlling catchment-derived nutrient loading is a prerequisite for lasting lake restoration efforts, otherwise internal stocks of nutrients will be replenished (Cooke et al., 2005).

As a result of the two above mentioned problems (the depletion of P reserves and the eutrophication of aquatic ecosystems), new and innovative methods are required. In this context, iron (Fe) magnetic microparticles (MPs) have been recently proposed as convenient P adsorbent (De Vicente et al., 2010; De Vicente et al., 2011; Merino-Martos et al., 2011). Briefly, MPs are used to adsorb P from aqueous solutions and after the adsorption is carried out, P loaded MPs can be separated by using a high gradient magnetic separation process. Later, P can be desorbed and potentially used as a fertilizer while the bare MPs can be reused. Next, we summarize the most relevant advantages for using MPs as P adsorbent for lake restoration (De Vicente et al., 2010; Merino-Martos et al., 2011; Funes et al., 2016, 2017; Álvarez-Manzaneda et al., 2017): (i) high P adsorption capacity under both batch and flow conditions; (ii) the insignificant dependence on physico-chemical conditions (redox and pH) of their P adsorption; (iii) the reduction in sedimentary P_{Mobile} concentrations caused by their addition (under both oxic and anoxic conditions), potentially contributing to a long-term reduction in P efflux; (iv) their lesser cost in comparison to other P adsorbents (e.g. $AlCl_3 \cdot 6H_2O$ and Phoslock®); and (v) the low toxic effects on plankton and benthic organisms. Accordingly, the use of MPs would help to counteract both the depletion of P reserves and the eutrophication of aquatic systems by removing P from eutrophicated systems and by using the recovered P as a fertilizer.

However, it is clear that before adding MPs in a whole-lake application strategy it is essential to gain more knowledge about MPs potential toxic effects on lake biota. The procedures currently in use for conventional risk assessment have a first step that consists in the identification and characterization of hazards based, among others, in basic toxicity tests (Amiard-Triquet et al., 2015). Accordingly, acute and chronic effects of MPs on *Daphnia magna* and on *Chironomus* sp. have been already evaluated (Álvarez-Manzaneda et al., 2017). However, and considering that MPs addition makes sense just in eutrophicated systems where the zooplankton community is dominated by rotifers instead of cladocerans (Gannon and Stemberger, 1978), it is essential to test MPs effects on rotifers. Apart from rotifers, algae were also chosen as test organisms in this study due to the following consideration: (a) they belong to the first level of the trophic chain and so, any change in the composition and density of the phytoplankton could change the biological and chemical quality of an ecosystem (Lewis, 1995); (b) they seem to be more sensitive for some contaminants than animal species (Hoffman et al., 2003) and (c) they have a short life cycle, allowing the evaluation of toxic effects over several generations (Silva et al., 2009). In addition to basic toxicity tests, experimental designs mimicking a natural environment (microcosms) are also recommended (Caquet, 2013). Therefore, by using microcosms from a hypertrophic coastal lake, potential changes on species composition and abundance of phytoplankton (del Arco et al., unpublished) and on zooplankton community (Álvarez-Manzaneda et al., unpublished) after MPs addition have been assessed.

Although the majority of standardized ecotoxicity tests and biomonitoring in aquatic systems are based on the active component of invertebrate communities, dormant egg banks are crucial for the long term survival and community dynamics of many aquatic organisms (Navis et al., 2013). In fact, the invertebrate dormant egg banks in the sediments of aquatic ecosystems constitute ecological and evolutionary reservoirs of species (De Stasio, 1989; Hairston and Munns, 1984; Hairston, 1996). Among invertebrate communities, rotifers are important components of such egg banks in freshwater systems. Most planktonic rotifers reproduce via cyclical parthenogenesis (Snell and Janssen, 1995), incorporating both asexual (amictic) and sexual (mimic) reproduction into their life cycle (Preston and Snell, 2001). The application of MPs for lake restoration may involve two kind of interaction with lake biota: i) direct and short-term effect caused by MPs and ii) indirect and long-term effect caused by the dissolved Fe (Tot-Fe_{dis}; after MPs removal). Therefore, and for the case of rotifers, it is essential to assess the potential effect of MPs and Tot-Fe_{dis} on both adult organisms and cysts.

In this context, our working hypothesis is that MPs addition for lake restoration cause lethal and sublethal effects on algae and on rotifers. Accordingly, in this paper we combine both acute, which are mostly based on mortality as endpoint, and sublethal toxicity tests looking at growth and/or reproduction of the biota. In particular, the general aim of this paper was to assess, by laboratory tests and following standardized Organization for Economic Co-operation and Development (OECD) protocols, the acute effects of MPs on *Chlorella* sp. (algal growth) and on the rotifer *Brachionus calyciflorus* (mortality). In addition, hatching test with *B. calyciflorus* cysts were performed for assessing the MPs indirect effects due to the Tot-Fe_{dis} increase. As MPs are efficient P adsorbent and they may therefore affect nutrient availability for phytoplankton, during the *Chlorella* sp. experiments a through monitoring of physico-chemical changes in the aqueous solutions was accomplished.

2. Material and methods

2.1. Test organisms

Laboratory experiments were carried out with two species belonging to two different trophic levels, the freshwater green algae *Chlorella* sp. and the rotifer *B. calyciflorus*.

Chlorella sp. (cell volume: 365 μm^3; diameter: 8.8 μm) was selected as the test species because this unicellular green alga has a good sensibility to toxicants and it is easily cultured at laboratory (Silva et al., 2009).

The stock culture of *Chlorella* sp., provided by the Department of Ecology of the University of Jaén, was cultivated in an 800 ml volume with Bold's Basal Medium (BBM; Bold, 1949). This freshwater algae medium was chosen based on previous studies who found that it is better than natural medium for toxicity tests with *Chlorella* sp. (Polonini et al., 2015). The culture was maintained in an isolated room at a temperature of 22 ± 0.5 °C and a cycle of light: darkness of 16: 8 h. In order to avoid the sedimentation of algae cells, the culture was shaken at 100 rpm and the cell density was estimated by using a Neubauer counting chamber.

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