**Title:** Acute and chronic effects of magnetic microparticles used in lake restoration on *Daphnia magna* and *Chironomus* sp.

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**Abstract**

Magnetic microparticles (MPs) have been recently proposed as a new and promising tool for restoring eutrophicated inland waters. In this study, we analyzed the acute and chronic effects of iron (Fe) MPs on *Daphnia magna* and on the benthic macroinvertebrate *Chironomus* sp. The endpoint in the acute toxicity tests was immobilization. In the chronic toxicity tests the offspring production (male and female) in *D. magna* and the mortality of larvae and pupae, and adult emergence in *Chironomus* sp. experiments were used as the endpoints. The concentration of MPs that caused 50% of immobilized individuals ($EC_{50}$) in the acute toxicity test was much higher in *D. magna* (0.913 g Fe $l^{-1}$) than in *Chironomus* sp. (0.445 g Fe $l^{-1}$), which is likely to be the result of differences in the lifestyle of these organisms, planktonic and benthic respectively. Considering the regular dose of MPs that could be used in a restoration plan, slight effects on organism immobilization are expected. The results of chronic toxicity tests in *D. magna* showed that in presence of dissolved Fe (dFe), parthenogenetic reproduction was significantly affected, while no significant effect on mortality of larvae and pupae and on adult emergence was detected in *Chironomus* sp. test. Taking into account that long-term exposure is not likely to occur under the regular procedure of MPs, we conclude that MPs is a riskless (no toxic effect on planktonic and benthic organisms) and efficient (high P adsorption capacity) tool for lake restoration.

**Keywords:** *Chironomus* sp., *Daphnia magna*, eutrophication, magnetic particles, lake restoration, toxicity

## 1. Introduction

Biogeochemical cycles are being dramatically and worldwide affected by human activities. For the case of phosphorus (P), human intervention has mobilized nearly half a billion tons of this element from phosphate rock into the hydrosphere over the past half century (Cordell et al., 2011). As a result, nowadays, we are facing two problems: the exhaustion of P reserves, essential for making fertilizers, and the P enrichment of inland aquatic ecosystems, which is the responsible for eutrophication. On the one hand, experts disagree on how much P is left and how quickly it will be exhausted but many argue that a shortage is coming and that it will leave the world's future food supply hanging in the balance (Gilbert, 2009). On the other hand, eutrophication is currently considered as a worldwide problem which affects 30% of the inland aquatic ecosystems (OECD, 1982; Sas, 1989; Cooke et al., 2005). As the main limiting nutrient of the primary production in aquatic ecosystems is P, it is essential to consider as a preliminary strategy the reduction in P concentration in the water column. To achieve this goal, three different but complementary approaches have been proposed (Hupfer and Hilt, 2008): (i) a reduction in P external loading, (ii) an increase in P retention by the sediment and (iii) an increase in P export from the system. Controlling the external load is an essential step to manage and restore the eutrophicated systems, in fact, it has been observed that an insufficient reduction in P external loading results in a long-term failure in lake restoration (8-10 yr; Smith, 2009).

Up to date, there is no a management tool as *panacea* for eutrophicated inland waters. Although chemical adsorbents such as Fe, aluminum (Al) and calcium (Ca) salts seem to be the most convenient, it is relevant to consider that, although inactivated, P remains in sediments and may be released to water column under changing physic-chemical and biological conditions such as temperature, pH, redox potential, biological activity or resuspension (Jensen and Andersen, 1992; Søndergaard et al., 1992; Rydin and Welch, 1998; Egemose et al., 2009; Funes et al., 2016). In order to by-pass these difficulties, great attention has recently been paid for developing new and efficient adsorbents that are able to reduce P levels in water bodies. One of the most promising methods is the addition of magnetic microparticles (MPs) for P removal to aquatic ecosystems as we get the P out of the system, so this method conducts to an increase in P export (P is removed from both lake water and lake sediment; Funes et al., 2016). Therefore, MPs are used to adsorb contaminants from aqueous effluents and after the adsorption is carried out, the adsorbent can be separated from the medium by a simple high gradient magnetic separation process (de Vicente et al., 2010). Once P is trapped, it can be later desorbed and recovered and simultaneously, MPs can be reused for adsorbing more P and they still maintain a high P adsorption capacity (de Vicente et al., 2010). All in all, several outstanding advantages of using these particles for lake restoration can be highlighted: (i) the high P: MPs molar ratio under both batch and flow conditions (de Vicente et al., 2010; Merino-Martos et al., 2011); (ii) the fast P adsorption process (in just 2 h under batch conditions; de Vicente et al., 2010); (iii) the ability for adsorbing P even in anoxic conditions (Funes et al., 2016); (iv) the recovery of MPs from the solution, reducing both economic costs and toxic effects on the biota and (v) the potential reusability of the recovered P as a fertilizer.

Despite of the excellent advantages of using MPs, before using them in a "whole-lake application", it is essential to assess their toxicological effects on both planktonic and benthic organisms. Up to date, there only exist studies focused on the toxicity of nano and no magnetic particles (Baun et al., 2008; Navarro et al., 2008; García et al., 2011; Keller et al., 2012; Shinde et al., 2012; Yah et al., 2012; Baumann et al., 2014) but no similar studies have been developed for the case of magnetic Fe MPs. Additionally, it is important to consider that in a whole-lake application, MPs would be removed after 24 h but dissolved iron (dFe) could be mobilized to the water column and stay longer time in contact with aquatic biota, with the subsequent potential toxic effects. In this context, 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 short- and long-term effects of magnetic MPs on both benthic and planktonic organisms. In particular, the specific aims were to evaluate both the acute effects (immobilization) of MPs on *D. magna* and *Chironomus* sp. and the chronic effects of dFe on *D. magna* and *Chironomus* sp.

## 2. Material and methods

### *2.1 Sampling and culturing of test organisms*

Experiments were carried out with *D. magna* and *Chironomus* sp. *D. magna* is a cladoceran which has been widely used in toxicity tests due to it sensibility to contaminants (e.g. Khangarot and Ray, 1987; García et al., 2011) and because of its size, high fecundity, parthenogenetic reproduction, short life-cycle and its relatively facility for culturing (Núñez and Hurtado, 2005). For this study, *D. magna* was isolated from Lake Grande (Jaén, Southern Spain). In the laboratory, a single clone from a parthenogenetic female was obtained. *Daphnia* cultures were maintained with densities ranging from 20 to 30 ind $l^{-1}$ (USEPA, 2002) in 1 l glass beakers containing hard (209 mg $l^{-1}$ of total hardness) commercial mineral water. Daphnids were fed *ad libidum* ($5 \times 10^4$ cells $ml^{-1}$, 0.0027 mg C) three times a week with a pure culture of the chlorophycean algae *Chlorella* sp. *Chlorella* sp. (365 $\mu m^3$, diameter: 8.8 µm), which was originated from a culture collection of the University of Granada, was maintained in an 800 ml volume with Bold's Basal Medium (BBM; Bold, 1949). Photoperiod was set to 16 h light: 8 h dark cycle and temperature at 22 ± 0.5 ºC. To avoid the sedimentation of algae's cells, the culture was shaken at 100 rpm. Algal cell concentration was estimated using Neubauer's counting chamber.

On the other side, benthic macroinvertebrates are a very suitable community to carry out ecotoxicological tests due to their easy collection, relatively slow mobility and their life expectancy, very useful for chronic toxicity tests (Iannacone and Alvariño, 2004). In particular, larvae of *Chironomus* sp. have a great ecological relevance for ecotoxicological researches (Iannacone and Alvariño, 2004). In the present study, up to 100 individuals of *Chironomus* sp. were collected from river Beiro (Granada, Southern Spain) using a kick net with 250 microns of mesh. Once in the laboratory, chironomid larvae were placed in a 50x26x36 cm aquarium, containing silica sand and three aerators to prevent anoxia. Hard (209 mg $l^{-1}$ of total hardness) commercial mineral water was used to fill the aquarium. Chironomids feeding was carried out three times a week by using fish flakes food (USEPA, 1993).

### *2.2.General characterization of magnetic microparticles*

Micronsized iron (Fe) particles were kindly supplied by BASF (Germany) and used without further treatment to make the suspensions. According to the manufacturer, the composition of this powder is 97.5% Fe, 0.9% C, 0.5% O and 0.9% N. Previous studies have characterized in detail their magnetization properties, electrophoretic mobility, particle size distribution and P adsorption properties (de Vicente et al., 2010; Merino-Martos et al., 2011; De Vicente et al., 2011). In brief, MPs used in this work are spherical in shape, relatively polydisperse and with a mean diameter of 805±10 nm. As expected, a ferromagnetic behavior was found for MPs with a negligible remnant magnetization. MPs do present a thin oxide surface layer and hence behave as amphoteric solids with surface charges controlled by the pH in the aqueous medium, with an isoelectric point around pH 6.5. Although MPs experienced a slight decrease in

P removal efficiency with increasing pH, P removal efficiency was larger than 85% at pH 7. Finally, reused MPs have a similar P maximum adsorption capacity (18.83 mg P $g^{-1}$ Fe) than bare MPs (15.80 mg P $g^{-1}$ Fe).

### *2.3.Toxicological tests with* Daphnia magna

Tests were made according to different OECD standardized protocols (2004, 2012) and using as reference 1 g Fe $l^{-1}$ concentration, which is the concentration with high P removal efficiency (de Vicente et al., 2010).

#### *2.3.1. Acute immobilization test with magnetic particles*

To run the immobilization test, 202 OECD Part I standardized protocol was followed (OECD, 2004). We used, < 24-h-old, F2-generation females of our clone of *D. magna*. Thirty five *D. magna* females were isolated and fed with 0.0035 mg C (37 000 cells) of *Chlorella* sp. $ml^{-1}$. Individually, female neonates were randomly distributed in groups of five individuals in 50 ml glass beakers containing the following MPs concentrations: 0.01; 0.05; 0.1; 0.5; 0.7; 1 and 2 g Fe $l^{-1}$ (concentration selection was carried out based on the results of a preliminary test as recommended by OECD protocol). All control and treatments were run in five replicates. All glass beakers were randomly placed in a culture chamber at 23ºC and a 14:10 light: dark cycle. After 24 and 48 h, mortality, immobilization (when animals are not able to swim within 15 seconds, after gentle agitation of the test vessel) and abnormal behaviors were recorded. As it is stated in the standardized OECD protocol, organisms were not fed during the experiment.

#### *2.3.2. Reproduction test with dissolved iron*

Following the 211 OECD (2002) standardized test, a reproduction test was run to assess sub-lethal effects of dFe on *D. magna* after 21 days. To carry out this chronic test, suspensions containing the following MPs concentrations were prepared: 0.01; 0.05; 0.1; 0.5; 1 and 2 g Fe $l^{-1}$. Similarly to a real lake-application (de Vicente et al., 2010;

Merino-Martos et al., 2011; Funes et al., 2016), after 24 h, MPs were removed by using magnetic techniques, and with the remaining solutions (containing dFe) the reproduction test was run. The reproduction test consisted of placing individually, < 24-h-old, F3-generation females of our clone of *D. magna* into 100 ml glass tubes containing 50 ml of the above mentioned solutions enriched in dFe. Daphnids were fed with 0.1 mg C *Daphnia*$^{-1}$ of algal concentration (1.8 x $10^6$ cells ml$^{-1}$) in an isolated room at 22 ± 0.5 ºC and a light: darkness cycle of 16: 8 h. Each treatment was run in ten replicates, and the medium was renovated three times a week. Every day, the number of female and male offspring and the survivorship of *D. magna* individuals were recorded. Every day, the offspring were removed. The survivorship of *D. magna* was always 100% in control and treatments.

### *2.4. Toxicological tests with* **Chironomus** *sp.*

#### *2.4.1. Immobilization test with magnetic particles*

Immobilization test was performed according to the 235 OECD standard method (2011) The experimental design consisted of adding five larvae of the same cohort to each 50 ml glass beaker. Four replicates per treatment, including the control, were considered. For this test, concentrations were the same as those used for *Daphnia* immobilization test (section 2.3.1): 0.01; 0.1; 0.5; 0.7; 1 and 2 g Fe l$^{-1}$. Each beaker was randomly placed in the laboratory at 23ºC and under a natural light cycle. After the 24 and 48 h-exposure, observations were carried out to each individual for 15 minutes. In this period of time the immobilization, as well as any signal of affectation, was recorded.

#### *2.4.2. Chronic exposure test with dissolved iron*

Solutions enriched in dFe were prepared similarly to those used for the reproduction test with *Daphnia* (section 2.3.2). Nominal MPs concentrations were:  0.01; 0.05; 0.1; 1 and 2 g Fe l$^{-1}$. As suggested by OECD for *Chironomus* sp., acute immobilization test, long-term test (30 days) was run with four replicates (control and treatments). An additional replicate for each concentration was used for measuring physic-chemical variables (temperature, conductivity and dissolved oxygen concentration with a multiparameter probe Eutech PCD650). The methodological approach consisted of placing, in each 50 ml glass beakers, five chironomids from the same cohort. They were fed three times a week with 2 ml of food flake fish diluted in 100 ml of mineral water. Beakers were placed randomly in the laboratory at 23ºC and under a natural light cycle. Every day, any signal of stress, adult emergency and physic-chemical variables were recorded.

### *2.5.Statistical analysis*

To estimate the MPs concentration that causes the immobilization of 50% of the individuals during the exposure period ($EC_{50}$-48 h), as well as its 95% confidence limits, a Probit analysis with the statistical program SPSS was carried out (OECD, 2004). This analysis is a kind of regression model to analyze a binominal response variable. To analyze the results of the chronic exposure tests in *Daphnia* and *Chironomus*, the R program was used considering the recommendations of Sokal and Rohlf (1995).

Normality and homogeneity of variances were checked by the Kolmogorov–Smirnov test and Levene's test, respectively (Sokal and Rohlf 1995). Our data did not satisfied normality and homocedasticity assumptions (Shapiro-Wilk and Levene tests, respectively with $p < 0.05$), and transformations did not achieve data to follow a normal distribution. In consequence, a non-parametric one way analysis of variance (Kruskal-Wallis ANOVA) (Quinn and Keough, 2002) was performed to test the effects of dFe on the number of *Daphnia* offspring (males and females), the number of dead larvae and pupae and the number of emerged adults of *Chironomus*. Mann–Whitney *U* tests, corrected for multiple testing with the sequential Bonferroni test (Rice, 1989) were used for examining differences in all these response variables between pairs of treatments.

# 3. Results

## *3.1 Toxicological tests with* **Daphnia magna**

### *3.1.1 Immobilization test with magnetic particles*

In the final immobilization test with *D. magna* no immobilization effects were registered in the control, as expected, while the percentage of immobilized organisms increased when increasing MPs concentration (Fig. 1). In addition, when organisms were exposed to 0.01 g Fe $l^{-1}$ no immobilization effect was recorded on the population, while in the highest concentration (2 g Fe $l^{-1}$) all animals were affected. The $EC_{50}$ (always referred to 48 h) was 0.913 g $l^{-1}$ (our data were adjusted to a normal distribution; Pearson's adjustment; p>0.05).

### *3.1.2 Reproduction test with dissolved iron*

Significant effects of dFe on the production of females offspring in *D. magna* have been found (Kruskal-Wallis ANOVA, $\chi^2$= 16.14, p<0.05). *Daphnia* raised in any concentration of dFe had significantly lower total number of female neonates than control did (Fig. 2a). In fact, the number of female neonates ranged from 0 to 19 (median value = 12) in controls while in treatments, female neonates were always lower than 11. In the presence of dFe, median values of female neonates ranged from 0 (2 g $l^{-1}$) to 4.5 (0.01 g $l^{-1}$). However, no differences were found between dFe concentrations, exclusive of control, for this trait (Fig. 2a). The Mann-Whitney U test showed significant differences between the control and any treatment (Mann-Whitney U with p<0.05 in all the cases) and marginal differences between 0.01 g $l^{-1}$ and 0.05 g $l^{-1}$ treatments (U=39.5, p<0.049), that after applying Bonferroni's correction did not show any significance.

For the case of male offspring, dFe did not stimulated their production in *D. magna* (Kruskal-Wallis ANOVA; $\chi^2$= 10.26; p>0.05; Fig. 2b). Median values was 0 in all control and treatments and the number of male neonates ranged from 0 to 8 (0.1 and 1 g $l^{-1}$).

### *3.2 Toxicological tests with* **Chironomus** *sp.*

#### *3.2.1 Immobilization test with magnetic particles*

As Fig. 3 shows, immobilization increased with MPs concentrations and the total immobilization in *Chironomus* sp. population was recorded at 2 g Fe $l^{-1}$. Data fit to a normal distribution (p>0.05; Pearson adjustment) and 0.445 g Fe $l^{-1}$ was identified as the concentration that caused the immobilization in half of the population ($EC_{50}$).

#### *3.2.2 Chronic exposure test with dissolved iron*

Table 1 summarizes physic-chemical variables recorded along the chronic experiment with *Chironomus* sp. In brief, pH was slightly basic and average values of electric conductivity, dissolved oxygen concentration and temperature ranged from 1.56 to 1.82 mS $cm^{-1}$, from 4.00 to 5.00 mg $l^{-1}$ and from 19.4 to 19.9ºC, respectively.

The long-term experiment results have evidenced the absence of any significant effect of dFe concentration on the number of dead larvae, dead pupae and emerged adults (Fig. 4 a, b and c respectively; Kruskal-Wallis ANOVA: for the number of dead larvae: $X^2$= 5.0327, p>0.05; for the number of dead pupae: $X^2$=6.602, p>0.05; and for the number of emerged adults: $X^2$= 4.251, p>0.0).

## 4. Discussion

### *4.1 Effects of MPs on the organism immobilization*

$EC_{50}$ referred to immobilization was notably lower in *Chironomus* sp. (0.445 g Fe $l^{-1}$) than in *D. magna* (0.913 g Fe $l^{-1}$), showing that the benthic organism was more sensitive than the planktonic one. This is likely to be the result of drastic differences in the lifestyle of these organisms. In fact, chironomids are benthic animals and hence they will be in contact with precipitated Fe all the moment, while *D. magna*, a planktonic organism, is much less time in contact with MPs as these particles rapidly settle down in the water column. At this point, it is relevant to note that considering the 53 mg MPs: mg P mass ratio as the adsorption efficiency ratio, reported in previous studies (de Vicente et al., 2010; Merino-Martos et al., 2011; Table 2), the addition of 0.4 g MPs $l^{-1}$ ($EC_{50}$ for *Chironomus* sp.) and 0.913 g MPs $l^{-1}$ ($EC_{50}$ for *D. magna*) would correspond to a scenario of 8.4 and 19.7 mg P $l^{-1}$, respectively, which are extremely high values for typical inland waters. Therefore, slight effects on immobilization of test organisms (*Chironomus* sp. and *D. magna*) are expected to be found when adding MPs in relation to P concentration in a restoration strategy.

Moreover, it is important to note that standardized OECD immobilization protocols with *D. magna* and *Chironomus* sp. are referred to an exposure of 24 h and 48 h. However, when applying this technique in a whole-lake experiment, MPs would be added to the lake water and after 24 h they would be removed as previous studies have found that maximum P adsorption occurred after this contact time (Funes et al., 2014). For this reason, toxic effects which may result from the application of MPs are likely to be even lower than those detected in these laboratory tests.

In order to compare toxicity of MPs with other P adsorbents (Phoslock, alum, Zeolites, calcite) used for lake restoration, a wide literature review has been done. We have focused our attention in *D. magna* and *Chironomus* sp. An evident scarcity in this type of toxicity and well standardized tests have been revealed making difficult to establish a thorough comparison (Table 2). If we compare MPs and Phoslock, $EC_{50}$, although referred to different endpoints, was in the same order of magnitude for both adsorbents.

However, it is crucial to take in consideration that P removal efficiency was half for Phoslock than for MPs; thus, it is expected major toxic effects of Phoslock on *D. magna* than of MPs in a whole-lake restoration project. In relation to the $EC_{50}$ for MPs and alum, it is clear from Table 2 that much higher values have been found for MPs reflecting the lower toxicity of this adsorbent compared to alum. In fact, Gostomski (1990) remarked that *D. magna* is one of the most sensitive invertebrate species to alum. Galvez-Cloutier et al., (2012) evaluated, by means of a laboratory microcosm experiment, the effect of adding alum, calcite and both alum + calcite on the survival of different planktonic and benthic species but no $EC_{50}$ values were reported. They found that in general, the restoration techniques had no acute neither chronic toxic effects on survival of *D. magna.* They also found that the alum + calcite technique impaired the survival of *Chironomus riparius*, and that the midge emergence was much higher compared to alum only and control. A very recent and interesting study was carried out by Clearwater et al., (2014) but no planktonic organisms were considered, just native benthic-dwelling macroinvertebrates and fish. These authors compared, by laboratory mesocosms, the lethal and sublethal effects of alum or Aqual-P (aluminum amended zeolite) and they found no significant effect of both adsorbents on survival or growth of the studied animals.

Currently, there is a completely lack of research about assessing the effect of magnetic Fe microparticles on aquatic organisms, while most of studies have focused on nanoparticles. Nanoparticles, with lower size than MPs used in our tests, restrict the access of food in some organisms, staying in their filtering systems (Traunspurger and Drews, 1996). Toxicological researches with nanoparticles have shown that particles size and their aggregation have an important role in the determination of toxicity (Keller et al., 2012). García et al. (2011) reported some data about the lethal concentration for half of the population ($LC_{50}$ = 0.23 mg $l^{-1}$) of *D. magna* of magnetite nanoparticles ($Fe_3O_8$). Although the end point of the test is different, immobilization *vs* mortality, we can infer that magnetite nanoparticles are much more toxic for daphnids than our MPs. More recently, Baumann et al. (2014) observed that coating Fe oxide nanoparticles drastically affected to daphnids mobilization, reporting $EC_{50}$ values which ranged from 27.9 (dextran coated nanoparticles) to > 100 mg $l^{-1}$ (polymer coated nanoparticles). These values are again far below $EC_{50}$ values obtained in our study, which lastly reflect the lower toxicity of our MPs for aquatic organisms.

***4.2 Long-term effects of dissolved iron on test organisms***

The adverse ecological effects of an exposition to a stress factor result in a response.

A typical example of response at organism level is the decrease in reproduction, resulting in a decrease in the size of the organism's population (Tannebaum, 2010). In some cases, a sublethal effect which result in a unable individual to produce viable offspring could be considered like a lethal effect because of the biological efficiency of the individual could be equal to a death individual (Newman and Unger, 2003). As heavy metals are very persistent in aquatic systems, they tend to accumulate, they are toxic on organisms and in high concentrations can affect in an adverse way to the structure and function of biotic communities (Boubonari et al., 2009).

Our results suggested that dFe 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. Contrarily to our results, other compounds such as an insecticide caused a great increase in the number of male neonates of *D. magna*, up to 91% (Olmstead and LeBlanc, 2003). It is necessary to consider that during our test, factors such as feeding or temperature, which in some way could affect to the organisms causing the production of male individuals (Koch, 2009), were very controlled, being the only unfavourable condition the presence of dFe coming in solution from MPs.

In a similar test carried out with *D. magna* for 21 days, it was observed the productivity in the number of descendants being in contact with different metals. Those results showed the lowest $EC_{50}$ for mercury (0.0013 mg $l^{-1}$) and the highest for arsenic (3.2 mg $l^{-1}$) (Enserink et al., 1991). More recently, Wollenberger et al., (2000) studied the effect of different veterinary antibiotics on *D. magna* reproduction, obtaining $EC_{50}$ values ranging from 4.6 mg $l^{-1}$ (Oxilinic Acid) to 40 mg $l^{-1}$ (Tiamulin). Other studies have shown, by means of reproduction tests, the existence of a 16% reduction in offspring for every female of *D. magna* maintained in contact with metals, (Biesinger and Christensen, 1972).

The outcome of this study is that the necessary dFe concentrations to negatively affect *D. magna* reproduction are higher than other metals reported in the literature (Enserink et al., 1991; Wollenberger et al., 2000). For the case of the long-term experiment with *Chironomus* sp., no effect of dFe on the number of dead larvae, dead pupae or emerged adult have been observed. Previous studies with *Chironomus riparius* and $Fe^{+2}$ observed, in a 48 h test, a significant mortality in larvae for concentrations up to 400 mg $l^{-1}$ (Rousch et al., 1997). It has been reported that indirect effects of dissolved colloids of Fe are more harmful than direct toxic impact of $Fe^{+2}$ (Linton et al., 2007). These authors found that the number of invertebrates decreased with increasing Fe concentration, detecting physiological stress (which conducts to a decrease in reproduction and growth), and being the most tolerant families Tipulidae and Baetidae. Rasmussen and Lindegaard, (1988) observed that a lot of invertebrates which can live in eutrophic environments can tolerate high concentration of Fe.

In relation to the emergency of adults, in a previous study with *Chironomus tentans* for 14 days exposed to cadmium (1030 mg $l^{-1}$), zinc (17.3 mg $l^{-1}$) and chromium (1640 mg $l^{-1}$), Wentsel et al., (1978) observed an emergency of around half of the total number of larvae. This percentage of emergency was higher than that observed in our study (20%). Wentsel et al., (1977) also reported that *Chironomus tentans* was absent in areas characterized by high concentrations of heavy metals (chromium, cadmium and zinc). These heavy metals inhibited the development of organisms, killed them or prevented the competence with other organisms properly. On the other hand, another study carried out with organisms of the family Chironomidae and metals such as copper, cadmium, zinc and lead, found that $LC_{50}$ was lower for cadmium and higher for lead (Anderson et al., 1980), being in any case much lower than those obtained in this study. Our results are opposite to those obtained with larvae of *Mytilus galloprovincialis* in aquatic environments in contact with soluble Fe, which caused acidity resulting in malformations and lethal effects on the animals (Traunspurger and Drews, 1996).

It has been found that several families of macroinvertebrates, in contact with $Fe^{+2}$ concentrations ranging from 0.2 to 1 mg $Fe^{+2}$ $l^{-1}$, evidenced no changes in the number of individuals while the species diversity was decreased (Rasmussen and Lindergaard, 1988). However, at higher concentrations (from 1 to 10 mg $Fe^{+2}$ $l^{-1}$), both the number of individuals and species decreased.

### *4.3 Implications for lake restoration*

If we consider a whole-lake application of MPs for removing P from both lake water and lake sediment, it is necessary to note the constrains for inferring MPs toxicity found in this study, under very controlled and simple conditions, to natural conditions. All the following features will evidence the overestimation of MPs toxicity in laboratory experiments compared to that expected under natural conditions: (i) in a real restoration project, MPs would be in contact with the plankton organisms for a very short time as MPs are characterized by a high settling velocity (considering MPs and water densities and following Stokes law, estimated value for MPs settling rate is 3.7 µm $s^{-1}$); (ii) in relation to MPs toxicity on benthic organism, it is also expected a lower affection as MPs will be in contact with them for just 24 h instead of the 48 h used in the standardized OECD toxicity tests; (iii) if we consider that the maximum P adsorption capacity by MPs (under batch conditions) was 18.83 mg P $g^{-1}$ MPs (de Vicente et al., 2010), and that 100% of immobilization in *D. magna* and *Chironomus* sp. have been reported in this study for 2 g MPs $l^{-1}$ (which correspond to 37.66 mg P $l^{-1}$), we can conclude that it is very unlikely to cause toxic effects on aquatic organisms under natural conditions as lower MPs concentration are likely to be necessary to apply and (iv) the complexity of the inland waters matrix may promote the occurrence of chemical reactions such as metal complexation which lastly may cause a reduction in dFe toxicity. In this sense, Sorvari and Sillanpää, (1996) found that after complexation of some metals such as $Fe^{+3}$ with free EDTA and DTPA the metal toxicity on *D. magna* was drastically reduced.

## 5. Conclusions

According to the results obtained in the immobilization test with *D. magna,* MPs concentration responsible for the immobilization in half of the population of daphnids was 0.913 g Fe $l^{-1}$ ($EC_{50}$). The presence of dFe (at any concentration) significant and negatively affected to the number of female neonates and, as a result, it affected to the reproduction of *D. magna.* However, no changes in the number of female offspring as a function of Fe concentration have been found. In addition, in the reproduction test with *D. magna*, no effect of dFe concentration on the number of male neonates was reported. The outcomes of this study is that MPs and dFe effects on immobilization and on reproduction, respectively, are lower than other reported in the literature for nanoparticles and for other metals. In relation to the toxicity assays with *Chironomus* sp., $EC_{50}$ for MPs was notably lower (0.445 g Fe $l^{-1}$) than that measured for *D. magna* (0.913 g Fe $l^{-1}$), which is likely to be the result of their different behavior (benthic *vs*. pelagic). Anyway, these MPs concentration are far above the MPs concentration required in a whole-lake restoration project if we consider the 53 mg MPs: mg P mass ratio reported in previous studies (de Vicente et al., 2010; Merino-Martos et al., 2011). The long-term exposition test on *Chironomus* sp. with dFe evidenced the absence of significant effect on larvae and pupae mortality and on the emergency of adults. Therefore, we can conclude that using MPs for reducing P concentration in lake water and lake sediment is a riskless (no toxic effect on planktonic and benthic organisms) and efficient (high P adsorption capacity) tool for lake restoration.


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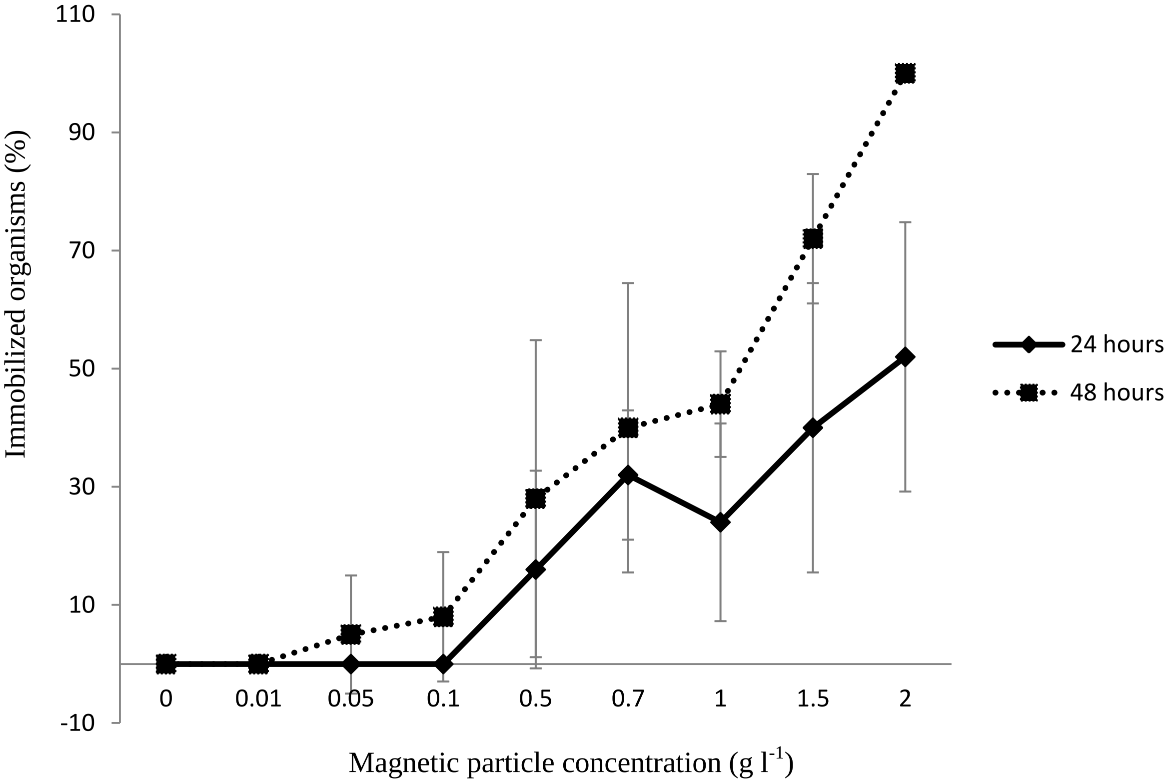


110
90
70
50
30
10
-10
Immobilized organisms (%)
0
0.01
0.05
0.1
0.5
0.7
1
1.5
2
Magnetic particle concentration (g l-1)
24 hours
48 hours


**Figure 1**

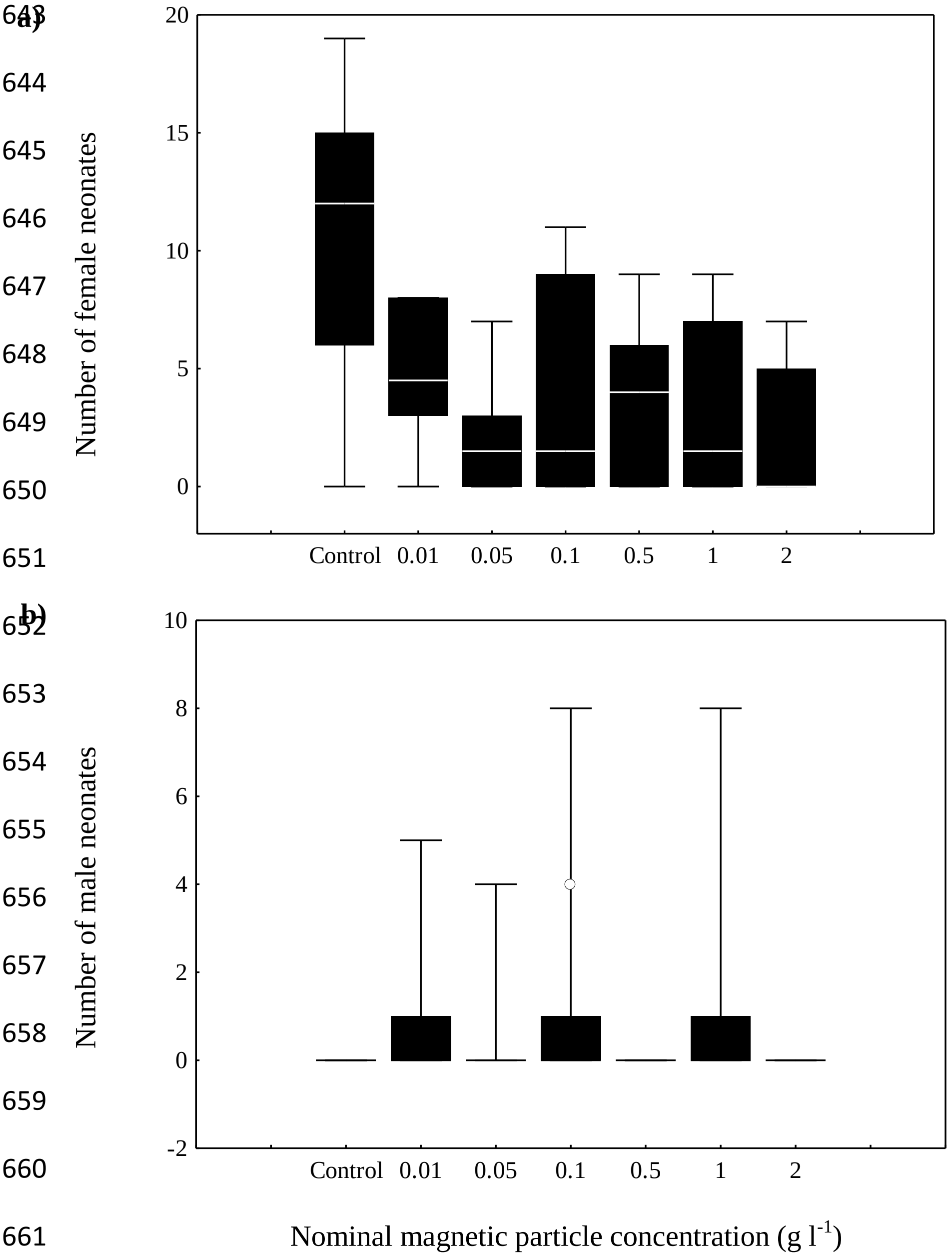
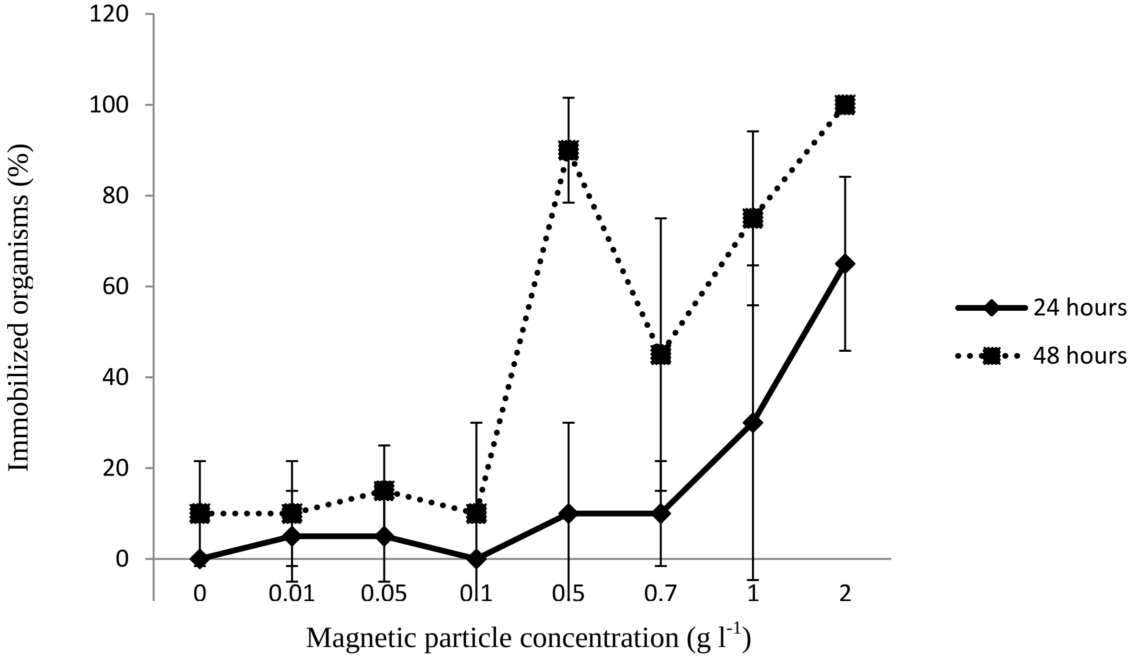
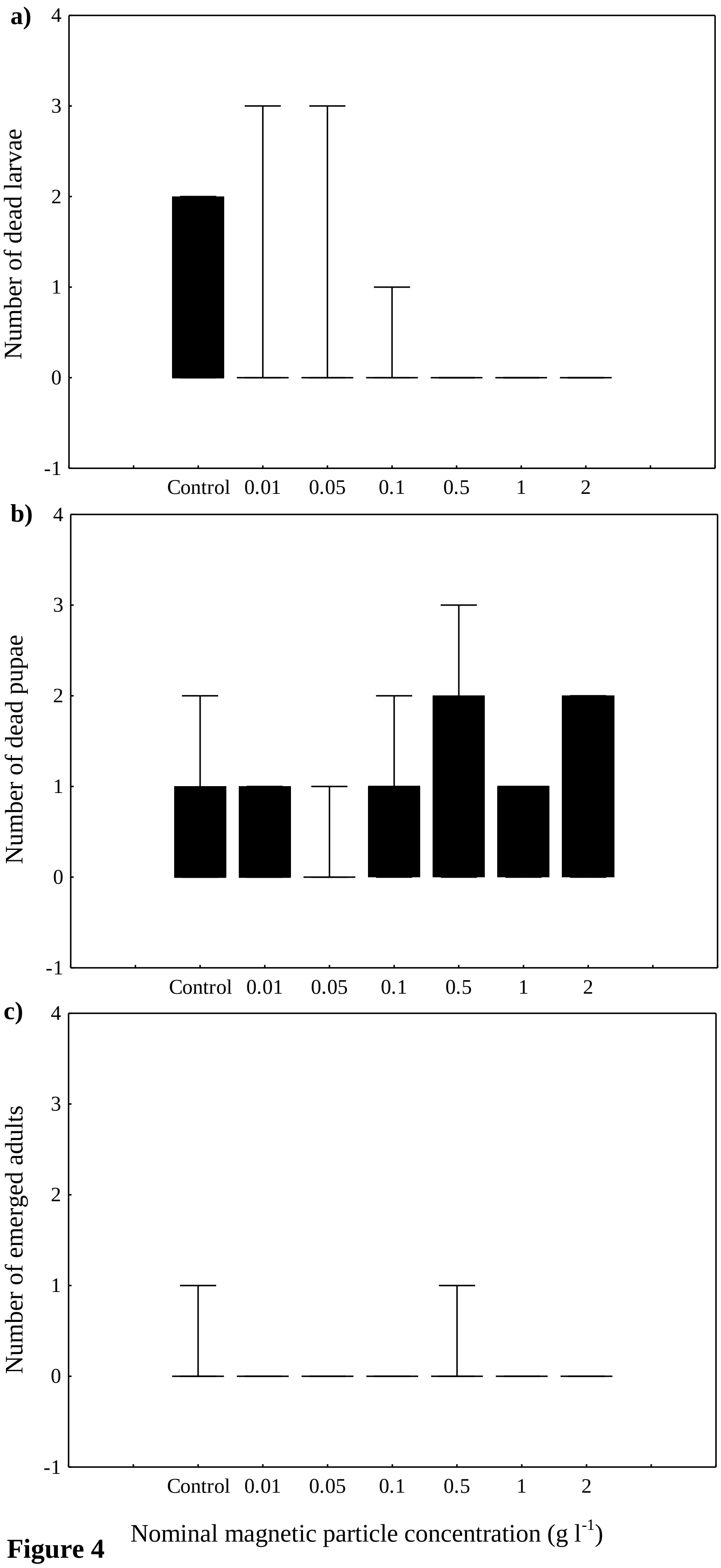

a)
Number of female neonates
20
15
10
5
0
Control
0.01
0.05
0.1
0.5
1
2
b)
Number of male neonates
10
8
6
4
2
0
-2
Control
0.01
0.05
0.1
0.5
1
2
Nominal magnetic particle concentration (g l-1)

**Figure 2**



120
100
80
60
40
20
0
Immobilized organisms (%)
0
0.01
0.05
0.1
0.5
0.7
1
2
Magnetic particle concentration (g l-1)
24 hours
48 hours


**Figure 3**



a)
b)
c)
Number of dead larvae
Number of dead pupae
Number of emerged adults
4
3
2
1
0
-1
Control
0.01
0.05
0.1
0.5
1
2
Nominal magnetic particle concentration (g l-1)


**Figure 4**

**Table 1.** Physic-chemical parameters recorded during the long-term experiment with *Chironomus* sp. Data are mean ± SD (min-max).

| MPs ( g $l^{-1}$) | pH | Conductivity (mS $cm^{-1}$) | $O_2$ (mg $l^{-1}$) | T (ºC) |
|---|---|---|---|---|
| **Control** | 8.30 ± 3.83 (7.04 - 8.81) | 1.77 ± 0.55 (1.01 - 2.73) | 4.30 ± 1.36 (1.39 - 7.43) | 19.9 ± 0.3 (18.8 - 20.8) |
| **0.01** | 8.48 ± 3.98 (7.8 - 9.05) | 1.82 ± 0.68 (1.02 - 3.40) | 4.93 ± 0.75 (3.33 - 7.48) | 19.6 ± 0.3 (18.6 - 20.8) |
| **0.05** | 8.48 ± 3.97 (7.8 - 9.07) | 1.66 ± 0.48 (1.00 - 2.63) | 4.72 ± 0.74 (2.94 - 7.55) | 19.5 ± 0.4 (19.2 - 20.2) |
| **0.1** | 8.57 ± 4.01 (7.79 - 9.03) | 1.67 ± 0.46 (0.99 - 2.63) | 5.00 ± 0.60 (3.75 - 7.54) | 19.6 ± 0.4 (19.2 - 20.5) |
| **0.5** | 8.58 ± 3.98 (7.76 - 9.02) | 1.67 ± 0.48 (0.99 - 2.70) | 4.66 ± 0.53 (3.61 - 7.29) | 19.4 ± 0.4 (18.6 - 20.3) |
| **1** | 8.54 ± 3.98 (7.66 - 8.99) | 1.62 ± 0.45 (0.99 - 2.72) | 4.23 ± 0.72 (1.57 - 7.18) | 19.5 ± 0.4 (18.8 - 20.3) |
| **2** | 8.54 ± 4.01 (7.66 - 9.37) | 1.56 ± 0.41 (0.99 -2.57) | 4.00 ± 0.56 (1.33 - 5.71) | 19.5 ± 0.5 (19.1 - 20.6) |

**Table 2.** Comparative values of $EC_{50}$ for *Daphnia magna* and *Chironomus* sp. for the most frequently used P adsorbent in lake restoration. P removal efficiency is also shown. [1]de Vicente et al. (2010); [2]Lürling & Tolman (2010) and [3]de Vicente et al. (2008). Mortality* reported in acute tests and Life cycle** in chronic tests.

| Adsorbent | Test species | End point | $EC_{50}$ (mg $l^{-1}$) | P removal efficiency (g product $g^{-1}$ P) | References |
|---|---|---|---|---|---|
| **MPs** | *Daphnia magna* | Immobilization | 1048 | 53[1] | This study |
| | *Chironomus* sp. | Immobilization | 445 | | This study |
| **Phoslock** | *Daphnia magna* | Growth (weight based rate) | 871 | 100[2] | Lürling & Tolman (2010) |
| | | Growth (length based rate) | 1557 | | |
| **Aluminum sulphate** | *Daphnia magna* | Mortality* | 38.2 | 66[3] | Kimball in Gostomski (1990) |
| | *Daphnia magna* | Life cycle** | 0.742 | | Kimball in Gostomski (1990) |
| **Aluminum chloride** | *Daphnia magna* | Mortality* | 25.3 | | Brooke et al. (1985) in Gostomski (1990) |