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ON THE USE OF IRON OXIDE COLLOIDAL SUSPENSIONS FOR IMPROVING WATER QUALITY

USO DE SUSPENSIONES COLOIDALES DE ÓXIDOS DE HIERRO PARA LA MEJORA DE LA CALIDAD DEL AGUA

Tesis Doctoral

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Fdo. Ana Inmaculada Funes Cabrerizo

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A mi familia,

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siempre estarán presentes

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Summary

Among all threats that impair water quality (acidification, salinization or contaminantion with xenobiotics), eutrophication has become one of the most common affecting lakes and reservoirs from all over the world. Enrichment of phosphorus (P) in water bodies, which is the growth-limiting nutrient of primary productivity in freshwater bodies, constitutes the main cause of eutrophication. Fertilizer run-off coming from agricultural areas and insufficient wastewater treatment are the main responsible for the increase of P inputs to the aquatic ecosystems leading to biodiversity loss, toxic cyanobacteria blooms and changes in the function of the system.

The use of lake restoration techniques in order to meet the requirements set by environmental policies is increasing from the last 50 years. In Europe, The Water Framework Directive (WFD) has established as a legal requirement to achieve a "good status" in all water bodies by 2027. Recently, the approaches that aim at reducing P availability in water column have been differentiated in three categories: reduction of external P inputs to the system, increasing P retention by the system and increasing P export from the system. Although a multi-approach is often required, the keystone of a lake restoration project is the reduction of external P load which could be accomplished by sewage diversion, natural/constructed wetlands or by phosphorous elimination plants (PEPs) situated at the main inflow of the lake. After a reduction of external P load, biological resilience and P release from sediment (internal P load) may be responsible for the delay in lake recovery. In such cases, the application of alum, iron (Fe) and calcium salts (P inactivation methods) is recommended to increase P adsorption capacity of the sediment. Nitrate addition, hypolimnetic aeration and sediment capping are other known techniques to reduce internal P load. Among the techniques intended to increase P export from the system can be highlighted hypolimnetic withdrawal, external elimination of P-enriched hypolimnetic waters and sediment dredging. The manipulation of the physical and also the biological structure of the aquatic ecosystem constitute other type of techniques to improve water quality.

A direct consequence of eutrophication is the solubilisation of heavy metals due to the low redox potential present in the anoxic bottom waters of lakes and reservoirs during summer stratification. Natural waters with high concentration of soluble manganese (Mn) may be pumped to water distribution network generating operational problems as the obstruction of pipes and the impairment of water quality for human consumption if they are not properly treated. Current techniques to remove Mn from aqueous solution are based on its oxidation and precipitation by adding chemical substances that alter water quality.

Nowadays there exists not a *panacea* in lake restoration since all the available restoration techniques present advantages and disadvantages depending on the characteristics of the system, but without doubt, a successful restoration strategy requires a substantial knowledge of the history lake and also the system ecology in order to anticipate to possible changes or responses. Due to the difficulties that present traditional P inactivation methods -redox sensitivity, biotoxicity in highly acidic or alkaline media, acidification of lake water due to floc formation, reduction of P adsorption capacity with ageing of the adsorbent- and given the unsuitability of traditional methods to remove Mn in water treatment plants, the development of new tools to combat eutrophication and to improve water quality is of special interest for water resources management.

Magnetic nano and microparticles, mostly based on Fe, have attracted special attention in areas such as biomedicine, industry, environmental remediation and the ones that concern the present study: water treatment and lake restoration. The reason why their use has become widespread in the last decades for adsorbing pollutants from aqueous media stems in their unique properties: fast adsorption kinetic due to their high surface reactivity, high adsorption capacity due to their high surface area and their magnetism which makes them different to other adsorbents. The latest property implies that magnetic particles can be removed from the media by applying a magnetic separation gradient once the target contaminant is adsorbed, thus minimizing contact time with biota and enabling their recovery for further reuse. One of the main advantages of using magnetic particles as adsorbents is the possibility of surface functionalization in order to increase the affinity for the adsorbate, reduce the biotoxicity or modify particle density for specific purposes.

A low-cost granulate material consisting of dried amorphous ferric oxy-hydroxide (CFH-12®, Kemira) has been recently proposed to adsorb P from wastewater with excellent results, being those low cost, high adsorption capacity and strong P-bonding with low desorption. However, those studies were only conducted at aerobic conditions and only for short exposure times (up to 2 weeks) for P adsorption. Further knowledge about long-term ageing effects or reductive effects on CFH-12® under anoxic conditions and their implications in P adsorption capacity is needed.

In this PhD dissertation the main purpose was to assess the performance of three different adsorbents to remove P from lake water and sediment in a context of lake restoration. Two of these compounds are magnetic as the case of commercial micronsized carbonyl Fe particles (grade HQ, BASF) and hybrid chitosan magnetic particles synthesized in laboratory whereas the other one is non magnetic and corresponds to CFH-12®. Apart from the removal of P, we also studied the feasibility of using micronsized carbonyl Fe particles to remove Mn from the aqueous solution in water treatment.

To accomplish that, firstly we studied the influence of pH on Mn removal efficiency by carbonyl Fe particles in batch conditions. In these experiments we obtained a strong influence of pH on Mn removal efficiency, being the removal efficiency extremely high at pH higher than 9 (>98%) as a result of chemisorption of Mn on magnetic particles surface but considerably lower (<30%) when decreasing pH value due to the presence of Mn as soluble cation. Secondly, we also studied the effect of other parameters such as contact time, adsorbent concentration and surface coating with Mn oxides [MnOx(s)] on Mn removal efficiency. Results demonstrated that MnOx(s) precipitate on magnetic particles surface in less than 1 hour at pH 9 and that Mn adsorption efficiency clearly increases when increasing adsorbent concentration (from 1 g L^{-1} to 2 g L^{-1}) and when coating magnetic particles with precipitated MnOx(s) which have high affinity for soluble Mn adsorption. The maximum adsorption capacity of MnOx(s)-coated magnetic particles was not reached since even working at very high Mn concentrations, adsorption sites were not saturated due to a multi layer adsorption process in which adsorption surface is continuously regenerated by overlapping MnOx(s) layers. The desorption of precipitated MnOx(s) from magnetic particles surface was tested as well as and the possibility of reusing the adsorbent. Results showed a low desorption rate (<10%) in a wide range of pH (6-9) as well as a high Mn removal efficiency (>99%) of reused magnetic particles, highlighting the stability of MnOx(s) precipitated on magnetic particles and the possibility of reusing them without losing adsorption capacity.

As a result of previous studies that highlighted the good performance of carbonyl Fe particles to remove P under batch and flow conditions -high P adsorption capacity (18.83 mg g^{-1}), fast adsorption kinetic (less than 2 h) and pH-independency of P removal efficiency (>80% in pH range 6-9)- we set up a core incubation experiment with sediment and water from a brackishwater lagoon (Laguna Honda, Almería, Spain) to see the effect of adding carbonyl Fe particles on P fluxes and sedimentary P pools. Cores were incubated in oxic and anoxic conditions with the purpose of assessing the effect of adding magnetic particles for 24 h on P efflux and sedimentary P pools. To do so, magnetic particles were added over the surface water of the cores and after 24 h they were removed by applying a magnetic separation gradient. Results showed a complete suppression of P efflux in the most unfavourable conditions that are usually present in hypertrophic lakes (anoxic conditions). Likewise, the application of magnetic particles resulted in an increase of P export from the system since mobile P was reduced 22-25 % in anoxic and 12-16 % in oxic conditions.

In another experiment, the effect of adding carbonyl Fe particles on P cycle ,but also on other nutrients such as carbon (C), reactive silicate (Si) and nitrogen (N) as well as other parameters related to water quality, was evaluated. For that, an enclosure experiment with large enclosures containing 6600 cm^3 of sediment and 40 L of water from Laguna Honda was set up. Carbonyl Fe particles were added to the enclosures in two different ways (1) above the surface water or (2) above the surface sediment. After a contact time of 24 h they were removed from the enclosures by a specially designed magentic rake. The enclosures were incubated for a 70-days oxic period and a 5-days anoxic period. We found that the addition of magnetic particles caused a reduction of more than 68 % of dissolved inorganic P (DIP) in both oxic and anoxic period as well as a reduction of 50% of Si and a 15 % of dissolved organic C (DOC), indicating that both Si and DOC interfere in P adsorption capacity by magnetic particles. Sedimentary P pools as the case of P bound to humic acids, NaOH extractable-P and easily degradable organic P were reduced by 11-21%, 15% and 12 %, respectively by the addition of magnetic particles. We concluded that adding the magnetic particles over the surface water was more recommendable due to a higher recovery efficiency of magnetic particles with the magnet (90%) against the 30% obtained when adding them over the surface sediment.

In the cores as well as in the enclosures experiment above mentioned it was evidenced the high density of carbonyl Fe particles which tend to penetrate deep in layer sediment. Deeper penetration of the adsorbent within the sediment implies less availability for P adsorption (since the main target is mobile P that concentrates in the first layers of sediment and decreases with depth), higher resuspension and less recovery efficiency of magnetic particles when removing them by applying a magnet. To overcome these drawbacks, we synthesized composite chitosan + magnetite particles. Resulting hybrid microparticles exhibited enough P adsorption capacity (4.84 mg g^{-1}) to be used in hypertrophic lakes and they were demonstrated to be excellent candidates to extract mobile P from the upper sediment layers because they sediment slower than traditional carbonyl Fe particles.

Finally, we set up a core incubation experiment with sediment and water from three Danish lakes in order to evaluate CFH-12® and the well-known freshly formed $Fe(OH)_3$ floc as P inactivation agents for lake restoration. The experiment was divided into an oxic and anoxic period in order to see effects of reducing conditions on the performance of the two compounds as well as the effect of ageing on P adsorption capacity. From results, we concluded that CFH-12® is a promising lake restoration tool which does not lead to a pH drop of lake water during addition and shows, with a Fe: mobile P binding molar ratio of 7.6-8:1, a significant reduction of DIP efflux. Moreover, CFH-12® showed, in contrast to freshly formed $Fe(OH)_3$ floc, no changes in its P binding capability with ageing and low redox sensitivity.

Resumen

De entre todas las amenazas que perjudican la calidad del agua (acidificación, salinización y contaminación con xenobióticos), la eutrofización se ha convertido en una de las más comunes que afecta a lagos y embalses de todo el mundo. El incremento de fósforo (P) en las masas de agua, el cual es el nutriente limitante para la producción primaria, constituye la principal causa de la eutrofización. El lavado de nutrientes que procede de la escorrentía de zonas agrícolas así como el tratamiento ineficiente de las aguas residuales son las principales causas del incremento de P en los ecosistemas acuáticos, lo cual se traduce en una pérdida de biodiversidad, *blooms* de cianobacterias tóxicas y cambios en la función general del sistema.

El uso de técnicas de restauración en los ecosistemas acuáticos ha incrementando en los últimos 50 años para cumplir los requerimientos establecidos en las políticas ambientales. En Europa, la Directiva Marco del Agua ha establecido como requerimiento legal alcanzar un "buen estado" en todas las masas de agua para el 2027. Recientemente, las medidas que tienen como objetivo la reducción de la disponibilidad de P en la columna de agua han sido diferenciadas en tres categorías: reducción de la carga externa de P al sistema, incremento de la retención de P en el sistema e incremento de la salida de P del sistema. Aunque a menudo un enfoque múltiple es requerido, la piedra angular de un proyecto de restauración de lagos es la reducción de la entrada externa de P al sistema la cual puede ser alcanzada mediante el desvío de aguas residuales, lagunas artificiales o naturales para la retención de contaminación difusa o mediante plantas de eliminación de P situadas en la principal entrada de flujo al ecosistema. Después de reducir la carga externa de P al sistema, tanto la resiliencia biológica como la liberación de P desde el sedimento a la columna de agua (carga interna de P) pueden retrasar la recuperación del lago. En tal situación, la aplicación de sales de aluminio, hierro (Fe) y calcio (métodos para la inactivación de P) se convierte en una opción recomendada para incrementar la capacidad de adsorción de P por parte del sedimento. La adición de nitrato, la aireación hipolimnética y el sellado del sedimento constituyen otro tipo de técnicas enfocadas a la reducción de la carga interna de P. De entre las técnicas destinadas al incremento la salida de P del sistema pueden señalarse la retirada de agua hipolimnética, el tratamiento de aguas hipolimnéticas enriquecidas en P así como el dragado de sedimentos. La manipulación de la estructura física y biológica del ecosistema acuático constituyen otras técnicas destinadas a la mejora de la calidad del agua.

Una consecuencia directa de la eutrofización es la solubilización de metales pesados debido al bajo potencial redox presente en las aguas anóxicas profundas de los lagos y embalses durante el período de estratificación estival. Las aguas naturales con altas concentraciones de manganeso (Mn) soluble pueden ser bombeadas a la red de distribución generando problemas operacionales como la obstrucción de tuberías así como la alteración de la calidad del agua destinada al consumo humano si no son debidamente tratadas. Las prácticas actuales para la eliminación de Mn de la solución acuosa se basan fundamentalmente en la oxidación y precipitación de dicho metal mediante la adición de sustancias químicas que alteran la calidad del agua.

En la actualidad no existe una panacea en la restauración de lagos ya que todas las técnicas de restauración disponibles presentan ventajas y desventajas dependiendo de las características del sistema a restaurar, pero sin ninguna duda, una estrategia de restauración exitosa requiere un conocimiento profundo de la historia del lago así como

de su ecología con el fin de poder anticiparse a posibles cambios o respuestas. Dadas las dificultades que presentan los métodos de inactivación de P tradicionales -sensibilidad redox, biotoxicidad en condiciones fuertemente ácidas o alcalinas, reducción de la capacidad de adsorción de P debido al envejecimiento de los adsorbentes- y dado que las actuales técnicas de eliminación de Mn en las plantas de tratamiento de aguas presentan resultados indeseables, el desarrollo de nuevas herramientas para combatir la eutrofización y mejorar la calidad del agua es de especial interés.

Las nano y micropartículas magnéticas, principalmente las constituidas por Fe, han suscitado especial atención en aéreas como biomedicina, industria, remediación ambiental y en las dos áreas que conciernen al presente estudio: tratamiento de aguas y restauración de lagos. La razón por la cual su uso se ha extendido tanto en las últimas décadas para la adsorción de contaminantes de medios acuosos radica en sus propiedades únicas: rápida cinética de adsorción debido a su elevada reactividad, alta capacidad de adsorción debida a su elevada área superficial y magnetismo, propiedad que las hace diferentes a otros adsorbentes. La última propiedad implica que las partículas magnéticas pueden ser retiradas del medio mediante la aplicación de un gradiente de separación magnética una vez el contaminante ha sido adsorbido, minimizando el tiempo de contacto con la biota y permitiendo la recuperación de las mismas para su posterior uso. Una de las principales ventajas de usar partículas magnéticas como adsorbentes es la posibilidad de funcionalizarlas con el fin de incrementar la afinidad por el adsorbato, reducir la biotoxicidad de la partículas o modificar su densidad para fines específicos.

Recientemente, un material granular de bajo coste compuesto de oxi-hidróxidos de Fe amorfos (CFH-12®, Kemira) ha sido propuesto para adsorber P de las aguas residuales con excelentes resultados, siendo éstos, un bajo coste, una alta capacidad de adsorción de P y un enlace fuerte de unión con el P mostrando una baja desorción. Sin embargo, estos estudios fueron llevados a cabo únicamente en condiciones óxicas y por cortos tiempo de exposición (hasta 2 semanas) para la adsorción de P. Por tanto, es necesario un conocimiento adicional sobre el efecto del envejecimiento del adsorbente y de la presencia de condiciones anóxicas sobre la capacidad de adsorción de P por el CFH-12®.

El principal propósito de esta tesis doctoral consiste en evaluar tres adsorbentes diferentes para eliminar P del agua y del sedimento lacustre en el contexto de la restauración de lagos. Dos de estos compuestos son magnéticos, como es el caso de las partículas de Fe carbonilo comerciales (grade HQ, BASF) y de las partículas híbridas de quitosano magnético sintetizadas en laboratorio mientras que el otro compuesto es no magnético y corresponde al CFH-12®. Aparte de la eliminación de P, también se ha evaluado la posibilidad de usar las partículas de Fe carbonilo para la eliminación de Mn de la solución acuosa en el tratamiento de aguas.

Para ello en primer lugar, se estudió la influencia del pH sobre la eficiencia de retirada de Mn por las partículas de Fe carbonilo en condiciones estancas. En estos experimentos se determinó una fuerte influencia del pH sobre la eficiencia retirada de Mn, siendo ésta extremadamente alta a pH mayores a 9 (>98%) como resultado de la quimiosorción del Mn sobre la superficie de las partículas magnéticas, pero considerablemente menor (<30%) a pH inferiores debido a la presencia de Mn como catión soluble. En segundo lugar, se investigó el efecto de otros parámetros como el tiempo de contacto, la concentración de adsorbente y el recubrimiento superficial de las partículas magnéticas con óxidos de Mn [MnOx(s)] sobre la eficiencia de retirada de Mn. Los resultados demostraron que los MnOx(s) precipitan sobre la superficie de las partículas magnéticas en menos de 1 h a pH 9 y que la eficiencia de retirada de Mn era claramente mayor cuando incrementaba la concentración de adsorbente (de 1 g L^{-1} a 2 g L^{-1}) y cuando las partículas magnéticas se recubrían con MnOx(s), los cuales poseen una alta afinidad para la adsorción de Mn soluble. La capacidad máxima de adsorción de las partículas magnéticas recubiertas con MnOx(s) no fue alcanzada ya que incluso trabajando a concentraciones muy altas de Mn, los sitios de adsorción no llegaron a saturarse debido a un proceso de adsorción en multicapa donde la superficie de adsorción se regeneraba a medida que se depositaban las capas de MnOx(s). La desorción de los MnOx(s) precipitados en la superficie de las partículas magnéticas fue evaluada así como la posibilidad de reutilizar las partículas magnéticas. Los resultados mostraron un bajo porcentaje de desorción (<10%) en un amplio rango de pH (6-9) así como una elevada eficiencia de retirada de Mn (>99%) de las partículas magnéticas reutilizadas, resaltando de esta forma la fuerte estabilidad de los MnOx(s) precipitados sobre las partículas magnéticas y la posibilidad de reutilizar las mismas sin perder capacidad de adsorción.

Como resultado de estudios previos que han resaltado el buen desempeño de las partículas de Fe carbonilo para eliminar P en condiciones estancas y de flujo continuo-alta capacidad de adsorción de P (18.83 mg g^{-1}), rápida cinética de adsorción (menos de 2 h) y alta eficiencia de retirada con independencia del pH de la solución (>80% en el rango de pH 6-9)- se estableció un experimento de incubación de cores con sedimento y agua procedentes de una laguna salobre (Laguna Honda, Almería, España). Los cores fueron incubados en condiciones óxicas y anóxicas con el fin de evaluar el efecto de añadir partículas de Fe carbonilo durante 24 h sobre los flujos de P y las fracciones sedimentarias de P. Para ello, las partículas magnéticas se añadieron sobre la superficie de la columna de agua y después de 24 h fueron retiradas de los cores mediante la aplicación de un gradiente de separación magnética. Los resultados mostraron una completa supresión del flujo de P en las condiciones más desfavorables presentes en los lagos hipertróficos (condiciones anóxicas). Asimismo, la aplicación de las partículas magnéticas ocasionó una retirada de P de los cores ya que la fracción de P móvil también se redujo en un 22-25% en condiciones anóxicas y un 12-16% en condiciones óxicas.

En otro experimento, el efecto de añadir partículas de Fe carbonilo sobre el ciclo del P pero también sobre otros nutrientes como el carbono (C), sílice reactivo (Si) y nitrógeno (N) así como sobre determinados parámetros relacionados con la calidad del agua fue evaluado. Para ello, se estableció un experimento empleando cerramientos que contenían 6600 cm^3 de sedimento y 40 L de agua de la Laguna Honda. Las partículas de Fe carbonilo fueron aplicadas a los cerramientos de dos formas diferentes: (1) sobre la superficie de la columna de agua o (2) sobre la superficie del sedimento. Después de un tiempo de contacto de 24 h, las partículas magnéticas fueron retiradas de los cerramientos mediante un dispositivo magnético especialmente diseñado para ello. Los cerramientos fueron incubados durante un período óxico de 70 días y un período anóxico de 5 días. De los resultados se concluyó que la adición de partículas magnéticas causó una reducción del 68% del P inorgánico disuelto tanto en condiciones óxicas como anóxicas así como una reducción del 50% del Si y un 15% del C orgánico disuelto (DOC), indicando que ambos, Si y DOC interfirieron en la capacidad de adsorción de P por las partículas magnéticas. Las fracciones de P sedimentario como el caso del P unido a ácidos húmicos, el P extraíble en NaOH y el P orgánico fácilmente degradable fueron reducidos en un 11-21%, 15% y 12%, respectivamente por la adición de las partículas magnéticas. En este experimento, se concluyó que la adición de partículas magnéticas sobre la superficie de la columna de agua fue más recomendable debido a que se alcanzó una mayor eficiencia de recuperación de las mismas con el imán (90%) frente al 30% obtenido cuando se añadieron sobre la superficie del sedimento.

Tanto en el experimento de los cores como en el de los cerramientos mencionados anteriormente, se puso de manifiesto la elevada densidad de las partículas de Fe carbonilo, las cuales tendían a penetrar hacia capas más profundas del sedimento. Una mayor penetración del adsorbente en el sedimento implica una menor disponibilidad para la adsorción de P (ya que el P móvil se concentra en las capas más superficiales del sedimento y su concentración decrece en profundidad), una mayor resuspensión y una menor eficiencia de retirada de las partículas magnéticas cuando éstas son eliminadas mediante la aplicación de un imán. Con el fin de superar esta limitaciones, se sintetizaron en laboratorio partículas de magnetita modificadas con quitosano. Las micropartículas híbridas resultantes exhibieron suficiente capacidad de adsorción de P (4.84 mg g^{-1}) para ser usadas en lagos hipertróficos y se demostró que eran excelentes candidatas para retirar P móvil de las capas más superficiales de sedimento ya que sedimentan más despacio que las partículas de Fe carbonilo.

Finalmente, se llevó a cabo un experimento de incubación de cores con sedimento y agua de tres lagos daneses con el fin de evaluar el CFH-12® y el bien conocido flóculo de $Fe(OH)_3$ como agentes para la inactivación de P en la restauración de lagos. El experimento se dividió en un período óxico y otro anóxico para estudiar los efectos de las condiciones reductoras sobre los dos compuestos de Fe así como el efecto del envejecimiento de ambos adsorbentes sobre la capacidad de adsorción de P. De los resultados, se concluyó que el CFH-12® es una herramienta prometedora para la restauración de lagos ya que su adición no implicó una reducción en el pH del agua del lago y mostró, con un ratio molar Fe:P móvil de 7.6-8:1, una reducción significativa del flujo de P inorgánico disuelto. A diferencia de lo que ocurrió con el flóculo de $Fe(OH)_3$, el CFH-12® no mostró sensibilidad a cambios en las condiciones redox así como no mostró pérdida de la capacidad de adsorción de P con el envejecimiento del compuesto.

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General introduction

1. Eutrophication: a worldwide affection of water resources

From ancient time, human society has realized that they depend on the natural world. However, the value of natural ecosystems has been ignored until their degradation has highlighted their importance (Daily, 1997). It is well known that natural ecosystems provide economical, ecological and social benefits essential for humans, the so called "ecosystem services" (Costanza et al., 1997). Aquatic ecosystems in particular, play a vital role in human existence and specifically, freshwater bodies such as lakes and reservoirs, which are important sources of nature conservation, water for irrigation, drinking, fishing, recreation and transportation (Hein, 2006; Jeppesen et al., 2009). They have also a spiritual and cultural intangible value and constitute good regulators of climate change (Williamson et al., 2009).

Amongst all threats that impair water quality such as acidification, salinization or contaminant with xenobiotics, eutrophication has become in one of the most common problems in the last 50 years affecting 30-40% of lakes and reservoirs from all over the world (Hupfer and Hilt, 2008). Eutrophication is the increase of primary productivity in aquatic ecosystems due to nutrient (P and N) enrichment (Carpenter et al., 1999). External nutrient inputs to inland waters are derived from several sources such as geological weathering, atmospheric deposition, point sources such as treated/untreated wastewater and industrial effluents, but most of all, from non point sources related to surface runoff of agricultural and urban areas (Smith, 2009). The latest type of pollution is the most difficult to quantify and regulate because of its diffuse character. Cultural eutrophication must be differentiated from natural eutrophication that occurs with aquatic ecosystem ageing over time (Hupfer and Hilt, 2008). Surface waters have been classified into different categories according to their supply of nutrients giving way to the "trophic state" concept. In this sense, the trophic state range from oligotrophic to hypertrophic when receiving low or high nutrient inputs, respectively (Nürnberg, 1996).

Eutrophication promotes negative effects both in the ecological and economical dimension. The main consequences of eutrophication process are illustrated in Table 1.

Table 1: Ecological and socio-economical effects of eutrophication (from Carpenter et al., 1999; Burkholder and Glibert, 2000; Smith, 2003, 2009).

Economical effects	Socio-economical effects
• Increase in primary productivity and biomass of phytoplankton and suspended algae	• Higher water treatments costs
• Shifts in algal composition with frequent algal bloom episodes, many of which are toxic species	• Technical problems in water treatment plants
• Shifts in vertebrate and invertebrate consumers species composition, mostly undesirable species	• Consumer complains about organoleptic characteristics of final drinking water
• Decrease in water transparency	• Increase of disease risk and its intensity
• Depletion of oxygen in the hypolimnion	• Difficulties in meeting standards of disinfections by-products
• Higher probability of fish kill during summer anoxia	• Decrease in the perceived aesthetic value of water bodies
• Accumulation of nutrients and toxic substances such as metal in deep waters	• Reduction in recreational uses
• Decrease in submerged aquatic vegetation	
• Reduction of the overall biodiversity	
• Loss of sensitive species and increase of opportunistic species	

The sensitivity of aquatic ecosystems to nutrient enrichment depends on several factors such as physico-chemical parameters of water column, water resident time, biological community or food web structure which are closely influenced to lake morphometry (Burkholder and Glibert, 2000; Håkanson, 2005; Smith, 2009). There are 304 million natural lakes in the world and their extension covers about 4.2 million km^2. Although the total freshwater volume of Earth´s surface is contained in few deep lakes, the total area is strongly dominated by shallow and small systems (Jeppessen et al., 2009; Downing, 2010). The intensive activity of small aquatic ecosystems and their dimensions make them more dynamic in time than large water bodies. Aquatic rates, processes and quantities are more complex or abundant in small lakes than in larger lakes. For instance, small lakes present higher species diversity than larger ones (Downing, 2010). Similarly to the worldwide tendency, shallow and small lakes dominate the Mediterranean landscape. The high catchment area to volume ratio makes shallow lakes especially vulnerable ecosystems to the eutrophication process due to their lower capacity for diluting substances (Smith, 2009). Apart from important external P inputs, shallow lakes suffer high internal loading triggered by wind-induced resuspension that continuously mixes the water column (Sondergaard et al., 1992). The response of shallow lakes to eutrophication may lead to a regime shift from clear water state, macrophytes-dominated, to turbid water state, phytoplankton-dominated (Scheffer and Carpenter, 2003; Hein, 2006). Although deep lakes are scarce in Mediterranean areas, most of reservoirs in this area present similar characteristics to deep lakes in their deeper part near the dam (Cooke et al., 2005). In deep lakes and reservoirs the thermal stratification developed during summer isolates epilimnion (surface warm water) from hypolimnion (deep cold water) promoting oxygen depletion due to organic matter break down with negative impacts on biota and water quality (Davison, 1993). As well as shallow lakes, reservoirs are also likely to present water quality problems due to their large watersheds and morphometric configurations (Cooke et al., 2005).

As occurs in terrestrial ecosystems, N and P constitute the growth-limiting nutrients for primary production in aquatic ecosystems. While in polar and temperate oceans N is the main nutrient limiting primary production, P is the growth-limiting nutrient in freshwater bodies (Burkholder and Glibert, 2000). In this sense, most of the efforts intended to combat eutrophication of inland waters are focused on P reduction instead of N (Hupfer and Hilt, 2008).

2. State of the art: traditional measures to combat eutrophication

The Water Framework Directive (WFD), that provides a common framework to protect, manage and restore surface and groundwater in Europe, has prescribed as a legal requirement by 2027 that all water bodies must achieve "good status". Surface waters status is measured by both its ecological and chemical status assessed in the scale of "high", "good", "moderate", "poor" and "bad" based on the Ecological Quality Ratio, which is a ratio between the reference conditions and measured status of the biological quality conditions (Heiskanenen, 2004;Willaarts et al., 2014). In the last report of European Commission about the implementation of WFD in Spain it was stated that although 43% of surface water bodies present "high" or "good" ecological status, a very high proportion (39.2 %) were classified as "moderate" to "bad" status and a large number of surface water bodies (>727) are classified as “unknown" status representing 17 % of the total (EC, 2015).

In order to achieve the requirements, implementation of appropriate tools and effective restoration methods are indispensable. The National Research Council (NRC, 1992) defined restoration as "the return of an ecosystem to a close approximation of its condition prior to disturbance" whereas the Society for Ecological Restoration (SER, 1995) defined ecological restoration as "the process of renewing and maintaining ecosystem health". Another definition of ecological restoration that differs from the above mentioned, because it emphasizes the anthropogenic intervention as a source of the disturbance, is the one proposed by Jackson et al. (1995): "The process of repairing damage caused by humans to the diversity and dynamics of indigenous ecosystems". One of the most important challenges in the field of aquatic ecosystems restoration is the establishment of the pre-disturbance condition as a frame of reference, which can be difficult due to unavailable ecological records or the absence of reference areas (Cairns and Heckman, 1996).

Large-scale restoration projects of eutrophic lakes started during the 1980s (Istvanovics and Somlyody, 2001). A successful restoration strategy requires a substantial knowledge of the history lake and also the system ecology in order to anticipate to possible changes or responses (Jørgensen et al., 2005). Although a multi-approach is often required, the keystone of lake restoration is the reduction of external P load to annual average inlet concentration lower than 0.1 mg P L^{-1} (Reitzel et al., 2003; Cooke et al., 2005; Jeppensen et al., 2009). However, most of the cases, the shift to a good ecological status requires a reduction of external P inputs to lower levels than found before the collapse (restoration hysteresis). This is a requirement that may be not feasible due to the dominance of non point sources or the high cost implied (Deppe and Benndorf, 2002; Scheffer and Carpenter, 2003).

Apart from the biological resilience (the ability or tendency of the system to maintain its existing structure), the internal P loading (P release from sediment to water column) is a important factor explaining the delay in lake recovery after a reduction of external P load (Søndergaard et al., 2003). The P exchange between sediment and water column is influenced by biological (bacterial activity, mineralization processes, and bioturbation), chemical (redox potential, pH, Fe:P ratio, nitrate availability), and physical factors (resuspension and sediment mixing) (Sondergaard et al., 2001). Apart from Fe, P also combines in sediments with carbonates, clays and organic matter which can be temporary (potentially bioavailable) or permanent sinks for P (not bioavailable).

Lake water P concentration can be reduced by three different methods as shown in Fig. 1: (1) Reductions of external P loads, (2) Increase of P retention capacity by sediment, and (3) Increase of P export (Hupfer and Hilt, 2008). In the following paragraphs a brief description of these methods is exposed.

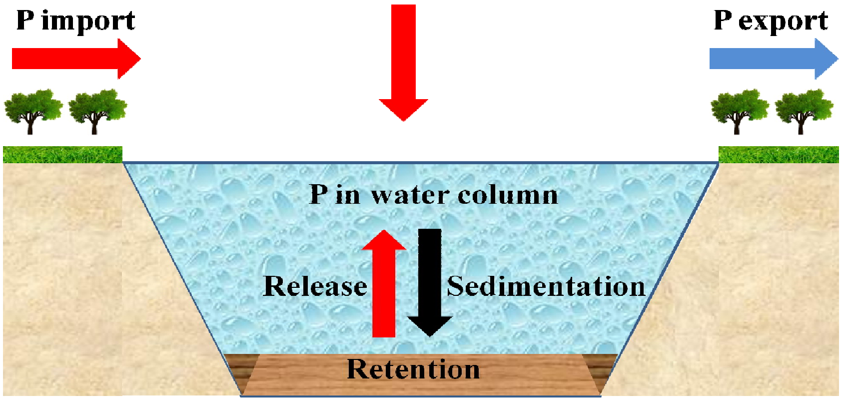

Fig. 1. P balance in aquatic ecosystems (modified from Hupfer and Hilt, 2008)

2.1 Measures directly focused on reducing lake water P concentration

2.1.1 Reduction of external P inputs

This approach includes four blocks of preventive measures which are more cost-effective than corrective measures to reduce P inputs to the aquatic ecosystems: (i) reduction of P emissions by promoting a cleaner production model and by implementing advanced treatments of urban and industrial sewage, (ii) diversion of urban and industrial sewage (raw and treated), (iii) limitation of inputs of diffuse pollution from agriculture by reducing water and wind erosion of soils, preserving the land-lake transition buffer zone or by natural/constructed wetlands, ponds or pre-dams and (iv) treatment of storm water by P elimination plants (PEPs) at the main inflow of the lake (Hupfer and Hilt, 2008; Gołdyn et al., 2014).

The response to a reduction in external P loading varies enormously between lakes. Some cases of drastic reduction of chlorophyll *a* after a reduction of external P loading are Lake Washington (USA) or Lake Constance (Germany) that can be explained by their fast renewal rate, oxic hypolimnion and great depth (Jeppesen et al, 2003). A study based on data of 25 Danish lakes showed a substantial reduction of phytoplankton and zooplanktivorous fishes as well as a concomitant increase in piscivorous fishes after reducing external P inputs (Jeppesen et al., 2002). However, many lakes have exhibited delayed recovery or have not responded at all to significant reduction in external P inputs as the cases of Lake Søbygard (Denmark) and Lake Shagawa (USA) mostly due to the high internal P loading (Cooke et al., 2005).

2.1.2 Increase of P retention capacity by sediment

P retention by sediment can be achieved by mechanical, chemical or biological methods and could be divided into two main categories: (1) P inactivation with salt addition and (2) Reduction of P release from sediment.

➢ P inactivation with salts addition

P inactivation mechanism by salts addition consist on spreading alum (Al), iron (Fe) or calcium (Ca) salts into a lake with pipes, by boats and airplanes or aeration devices to increase the number of adsorption sites for P uptake (Hupfer and Hilt, 2008). Soluble P is precipitated as low soluble salts or adsorbed by colloidal aggregates whereas particulate P (algal biomass) is coagulated and settle to the lake bottom sediments. A summarize of advantages, disadvantages, recommendations for their use as well as recommended dose is shown in Table 2.

Table 2. Some advantages, disadvantages, recommendations for whole-lake applications and dose required when using traditional salts (Jensen et al., 1992; Hansen et al., 2003; Anderson, 2004; Cooke et al., 2005; de Vicente et al., 2008; Kleeberg et al., 2013; Reitzel et al., 2013b)

Table 2. Some advantages, disadvantages, recommendations for whole-lake applications and dose required when using traditional salts (Jensen et al., 1992; Hansen et al., 2003; Anderson, 2004; Cooke et al., 2005; de Vicente et al., 2008; Kleeberg et al., 2013; Reitzel et al., 2013b).

Adsorbent	Advantages	Disadvantages	Recommendations for whole-lake applications	Recommended dose
Al	High affinity and P adsorption capacity	Restricted pH range (6-8) to avoid Al biotoxicity	Lake water alkalinity higher than 75 mg L^{-1} and pH of lake water in the range 6-8	Al: P_{Mobile} >10:1 (molar ratio)
	Non redox sensitive	Al is toxic for macroinvertebrates and fish at concentrations as low as 0.1-0.2 mg L^{-1}	Lake water with high concentrations of Si, Ca, SO_4^{2-} and TOC as ligands of Al reduce its toxicity	
	Long-term effect (5-14 years)	Low temperatures in lake water difficult Al floc formation increasing risk of Al toxicity	Lakes with significant vertical P distribution and/or diffusion and polymictic lakes	
		Decrease in pH value of lake water due to formation of Al floc	Lakes in which external P load has been previously reduced	
		Reduction of sediment stability due to Al floc presence being more susceptible to resuspension	No visible effects of long-term effects or biomagnification due to short-time exposure of Al floc (it forms in less than 1 h and settles)	
		Reduction of P adsorption efficiency with ageing of Al floc		
		Chemical interferences with silicate and humic substances reduce P adsorption capacity		
		Additional buffer substances are required in low or moderate-alkalinity lakes to avoid Al toxicity due to solubilisation of the floc		
		Al treatment is not effective in littoral zones due to presence of macrophytes		
		Al can be also toxic even at pH 6-8 if exposures are prolonged		
Fe	Natural P binder in aquatic ecosystems	Redox sensitivity	Well oxygenated water column with Fe:P ratio < 15:1 and Fe:S ratio < 1.2-1.8 (by weight)	Fe:P_{Mobile} >8:1 (molar ratio)
	High affinity and P adsorption capacity	Decrease in pH value of lake water due to formation of Fe oxy-hydroxides	Waters in which P release from sediment is mostly determined by Fe cycle	
		Chemical interferences with humic substances		
		Reduction of P adsorption in anoxic sulphide-rich ambients		
		Reduction of P adsorption capacity due to competition with OH groups at high pH values		
		Additional and repetitive management required to ensure oxic conditions		
		No long-lasting effect due to redox sensitivity		
Ca	No direct adverse effect on macroinvertebrates although indirect effect cannot be rejected	Calcite dissolution at low pH values that reduces P adsorption capacity		No dose determined. Maximum P adsorption capacity of $CaCO_3$: 2.25 mg P g^{-1} $CaCO_3$)
	Relatively inexpensive	Recent technique with more experimentation needed		
	Safe to apply			

The first application of Al to control eutrophication goes back to 1968 in Lake Langsjön (Sweeden) and since then it has become the most popular lake management tool (Cooke et al., 2005). The addition of different Al salts ($Al_2(SO_4)_3$, $Na_2Al_2O_4$ or $AlCl_3$) into a lake results in the formation of a $Al(HO)_3$ floc which presents a high affinity for dissolved and particulate P from water column and sediment (Reitzel et al., 2009). An increase in orthophosphate but also in organic P compounds (phosphate monoesters, DNA-P and P lipids) in sediment after Al treatment has been determined when analyzing sediment samples with ^{31}P NMR spectroscopy suggesting Al floc affinity by both inorganic and organic P compounds (Reitzel et al., 2006). Al is generally preferred to Fe because it is not redox-sensitive. Its potential toxicity at pH below 6 and above 8 implies the restriction of its use to high-alkalinity lakes or the necessity of additional buffered substances to increase neutralizing capacity of the lake (Anderson, 2004). The recommended Al dose was initially based on lake alkalinity (Rydin and Welch, 1998) but recently, the addition of Al relative to P that potentially contributes to internal loading (sedimentary P_{Mobile}) has been reported to increase treatment longevity in terms of TP reduction (Rydin and Welch, 1999; Reitzel et al., 2003; Reitzel et al., 2005; Huser et al., 2015). Fe is considered to be a natural binder of P (Boers et al., 1992) and was firstly used as P adsorbent in Lake Dordrecht (Germany) in 1969. Most common salts used for P adsorption are $FeCl_2$, $FeSO_4$, $FeCl_3$ or $FeClSO_4$ (Hupfer and Hilt, 2008). Although there is evidence that Fe enrichment of sediments may reduce P release from sediment even in anoxic conditions (Quaak et al., 1993) and a ferrous phosphate mineral, vivianite ($Fe_3(PO_4)_2$, can be a long term sink for P retention in anoxic sedimentary conditions (Rothe et al., 2014), the redox sensitivity of Fe and the strong influence of strong alkaline conditions on its P binding capacity frequently require complementary costly techniques (as aeration or artificial circulation) to ensure its long- term efficiency (Mortimer, 1941, 1971; Cooke et al., 2005). Addition of Ca salts [calcite($CaCO_3$) and lime ($Ca(OH)_2$)] induces calcite precipitation which either adsorbs P on its surface or can co-precipitate with it to form minerals such as hydroxyapatite (a calcium phosphate mineral naturally occurring in lake sediments). These minerals constitute strong bounds to P due to a low solubility at pH higher than 9 (Hupfer and Hilt, 2008), however, solubilisation of these minerals at low pH values normally found in anoxic bottom waters due to organic matter breakdown requires additional management (aeration or complete mixing) similarly to the case of Fe addition to avoid internal P loading (Cooke et al., 2005; Dittrich et al., 2011).

Although all in-lake P binding-salts have in common a high affinity for P adsorption and could be a long-lasting sink for P, diverse drawbacks of their application are (i) reduction of P adsorption efficiency with ageing (Al) and the increase of toxicity at high pH values found in eutrophic waters due to solubilisation (Al) or competition with hydroxyl groups (Fe and Al) (Marsden, 1989; de Vicente et al., 2008; Egemose et al., 2009; Reitzel et al., 2013b), (ii) redox sensitivity of Fe (Kleeberg et al., 2013), (iii) reduction of the lake water pH due to formation of Al and Fe flocs (Hupfer and Hilt, 2008; Reitzel et al., 2013b), (iv) reduction of sediment stability hindering macrophytes colonization by the presence of fresh Al floc (Egemose et al., 2009; Egemose et al., 2010), (v) complementary measures such as in-lake addition of buffer substances or frequent aeration and oxygenation are often needed (Hupfer and Hilt, 2008), (vi) short/medium-term effectiveness of adsorbents, being more long lasting Al (5-15 years) than Fe effects (Huser et al., 2015), and (vii) the potential mobilization of inactivated P under changing physico-chemical conditions such as pH, redox potential, temperature, biological activity or resuspension (Sondergaard et al., 1992; Perkins and Underwood, 2001).

In-lake addition of adsorbents whose main objective is to reduce lake water P concentration entail the permanence of the adsorbent within lake sediment and thus, their longevity and effectiveness strongly depend on multiple factors such as: water residence time, water column stability, relative magnitude of internal to external P load, lake morphometry, macrophytes and benthic presence, bioturbation, physic-chemistry of water column, ageing and crystallization of flocs, lake bathymetry, applied dose and watershed to lake area ratio (Huser et al., 2015).

- **Reduction of P release from sediments**

Both hypolimnetic aeration/oxidation (introducing oxygen as air or liquid in the hypolimnion while preserving thermal stratification) and nitrate addition (*in situ* oxidation of surface sediment) aim at reducing organic matter content which is directly related to oxygen depletion, thus increasing P retention capacity and avoiding the presence of reduced species such as Fe^{2+} and Mn^{2+} (Foy, 1986; Cooke et al., 2005). In cases in which Fe:P ratio is not enough to suppress P release from the oxic sediment and the low pH values difficult denitrification it can be necessary the addition of Fe^{3+} and/or $Ca(OH)_2$, which is known as Riplox method (Ripl, 1976). These techniques are mainly recommended in sediments in which P exchange between sediment-water interface is controlled by Fe redox reactions and managers must be aware of their short-term effectiveness since the effects on P cycle are restricted to the period immediately after the application (Hupfer and Hilt, 2008).

Sediment capping is another cost effective method based on the application of a passive or active layer (if additionally binds P) of clean materials (zeolites, sand or calcite) over the contaminated sediment (Jacobs and Förstner, 1999). It primarily conducts to the physical isolation of the contaminated sediment from the water column to stabilize the sediments, prevent resuspension and reduce the release of dissolved contaminants (P and harmful substances) into the water column (Xu et al., 2012). This technique is not recommended in shallow lakes due to the frequent resuspension events (Hupfer and Hilt, 2008) and because its application hinders macrophytes growth, which play a key role in maintaining a clear water state by reducing both nutrient availability for phytoplankton growth and sediment resuspension, providing refuge for zooplankton against planktivorous fish and competing with phytoplankton for light and nutrients (Scheffer et al., 1993).

2.1.3 Increase of P export

Hypolimnetic withdrawal is an in-lake technique based on the selective discharge of bottom water to enhance the removal of nutrients and reduced substances that are released in the anoxic hypolimnion of stratified lakes (Nürnberg, 2007). Main disadvantages of this technique is the high investment required and its impact on downstream waters including eutrophication, temperature increase, unpleasant odour and oxygen depletion (Nürnberg, 2007; Zamparas and Zacharias, 2014). Its effectiveness depends on the water-retention time and the differences in P concentration between the epilimnion and hypolimnion (Hupfer and Hilt, 2008).

External P elimination is another technique that results in an effective removal of P and other toxic substances from deep bottom waters. P-rich bottom waters can be treated in a plant near the shore by different treatments such as precipitation, flocculation, adsorption or filtration. In contrast to hypolimnetic withdrawal, this technology does not cause hydrological imbalance since treated water is returned to the lake by using pipes (Hupfer and Hilt, 2008).

Finally, sediment dredging is the partial or complete removal of sediment layers to remove nutrients, organic matter or toxic substances and it can be intended to control both phytoplankton and macrophytes (Hupfer and Hilt, 2008). Although an efficient and long-lasting reduction on internal P load has been reported in some cases (Welch and Cooke, 2005), this technique has practical considerations such as how much sediment is needed to be to removed, where to put contaminated sediment following removal, and the high cost of the disposal of considerable amounts of sediment (Hickey and Gibbs, 2009). In addition, the increase of turbidity and release of toxic substances during dredging operation are potential drawbacks of this technique (Hupfer and Hilt, 2008).

2.2 Control of physical and biological structure

In deep lakes where summer stratification normally occurs, destratification or artificial mixing of the water column can be used to restore oxygen levels in the whole water column. This technique uses a compressor situated at the lake bed to blow air. The rising bubbles disrupt the thermocline so that deep waters with high content of nutrients are mixed with epilimnetic waters (Klapper, 2003). Although mixing of lakes is rapid, especially if they are small, the release of harmful reduced substances to epilimnion may cause significant ecological impacts like fishkill (Hickey and Gibbs, 2009). The main benefits of artificial mixing are: (i) enlarging the suitable habitat for aerobic animals, (ii) reducing P internal loading and (iii) reducing algal biomass by increasing the mixed depth, thereby reducing available light, and by subjecting mixed algal cells to rapid changes in hydrostatic pressure (Cooke et al., 2005).

Two main strategies that aims at modifying the biological structure can be applied for improving lake water quality: (i) food web manipulation and (ii) macrophytes biomass control. Food webs are either regulated by resources (‘bottom-up’) or by predation (‘top-down’). A reduction of planktivorous fish stock may led to cascade effect on phytoplankton (top-down control) whereas reduction of benthivorous fish minimizes resuspension and internal P load (bottom-up control) (Meijer et al., 1994; Hupfer and Hilt, 2008) and ultimately both led to a shift from turbid to clear water. Biological manipulation has been substantially more successful in shallow lakes than deep lakes, where top-down control is more important and resuspension have more influence in water quality than in deep-lakes (Jeppesen et al., 1997). The more applicable recommendations for biomanipulation include (Hansson et al., 1998): (i) the reduction in the biomass of planktivorous fish should be 75% or more; (ii) the fish reduction should be performed efficiently and rapidly (within 1–3 years); (iii) efforts should be made to reduce the number of benthic feeding fish; (iv) the recruitment of young of the year (YOY) fish should be reduced; (v) the conditions for establishment of submerged macrophytes should be improved and (vi) the external input of nutrients (P and N) should be reduced as much as possible before the biomanipulation.

3. Increase of soluble manganese in lakes and reservoirs as a direct consequence of eutrophication

In many countries heavy metals emissions have diminished due to legislation restrictions and the application of clean technologies (Karvelas et al., 2003). However, the number of anthropogenic sources of heavy metals has increased with rapid developing of industrial and urban areas (Li et al., 2012). Although water treatment plants are expected to control the discharge of metal-contaminated waters to the environment, the reality is that 80-90 % of wastewater generated in developing countries is directly discharged into surface water bodies without any previous treatment (Karvelas et al., 2003; UN Water, 2008) . The inadequate (under-dimensioned or aged) wastewater treatment or its absence may lead to an increase of heavy metal concentration in lakes and reservoirs resulting in hazard effects for human health and ecosystems. Because drinking water is obtained from surface waters (rivers and reservoirs) and groundwater, an optimized drinking water treatment is of fundamental importance for health safety (Fawell and Nieuwenhuijsen, 2003).

Manganese (Mn) is a natural occurring metal present in soils and rocks that constitutes a micronutrient necessary for metabolism of plants and animals (ATSDR, 2000). Mn is found in surface and groundwater due to weathering and leaching of Mn-containing minerals (Bouchard et al., 2011). However, anthropogenic sources such as steel production, fireworks, dry-cell batteries, paints, cosmetics, ceramics, fertilizers, unleaded gasoline and pharmaceutical uses may increase its presence in aquatic ecosystems (ATSDR, 2000). As other metals, Mn does not break down in the environment but it changes its form. The speciation of Mn is of special importance to understand its toxicity, biological activity and its fate in the environment (Chiswell and Mokhtar, 1986). Mn can be found as free metal surrounded by water molecules, forming complexes with inorganic or organic substances or associated with colloids or particulate substances such as clays, Fe oxides or biota. The abundance of each fraction is determined by environmental factors such as potential and abundance of cations, anions, colloids and particulate matter being the most influencing ones pH and redox potential (Chiswell and Mokhtar, 1986). More recently, Peters et al. (2011) developed biological ligand models which assessed Mn biotoxicity as a function of water quality parameters (pH, alkalinity, dissolved organic carbon (DOC), competing ions). These authors found a higher risk of Mn toxicity for fish and invertebrates in acidic and low alkaline waters because of the enhanced biological uptake of Mn in these conditions.

Although Mn may exist in several oxidation states, in freshwater only three are important (II, III, IV) (Davison, 1993). Mn is present in anoxic waters as soluble cation (Mn II) whereas in well oxygenated waters it exists as oxidized and insoluble form (mainly as MnO_2) (Chiswell and Mokhtar, 1986; Chiswell and Huang, 2006). Increase of pH and redox potential lead to precipitates formation due to the oxidation of Mn (II) to Mn (III)/(IV) oxyhydroxides which are less toxic and bioavailable (Harford et al., 2015). Fig. 2 represents the diagrams of different species of Mn as function of pH and redox potential:

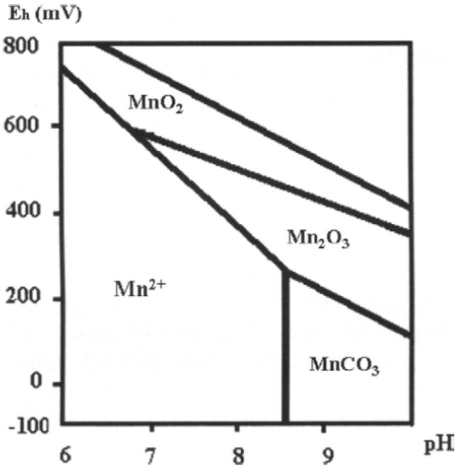

Fig. 2. Eh versus pH for Mn (from Chiswell and Huang, 2006).

A direct consequence of eutrophication-induced anoxia is the enhanced flux of soluble Mn from sediment to water column. That is explained by the reduction of Mn to its soluble form (Mn II) at low redox potential in anoxic bottom waters (Baden et al., 1995). Although soluble Mn is normally found at the anoxic hypolimnion of lakes during summer stratification and in groundwaters, it can also be present in surface waters of deep lakes (i.e Rostherne Mere Lake) during winter due to water column mixing after the complete turnover in autumn (Davison, 1993; Ellis et al., 2000). The low bacterial activity when temperatures are low, the low oxidation rate of Mn in the absence of catalysts or low pH values may explain its presence in well oxygenated surface waters (Harford et al., 2015).

Natural waters with high concentration of soluble Mn may be pumped to water treatment plants and if not oxidized during water treatment, Mn may reach the distribution network generating several technical problems, impairment of water quality and effects on human health (Roccaro et al., 2007). Operational difficulties in water supply network arise when Mn is oxidized by bacterial metabolism or chemical products used for disinfection giving rise to the formation of insoluble deposits that obstruct pipes (Roccaro et al., 2007). Consumers complaints are often received due to staining of laundry, turbidity or unpleasant metallic taste and odour (Bamforth et al., 2006). Manganism, the term for Mn toxicity was originally described by Couper in 1837. From then, extensively reports about neurotoxic effects of Mn have been reported in the literature that comprise memory impairments, deficit in intelligence function in children, insomnia, trembling of fingers and equilibrium loss among others affections (Woolf et al., 2002; Wasserman et al., 2006; Menezes-Filho et al., 2009; Bouchard et al., 2011). Scarce studies about ecotoxicological effects of Mn on aquatic biota are available in the literature since it has been determined to be less toxic than other metals. However, some studies have reported that exposures of fish to Mn cause anemia (Sanders et al., 1998).

Concentrations of this metal in natural waters rarely exceed 1.0 mg L^{-1} being more usual 0.2 mg L^{-1} or less (Sanders et al., 1998). In the last edition of *Guidelines for Drinking-Water Quality*, the World Health Organization (WHO, 2011) did not establish a formal guideline value for Mn concentration in drinking water but it set a health-based value of 400 $\mu g\ L^{-1}$ arguing that this value is "well above the Mn concentration normally found in drinking waters". However, there is a large number of water supplies from many countries affected by Mn concentration higher than 400 µg L^{-1} as the case of Greece, Japan, Bangladesh, Bolivia, and so on (Frisbie et al., 2012). Because all of this, it is important to revise the health-based guideline of 400 µg L^{-1} proposed by WHO bearing in mind that another more conservative maximum level of 50 µg L^{-1} has already been set by the European Directive 98/83/CE and the Environmental Protection Agency (EPA). A lower threshold (10 µg L^{-1}) was even proposed by Chiswell and Huang (2006) since brown-deposits of Mn were observed to occur with values as low as 20 µg L^{-1}.

Several methods can be applied to reduce Mn in drinking water and some of them are the same to those to control eutrophication. In-source water treatments includes techniques such as destratification or hypolimnetic oxygenation that aims at oxidizing dissolved Mn and reduce Mn release from sediment to water, respectively. The first one tends to increase the temperature of water body leading to undesirable effects on phytoplankton species composition and may increase insoluble species of Mn in water column (Chiswell and Huang, 2006). In-plant treatments focus on Mn oxidation with air or oxygen followed by clarification and filtration (Katsoyiannis and Zouboulis, 2004). Because Mn oxidation is slow at pH normally found drinking waters (6-8), the addition of chemical substances such as chlorines, ozone, hypochlorite, or potassium permanganate are often required to achieve its precipitation (Aziz and Smith, 1996). However, the addition of chemical products for disinfection in water treatment processes should be minimized due to the high cost implied, the alteration of water quality (coloration of water and skin irritation when overdosing) and the formation of undesirable by-products harmful for humans such as trihalomethanes (Gallard and Von Gunten, 2002; Roccaro et al., 2007). Biological oxidation of Mn by bacteria of the genera *Leptothrix*, *Crenothrix*, *Hyphomicrobium*, *Siderocapsa* and *Metallogenium* represent a less aggressive water treatment but strongly dependent on the pH of solution and redox potential which must be strongly oxidizing (Mouchet, 1992; Burger et al., 2008). Because of the numerous disadvantages of using traditional techniques to remove Mn from solution, the development of new adsorbents that do not alter water quality and do not depend of redox conditions is of great interest.

4. New tools for improving water quality and lake restoration

To counteract disadvantages of traditional salts, in the late 1990s, Phoslock®, a lanthanum (La)-modified bentonite clay was developed (CSIRO, Australia) as an innovative synthetic material with high affinity to adsorb dissolved P from the water column and increase the sediment P-sorption capacity in a wide range of environmental conditions (pH 5-9) including anoxic conditions (Robb et al., 2003; Meis et al., 2012). By contrast, some studies have reported a low soluble reactive P (SRP) reduction (35-40%, Ross et al., 2008) and even P release from sediment after Phoslock® application (Egemose et al., 2010). Although the affinity of La to bind P is less affected by deviation from neutral pH as compared to Al and Fe (Lürling and Faassen, 2012), a significant reduction of P adsorption efficiency occurs at pH larger than 8 and when increasing alkalinity, having some limitations in hardwater systems (Ross et al., 2008; Reitzel et al., 2013a). Indirect or direct ecotoxicological effect of dissolved La and Phoslock® have been scarcely studied (Barry and Meehan, 2000; Lürling and Tolman, 2010; Spears et al., 2013). Combination of Phoslock® with other techniques with either Al floc (Floc & Lock) or with sediment dredging have been determined to be more effective than when they were individually applied (Lürling and Faassen, 2012; Lürling and Oosterhout, 2013).

Previous studies have shown that the natural zeolites had negligible affinity for P due to the presence of structural negative charges on the zeolite framework (Hrenovic et al., 2008; Lin et al., 2011). However, modification of zeolites with components with high affinity for P may increase their adsorption capacity. Recently, an Al-modified zeolite (Z2G1 or Aqual-P®, Blue Pacific Minerals, Matamata, NZ) has been tested to suppress P fluxes from sediment cores as a trial prior to whole-lake application with good results in aerobic and anoxic conditions showing no toxic effects on phytoplankton and zooplankton species compositions or on freshwater crayfish and fish (Gibbs and Özkundakci, 2011; Landman and Ling, 2011; Özkundakci et al., 2011; Parkyn et al., 2011). Batch experiments carried out by Yang et al. (2014) with raw and zirconium-modified zeolite (ZrMZ) amended sediment determined that treated sediment increased its P adsorption capacity and showed a low desorption of P bound to ZrMZ under common pH (5–9) and reducing conditions. However, P adsorption capacity was highly dependent on pH being ZrMZ only efficient for P removal in neutral or low alkaline conditions. Finally, La oxide-modified zeolite obtained from fly ash was successfully employed by Wang et al. (2016) to reduce P from natural lake waters below the detention limit in recent laboratory scale experiments.

Bephos® is a very novel P adsorbent prepared by embedding Cu, Fe and humic acids in the interlayer of a natural bentonite (Zamparas et al., 2013). The presence of positive and negative charges makes it suitable for the adsorption of anions and cations. Although it presents high P adsorption capacity and high P removal efficiency (80%) whatever the pH between 5-9 (batch adsorption experiments), further research is needed about its performance in natural conditions (lake water and sediment, anoxic conditions) and its potential toxicity.

Previous studies have proposed the use of drinking water treatment residuals (WTRs) to control P from run-off or internal P loading. The high affinity for P is because they are mostly constitutes by Al and Fe oxides used in water purification. Oliver et al. (2011) demonstrated that Fe oxides-based WTRs are able to retain P in both aerobic and anaerobic conditions when performing batch adsorption experiments, however redox sensitivity of these by-products has been proven with the increase of P and Fe in the overlying water under low oxygen saturation (Oliver et al., 2011; Wang et al., 2013) as well as a potential leaching of heavy metals into water column (Boyer et al., 2011).

4.1 Non magnetic iron oxides: the case of CFH-12®

Recently, Lyngsie et al. (2014a) proposed a Fe oxide-based sorbing material (CFH-12®; Kemira Oyj, Finland) to remove P from drain outlets coming from agricultural areas that was determined to have a high P adsorption capacity, fast adsorption kinetic and high stability of P bonding (low desorption). It consists of poorly ordered Fe oxides (oxalate-extractable) and small amounts of Mg and Ca carbonates and Al oxides which are all of them well-known effective P sorbents. Chemical composition along with a high specific surface area (32-44 $m^2 g^{-1}$) that increases with decreasing grain size (from 1 to 0.05 mm) are the main responsible for its high P adsorption capacity (27.59 mg P g^{-1}, Lyngsie et al., 2014b). Apart from high adsorption capacity and fast reactivity, CFH-12® has demonstrated to strongly adsorb P with little P desorption expected when changing physico-chemical conditions. This conclusion stems from the results obtained in the sequential extraction procedure of P-saturated CFH-12® in which less than 5% of P was extracted in the labile pool (distilled water and $NaHCO_3$) whereas P extracted in non labile pool (NaOH and HCl) represented the largest fraction (14-18% and 75-80%, respectively).

4.2 Magnetic microparticles

4.2.1 General considerations and theoretical background

Magnetic nano and microparticles are of great interest today, and Fe is among the most useful magnetic materials (Huber, 2005). Magnetic particles can be synthesized by different methods, being the most important thermal decomposition, sonochemical decomposition, microemulsion, vapor phase, hydrothermal synthesis and co-precipitation. Among all of these methods, thermal decomposition and hydrothermal synthesis provide the best results with a very narrow size distribution and very good shape control of the synthesized particles although high reaction temperatures and pressures are required (Huber, 2005; Lu et al., 2007).

There exists a widespread use of magnetic particles in different areas such as industry, biomedicine, biotechnology, water treatment and bioremediation due to their promising performance. Among all of these applications, the use of magnetic particles to remove contaminants from the environment has attracted special attention (de Vicente et al., 2010; Tang and Lo, 2013). Some examples of this technology is the injection of zero-valent Fe (ZVI) into subsurface forming reactive barriers for the remediation of groundwater (Mueller et al., 2012; Fu et al., 2014) or the use of functionalized/non functionalized magnetite (Liu et al., 2008; Huang and Chen, 2009; Mahdavian and Mirrahimi, 2010; Wang et al., 2010; Warner et al., 2010) or maghemite (Hu et al., 2005; Hu et al., 2006) particles for heavy metals removal in wastewater treatment. Current application of magnetic particles in wastewater treatment could be done either by using magnetic particles as adsorbents for contaminants removal or by using magnetic particles as photocatalysts to convert contaminants into less toxic (Xu et al., 2012).

Apart from high adsorption capacity, fast kinetics and high reactivity for contaminants removal due to their small particle size and high surface area to volume ratio, magnetic nano and microparticles present an important property that makes them different from traditional adsorbents, magnetism (Tang and Lo, 2013). Particle size has important implications in magnetic properties. Larger particles (micrometers) possess a magnetic multidomain structure consisting of regions of uniform magnetization separated by domain walls. By contrast, small particles (nanometers) possess a single domain structure where groups of spins are all pointing in the same direction and acting cooperatively (Philippova et al., 2011). In the absence of magnetic field, the domains are randomly oriented and hence the net magnetization M (sum of all magnetic moments) is zero. As the magnetic field strength H increases, the domain walls begin to move and domains whose moments are aligned with the external field grow at the expense of others being M no longer zero. At very high fields, when all material is occupied by a single domain in which all atomic magnetic moments are aligned with the field, the material becomes saturated. Contrarily to larger particle sizes, very small particles show superparamagnetic behaviour which entails high magnetic susceptibility (high magnetization capacity at low magnetic field strength), negligible remanence (magnetization becomes zero when removing magnetic field) and coercivity (the field required to bring magnetization to zero).

In spite of being effective adsorbents, small magnetic particles have the disadvantage of being kinetically unstable. This affects their long-term efficiency and magnetic properties (Gómez-Pastora et al., 2014). Briefly, small particles tend to form agglomerates due to attractive and van de Waals forces to reduce the energy associated with the high surface area to volume ratio leading to a reduction in surface area and adsorption capacity (Chen et al., 2007; Lu et al., 2007). Likewise, naked metallic nanoparticles are easily oxidized in contact with air due to their high reactivity, resulting generally, in loss of magnetism and dispersibility (Lu et al., 2007). In order to protect them against aggregation and oxidation, magnetic particles can be modified by surface coatings with surfactants or polymers (organic or inorganic). The external coating not only stabilizes the nanoparticles, but can also be used for surface functionalization which aims at increasing the affinity with the adsorbate (Gómez-Pastora et al., 2014).

The use of magnetic particles to adsorb contaminants from the aqueous media is based on the adsorption of the target contaminant on the magnetic particle with its subsequent removal from the media by applying a magnetic separation gradient. Magnetic separation technique is based on the following theoretical background (de Vicente et al., 2010). In the presence of an external magnetic field two types of magnetic interactions occur: particle-particle interactions and magnetic field-particles interactions. The last type of interaction is the one of concern in our study and can be described by the following equation:

$$\overrightarrow{F_m} = (\vec{m}\,\vec{\nabla})\vec{B}$$

where $\vec{B}$ is the magnetic induction or magnetic flux density, $\vec{m}$ is the dipole moment of the magnetic particle. Assuming that the particles are embedded in a non magnetic medium, the magnetic dipole moment can be written as $\vec{m} = \vec{H}\chi V$, where $\vec{H}$ is the magnetic field strength, χ is the magnetic susceptibility of the particles and V is the volume of the particle. Assuming that there are no time-varying electric fields or currents in the medium, $\vec{\nabla}\,\vec{B} = 0$ the magnetic force on particles can be written as:

$$\overrightarrow{F_m} = V\chi\nabla\left(\frac{1}{2}\vec{B}\,\vec{H}\right)$$

where $\frac{1}{2}\vec{B}\,\vec{H}$ is the magnetostatic field energy density. Thus, if $\chi > 0$, the magnetic force acts in the direction of steepest ascent of the energy density scalar field and it is the basis for any magnetic separation application (de Vicente et al., 2010; Lukehart and Scott, 2013).

A deep knowledge about the electrokinetic characteristics of magnetic particles as colloidal systems is also needed in order to understand their behaviour as adsorbents and their potential applications. A colloid is a system consisting of small particles (dispersed phase) uniformly distributed in a dispersant phase (usually a liquid). To consider a system as colloidal, the medium particle size should be of the order or inferior to 1 μm. Most substances including colloidal particles, acquire a surface electric charge when brought into contact with a polar medium due to several causes: (i) differential dissolution of certain ions from the surface, (ii) ionization of surface groups, (iii) replacement of superficial ions, (iv) adsorption of specific ions, and (v) isomorphous substitution (Myers , 1999). As a result of the presence of electrical charges, surfaces exhibit various properties (i.e. electrokinetic properties) that are not present in systems having no surface charge (Myers, 1999). Surface charge of colloidal particles affects the distribution of the ions immersed in the suspension near the surface. Counterions in solution will be strongly adsorbed on the particle surface (by electrostatic or van der Waals forces), forming the so-called *Stern Layer* (Fig. 3). The rest of counterions that are not adsorbed on the particle surface but are distributed in its vicinity influenced by electrostatic forces (attraction of counterions and repulsion of ions of the same charge) and Brownian motion form what is *called Diffuse Layer*. *Stern Layer* and *Diffuse Layer* constitute the so-called *Diffuse Double Layer* (DDL), in which the ion concentration decreases with increasing the distance to the charged particle (Shaw, 1992). The extension of DDL is inversely proportional to the ionic strength of the medium. A high ionic strength in the colloidal system generates a compressing of DDL resulting in decreased repulsion between particles barrier (formation of aggregates) and thus a lower kinetic colloid stability.

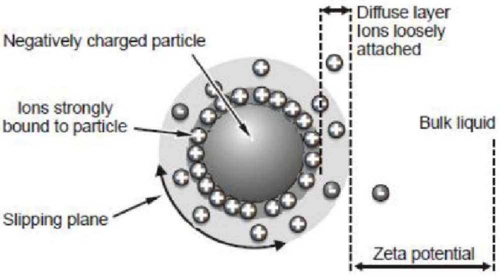

Fig. 3. DDL and zeta potential of a charged particle. Zetasizer nano user manual (Malvern, 2007).

Within the *Diffuse Layer* there is a boundary called *slipping plane*. Any ions within this boundary will move with the particle when it moves in the liquid but any ions outside the boundary will stay where they are. The value of electrical potential at this plane is called *Zeta potential* (or electrokinetic potential) which is a concept of particular importance for understanding adsorption processes and mechanisms of colloidal stability since it determines the value of the electrostatic repulsive forces between the particles. Zeta potential of colloidal particles cannot be experimentally measured but it is inferred from their electrophoretic mobility which can be measured by Laser Doppler Electrophoresis among other techniques. In this technique an electric field is applied to a cell-containing the colloidal dispersion. Charged particles migrate to the oppositely charged electrode and its velocity is measured with the well-known Doppler effect: a particle that is in movement scatters light with a different frequency to the incident beam applied. By measuring this frequency shift, one obtains the particle velocity which is expressed in units of field strength as their mobility (Goff and Luner, 1984).

4.2.2 Magnetic particles as a tool for P removal from aquatic ecosystems

P removal from aquatic ecosystems by magnetic separation techniques can be considered as a promising tool for lake restoration. Magnetic nano and microparticles proposed for P adsorption in this PhD dissertation exhibit Fe oxides on the surface due to their high reactivity when put in contact with air or water. Fe oxides (along with Al oxides) are the most important P adsorbents in natural soils. The hydroxyl (-OH) functional groups of the surface of Fe oxides are the reactive sites in which interactions occur with other atoms or molecules of the media (Cornell and Schwertmann, 1996). The surface charge of Fe oxides is determined by dissociation of these functional groups, which are protonated (acidic media) or deprotonated (basic media) depending on the pH of the medium (Hunter, 1993). P adsorption mechanism on Fe oxides is an exchange reaction where coordinated -OH on the oxide surface (adsorption sites) are replaced by phosphate ions (Borggaard et al., 1990).

Several studies have reported a high adsorption capacity of magnetic particles such as bare magnetite (Dixon, 1984; Shaikh and Dixit, 1992; Daou et al., 2007; Choi et al., 2016) or functionalized magnetite (Lai et al., 2016). Fe carbonyl magnetic microparticles (800 nm), spherical in shape and polydispersed in sized, has been previously determined to have high P adsorption capacity (18.83 mg g^{-1}) and high P removal efficiency which was higher than 80% whatever the pH (5-9) when testing them in batch (de Vicente et al., 2010) and flow conditions (Merino-Martos et al., 2011). Also, these magnetic particles have been demonstrated to be supermaramagnetic materials with high susceptibility and negligible remnant magnetization, both indispensable characteristics to their easy recovery from the systems and their further reuse.

The main advantages of using magnetic particles (in general) as P sorbing materials compared to other adsorbents previously suggested for lake restoration are: (i) magnetic particles can efficiently adsorb P under different working conditions (de Vicente et al., 2010; Merino-Martos et al., 2011) so they can be used for trapping P in rainfall ponds and hence for controlling P input through run-off. Also, they can be applied in water treatment plants and/or point sources under continuous flow conditions; (ii) magnetic particles are able to adsorb considerable amounts of P for pH > 7, despite being negatively charged, due to specific adsorption (Daou et al., 2007; de Vicente et al., 2010), (iii) P adsorption by magnetic particles does not depend on redox conditions (Funes et al., 2016); (iv) magnetic particles addition causes a reduction in sedimentary P_{Mobile} concentration (under both oxic and anoxic conditions) hence contributing to a potential reduction in long-term P efflux; (v) the recovery of P-loaded magnetic particles and its later P desorption (de Vicente et al., 2010) allow its potential further reuse as fertilizer, (vi) based on a preliminary economic balance, Funes et al. (2016) found that when re-using magnetic particles more than 2 times, it is economically worth to use this adsorbent instead of $AlCl_3•6H_2O$ or Phoslock® and (vii) no toxic effect on the planktonic and benthonic organisms are expected to be caused after magnetic particles addition (Álvarez-Manzaneda et al., unpublished results).

In view of the disadvantages of using traditional techniques to improve water quality and taking into account the potential advantages that offer new techniques, in this PhD dissertation we test the use of both CFH-12® and magnetic microparticles as in-lake techniques for adsorbing P in lake restoration. In the case of magnetic microparticles two possibilities of use are proposed: either as P sorbents in the inlets of lakes to reduce external P load or their in-lake addition as P sorbing materials to increase P export since P removed is not available in the systems anymore to be released under changing physico-chemical conditions. Likewise, we propose the use of magnetic microparticles as adsorbents to remove Mn in water treatment plants.

5. Economic balance of different adsorbents

For selecting the most appropriate P adsorbent for a whole-lake application, it is essential to get some knowledge about the economic cost of the lake restoration strategy. Next, we show the results of the economic balance for restoring the highly eutrophic Lake Honda (Almería, Spain) that is suffering high internal P loading. In particular, we have compared some of the most promising P sorbents for lake restoration such as Phoslock®, $AlCl_6$, carbonyl Fe microparticles and CFH-12® (Table 3).

Table 3. Economical balance of P sorbents materials. Please, note that the price of all adsorbents except for the CFH-12® (not available information) has been calculated for a real whole-lake application.

	Price (€ Kg^{-1} product)	**P adsorption capacity (Kg product Kg^{-1} P)**	**Economic cost (€)**
$AlCl_3$ $6H_2O$	4.4	66.4	233922
Carbonyl Fe	6.5	55.0	275827
Phoslock®	2.6	100.0	212000
CFH-12®	4.3	36.3	123943

The amount of each adsorbent to add has been calculated in terms of their P adsorption capacity and the amount of sedimentary P_{Mobile} present within 10 cm depth (8.52 g m^{-2}), taking into account the whole lake area (94000 m^2). The total cost of adding an adsorbent in particular to the lake is directly related to its price and maximum P adsorption capacity. From results we can conclude that the cost of lake restoration decreased in the order carbonyl Fe microparticles, $AlCl_3$, Phoslock® and CFH-12®, being the most expensive technique that based on the addition of carbonyl Fe microparticles and the cheapest CFH-12® (Table 3). However, we have to keep in mind that when re-using carbonyl Fe microparticles more than two times, we get the most profitable P sorbing material.

Objectives

The two primary concerns of this PhD dissertation were: (i) To assess the use of iron (Fe) oxide colloidal suspensions: magnetic particles (commercial carbonyl Fe microparticles and synthesized chitosan magnetic microparticles) and non magnetic Fe oxy-hydroxide (CFH-12®) to remove phosphorus (P) from inland waters; (ii) To evaluate the feasibility of using carbonyl Fe microparticles to remove manganese (Mn) from solution for wastewater treatment.

Specific objectives are as follows:

1. To investigate the effect of pH on Mn removal efficiency in the absence and in the presence of carbonyl Fe particles as well as to describe the mechanism of Mn adsorption by carbonyl Fe particles (*Chapter I*).

2. To identify the optimal conditions for Mn removal by carbonyl Fe particles: (i) contact time, (ii) adsorbent concentration, (iii) effect of surface functionalization on Mn adsorption capacity, (iv) desorption conditions and (v) to test the possibility of reusing carbonyl Fe particles (*Chapter I*).

3. To evaluate the effect of adding carbonyl Fe particles to sediment cores on P efflux and sedimentary P pools as well as on Fe concentration in water column and sediment under oxic and anoxic conditions (*Chapter II*).

4. To set up a long-term enclosure experiment (84 days) as a more realistic scenery to examine the effectiveness of adding carbonyl Fe particles for improving water quality. Specifically, to study (i) the effect of magnetic particles addition on water column P concentration and sedimentary P pools, (ii) the effect of applying magnetic particles on nutrients cycling (carbon, reactive silicate and nitrogen) and (iii) the best working conditions of magnetic particles application to achieve the highest magnetic particles recovery efficiency and the maximum improvement of water quality (*Chapter III*).

5. To design and construct a large-scale magnetic rake to remove magnetic microparticles from the system (in this study, enclosures) with the purpose of minimizing resuspension (*Chapter III*).

6. To synthesize a biocompatible and low density magnetic P adsorbent with the ability of remaining in the upper layers of sediment to be available for P adsorption and being easily recovered by magnetic separation techniques (*Chapter IV*).

7. To assess the performance of two Fe oxy-hydroxide-based compounds, the $Fe(OH)_3$ floc and the dried granulated CFH-12® to reduce internal P loading. Specifically, to examine: (i) the effect of Fe compounds addition on P and Fe efflux in oxic and anoxic conditions, (ii) the effect of Fe compounds addition on other electron acceptors (sulphate, nitrate and Mn), (iii) the effect of the ageing of Fe compounds on P adsorption capacity, (iv) the distribution of CFH-12® within the sediment and (v) the extraction of P and Fe from CFH-12® under strong reducing and acidic conditions (*Chapter V*).

Chapter I : The influence of pH on manganese removal by magnetic microparticles in solution

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Abstract

Manganese (Mn) removal from aqueous solution by carbonyl iron microparticles using magnetic separation techniques. A set of batch experiments are performed to explore the effect of pH, adsorbent concentration, surface coating and contact time for achieving the highest Mn removal efficiency. Mn removal efficiency is extremely high (>98%) for pH values larger than 9 as a result of the chemisorption of Mn oxides onto magnetic microparticles. In contrast, Mn removal efficiency for pH < 9 was significantly reduced as Mn remains as a soluble cation. In this manuscript we demonstrate that the efficiency clearly increases when increasing the adsorbent concentration and when using MnOx(s) coated magnetic particles instead of bare particles. Desorption rates from Mn-loaded magnetic particles at different pHs were always lower than 15%. Furthermore, Mn removal efficiency remained at a very high value (>95%) when reused particles were employed in the adsorption process.

1. Introduction

High concentrations of Manganese (Mn) can be found in water supply network because of its solubilization under hypoxic conditions (<16% oxygen saturation) (Baden et al., 1995; Zaw and Chiswell, 1999). Some of the problems associated with high Mn levels are the obstruction of pipes, addition of unpleasant taste to water, staining of laundry (Bamforth et al., 2006; Tekerlekopoulou et al., 2008) or toxicity for human health (Elsner and Spangler, 2005).

Mn concentration in fresh waters broadly ranges from 1 to 200 mg L^{-1}, being more usual values below 10 mg L^{-1} (Barceloux, 1999; Keen and Lonnerdal, 1986). European Directive 98/83/CE as well as the Environmental Protection Agency (EPA) set a maximum Mn concentration level of 50 mg L^{-1} (U.S Environmental Protection Agency, 2012). On the other hand, World Health Organization (WHO) suggests a health-based value of 400 mg L^{-1} (WHO, 2011). Nowadays, a large number of water resources all over the world are being affected by Mn concentrations that exceed 400 mg L^{-1} such as Greece, Japan or Australia, among others (Frisbie et al., 2012).

In order to meet the quality standards for drinking water, a wide variety of physical and chemical methods, or combinations of both of them, have been used for reducing Mn concentration of polluted waters. Since Mn remains as soluble cation in a wide pH range (4.5-8) it is a difficult and costly process to remove it from solution (Bamforth et al., 2006). Up to now, natural adsorbents have been used, including activated carbons (Emmanuel and Veerabhadra Rao, 2009; Jusoh et al., 2005), zeolites (Doula, 2006, 2009; Erdem et al., 2004; Taffarel and Rubio, 2009) and kaolinites (Yavuz et al., 2003). The use of strong oxidizing chemicals (potassium permanganate, ozone, etc) for oxidation and precipitation of Mn are also common techniques used in water treatment plants (Gantzer et al., 2009; Kohl and Medlar, 2006). In addition, there exist biological treatments usually based on the use of Mn oxidizing bacteria coated beds that promote Mn biofiltration under oxic conditions and its removal from the solution (Burguer et al., 2008; Pacini et al., 2005; Tekerlekopoulou et al., 2008). Most of these techniques are not profitable since in many cases exists high operational cost, insufficient adsorption capacity, difficulties of separation and regenerations of the adsorbents or formation of undesirable by-products that can alter water quality (Katsoyiannis and Zouboulis, 2004; Yong-Mei et al., 2010).

In recent years, the application of magnetic particles for removing contaminants from the aqueous effluents has attracted special attention (Ngomsik et al., 2005). This technology involves the separation of the adsorbent (magnetic particles) from the medium in which is immersed by applying a gradient magnetic separation, once the contaminant is adsorbed on its surface. Magnetic field generated by a magnet exerts a force on magnetic particles proportional to the volume of the particles, magnetic susceptibility of the particles, magnetic induction and magnetic field strength (de Vicente et al., 2010; Merino-Martos et al., 2011). A recent example of the application of this innovative technology is the use of magnetic particles for Phosphorous (P) removal from aqueous solutions, the main cause of eutrophication (de Vicente et al., 2010). In this study, authors reported a high efficient removal process (>80%) in a wide pH range (5-9). Other authors have used functionalized magnetic nanoparticles as adsorbents for the removal of heavy metals such as Cu^{2+}, Hg^{2+}, Cd^{2+}, Ag^{+}, Ni^{2+}, Pb^{2+} from the aqueous solution (Ge et al., 2012; Huang and Chen, 2009; Yong-Mei et al., 2010; Song et al.,

2011) or for the extraction of trace metals present in biological samples with excellent results (Huang and Hu, 2008).

For making best use of magnetic particles, and in particular, for guaranteeing the colloidal stability of the particles in the medium and for preventing their degradation in contact to oxygen or other chemicals, magnetic particles are frequently coated with organic or inorganic core-shell structures or dispersed in polymers-containing matrices such as silica or carbon (Lu et al., 2007). Moreover, shells provide the ability to functionalize the magnetic particles by specific ligands such as catalysts or special functional groups that enhance the affinity with the adsorbate (Philippova et al., 2011; Zhang et al., 2011). MnOx(s) minerals occur as coating or fine-grained aggregates in soils and aquatic ecosystems and play an important role in chemical reactions (Post, 1999). They are thought to be important resources for metalloids immobilization by mechanisms as adsorption or surface precipitation (della Puppa et al., 2013). MnOx(s) have a large surface area and a low pH_{pzc} (2.8-4.5) which result in a negative surface charge in a wide range of environmental conditions (Feng et al., 2007; Posselt et al., 1968).

Therefore, Mn removal by magnetic particles could be considered as a promising technology for water quality treatment. Among the most outstanding advantages of using these particles, it can be suggested: (i) high adsorption capacity due to high specific surface of the adsorbents, (ii) easy and efficient recovery of the adsorbents from the solution due to magnetic properties, minimizing alterations of water quality, (iii) the reusability of the adsorbents contributing to economic cost savings and (iv) the possibility to functionalize the particles for specific purposes (de Vicente et al., 2010; Singh et al., 2011; Zhang et al., 2011). Even more, due to their advantages as cation adsorbents, we hypothesize that covering the surface of magnetic particles with MnOx(s) can be an excellent optimization strategy for Mn removal from aqueous solutions.

In this context, the main aim of this study was to assess the suitability of using bare and MnOx(s) coated magnetic particles for Mn adsorption and its subsequent removal from aqueous solution by applying a high gradient magnetic separation technique. For this purpose, a set of batch experiments were carried out in order to determine the optimum pH for an efficient Mn removal and to obtain the maximum adsorption capacity. In addition, the Mn desorption from Mn-loaded magnetic particles, at different pH, and the reusability of magnetic particles was tested.

2. Materials and Methods

2.1 Materials

Iron (Fe) microparticles were supplied by BASF SE (grade CC, Germany) and used as adsorbents. The bulk composition of the particles consists essentially of iron (more than 99.5%). All chemicals were obtained from Panreac (Spain), except Mn stock solution ($Mn(NO_3)_2$) which was supplied by Merk (Germany). All suspensions were prepared by using deionized and filtered water (Milli-Q Academic, Millipore, France).

2.2 Characterization of magnetic particles

A LEO Gemini 1530 field-emission scanning electron microscope (FESEM) was used to estimate the size and morphology of the bare magnetic particles. The samples were prepared by drying a drop of a magnetic dispersion onto a glass substrate. After that, a thin coating of carbon was applied. To elucidate whether Mn is oxidized and/or adsorbed onto bare magnetic particles surface after an adsorption experiment at pH 9, surface morphology and qualitative chemical composition of the particles after adsorption tests was investigated using an Auriga Cross Beam (FIB)® workstation (Carl Zeiss SMT, Inc.) adapted to a field emission high resolution scanning electron microscope (FESEM) and equipped with energy-dispersive X-ray analysis (EDX) (INCA 300, Oxford Instruments). Spectra were obtained in a range of 0-20 keV. Quantitative chemical composition of magnetic particles surface (before and after adsorption tests) was analyzed using a Kratos Axis Ultra-DLD X-ray photoelectron spectrometer. The wide spectrum (pass energy of 160 eV) and individual spectra (pass energy of 20 eV) were obtained using an electrostatic lens. The samples were prepared for a microscopic inspection immediately after an adsorption experiment. Adsorption experiments typically comprised a 1 g L^{-1} of bare magnetic particles stock suspension and 100 mg L^{-1} of Mn stock solution that are then mixed at pH 9. After adsorption of Mn, samples were dried for 24 h at 60 ºC and analyzed.

The magnetization measurements of the bare magnetic particles were carried out at room temperature (25 ºC ± 5 ºC) as a function of the applied external magnetic field strength with a Quantum Design (San Diego, CA) MPMS-XL5.0 Tesla Magnetometer. The external field was swept from -1500 to 1500 kA m^{-1}.

Electrophoretic mobility of bare magnetic particles was determined using a zetameter (Zetasizer Nano Z, Malvern instruments, Germany) at 25 ºC ± 5 ºC using the Laser Doppler Micro-electrophoresis technology. Briefly, suspensions with bare magnetic particles and 3 mM $NaHCO_3$ were first agitated with an orbital shaker for 24 h at the desired pH value. After this time, pH value was readjusted just before measurement and the suspensions were made up to 50 mL of volume. To minimize sedimentation of magnetic particles under gravity, prior to the test, the samples were sonicated for 5 min. Each mobility data point reported in this work is the average of three measurements in the course of 10-40 "runs".

2.3 Preparation of magnetic colloidal dispersions

The stock dispersion of bare magnetic particles was prepared by adding 5 g of Fe particles to 100 mL of Milli-Q water, obtaining a final concentration of 50 g L^{-1} of magnetic particles. This stock suspension was thoroughly sonicated for 5 min to minimize colloidal aggregation and sedimentation under gravity.

Dispersions of MnOx(s) coated magnetic particles were also formulated. For this purpose, a volume of 1 mL of bare magnetic particles stock suspension (50 g L^{-1}) were shaked (150 rpm, in a horizontal shaker) for 24 h with 3 mM $NaHCO_3$ at room temperature 25 ºC ± 5 ºC. 2 mL of Mn stock solution (1000 mg L^{-1}) was added to the suspension and pH value was adjusted to pH = 9 with 1 N NaOH. After 24 h of agitation, pH was readjusted and suspension was made up to 50 mL of volume. The appearance of black-brown precipitates indicated presence of MnOx(s) (Lloyd et al., 1983). A magnetic separation gradient of approximately 12,400 kA m^{-2} (PASCO scientific; EM-8641) was applied to the suspension for 5 min and all the supernatant was removed. MnOx(s) loaded magnetic particles were washed twice with 1 N NaOH and once with 3 mM $NaHCO_3$. MnOx(s) coated magnetic particles were then dried in an oven at 60 ºC for 24 h and stored.

2.4. Kinetic stability of magnetic suspensions

Turbidity measurements were performed using a Turbiscan MA 2000 (Formulaction, Toulouse, France) in order to investigate the formation of MnOx(s) precipitates in solution and the kinetic stability of iron suspensions. In a typical essay, the dispersion is placed in a cylindrical plastic cell which is scanned from the bottom to the top with an incident near infrared light source (λ_{air} = 850 nm). A transmission sensor (180º from the incident radiation) receives the light transmitted through the sample and, a backscattering sensor (45º from the incident radiation) detects the light backscattered by the sample, obtaining transmission and backscattering data every 40 mm (Wulff-Pérez et al., 2009). Turbiscan software provides curves that correspond to transmission data as a function of sample height (mm) at a certain time. The measurements are repeated over time, resulting in several overlaid curves that provide information about the stability of dispersion over time. Measurements consisted in analyzing the optical transmission of the samples at different conditions (presence or absence of magnetic particles and Mn) and at different pH values (7 and 9).

2.5. Oxidation and adsorption experiments

To determine Mn oxidative removal by O_2 (that is, in the absence of magnetic particles), 0.1 mL of a Mn stock solution (1000 mg L^{-1}) was mixed with 40 mL of 3 mM $NaHCO_3$ and pH value was adjusted in the range of 6-10 by adding 1 N NaOH or HCl. The solution was left under shaking (150 rpm, in a horizontal shaker) for 24 h (after that time, black-brown precipitates indicated the presence of MnOx(s) in solution) and pH value was readjusted. Suspension was made up to 50 mL of volume and left to sediment for 5 min. After that, the supernatant was pipetted off and filtered (Whatman GFF) to be analyzed for Mn concentration following the spectrophotometric procedure using 1-(2-pyridyl-azo)-2-naphthol (PAN) colorimetric method, which is a slight modification of that proposed by Goto et al. (1977). Finally, Mn oxidative removal only by O_2 was calculated as follows:

$$Mn_R = \frac{C_0 - C_e}{C_0} 100 \qquad (1)$$

where C_0 is Mn initial concentration (mg L^{-1}) and C_e is Mn equilibrium concentration (mg L^{-1}), that is, at the end of shaking time.

The effect of pH on Mn removal by both bare magnetic particles and MnOx(s) coated magnetic particles was tested in batch mode as follows: (i) A mixture of 1 mL of bare/ coated magnetic particles stock solution (50 mg L^{-1}) and 40 mL of 3 mM $NaHCO_3$ was shaked (150 rpm) for 24 h. (ii) 0.1 mL of Mn stock solution was added to the magnetic suspension and pH was adjusted to different values (6-10 in case of bare magnetic particles and 6-9 in the case of coated magnetic particles) by adding 1 N NaOH or HCl.

Suspensions were shaked (150 rpm) for 24 h. After that time, pH was readjusted and made up to 50 mL of volume with 3 mM $NaHCO_3$. (iii) Magnetic separation was later carried out for each magnetic suspension. It consisted on applying a magnetic field gradient for 5 min (PASCO scientific; EM-8641) (de Vicente et al., 2010). Supernatant was pipetted off and filtered to measure Mn concentration in solution with the above mentioned spectrophotometric procedure. The similar methodology was repeated for different pHs in the range from pH 6 to pH 10. The efficiency of Mn removal process by magnetic particles, Mn_R, is defined as the relationship between adsorbed Mn concentration and initial Mn concentration according to equation (1).

The kinetics of the adsorption process by bare magnetic particles at pH = 9 was also measured in order to determine the necessary time to reach the equilibrium conditions at room temperature. For such study, a typical adsorption experiment was carried out with an initial Mn concentration of 40 mg L^{-1} keeping magnetic suspension and Mn solution under different contact times (1, 2, 5, 10 and 24 h) at pH 9. A magnetic field gradient was applied for 5 min and Mn concentration was determined as previously mentioned. Mn adsorbed per unit amount of adsorbent, q (mg g^{-1}), was estimated using the following expression:

$$q = \frac{C_0 - C_e}{M_a} V \tag{2}$$

where M_a is the mass of adsorbent in g (in our case, 0.05 g of magnetic particles) and V is the total volume of suspension (L). To ascertain the influence of adsorbent concentration (bare magnetic particles) in Mn removal and adsorption capacity, q, different volumes (2 and 4 mL) of a magnetic particles stock suspension (50 g L^{-1}) were put in contact with a Mn concentration of 2 mg L^{-1} in the pH range of 7-9 as in a typical adsorption experiment. Afterward, a magnetic separation gradient was applied and Mn concentration was determined.

Finally, and in order to achieve the maximum adsorption capacity of MnO(x) coated magnetic particles, the adsorption isotherm was obtained after running a typical adsorption experiment, as it was described above, at the following operational conditions: pH = 7 and initial Mn concentrations: 0; 0.2; 2; 5; 25; 50; 100; 200 and 500 mg L^{-1}. Adsorption isotherms were later obtained by plotting q against C_e. The most commonly used equations for describing P sorption (adsorption and precipitation) are Langmuir and Freundlich models, characterized each one by different assumptions. As Langmuir equation assumes that a monolayer of adsorbed molecules is formed on the surface and an adsorption maximum is achieved as the monolayer becomes filled by the adsorbate (which is actually not our case); our data were fitted to the Freundlich equation:

$$q = K_{F\,C_e}{}^{n} \tag{3}$$

where K_F and n are positive-valued adjustable parameters.

2.6. Desorption study

Firstly, an adsorption experiment with 1 g L^{-1} of magnetic particles and 100 mg L^{-1} Mn was carried out at pH 9. After the extraction of supernatant, magnetic particles were left for shaking (24 h) with 3 mM $NaHCO_3$ at different pH values (6, 7, 8 and 9). After making up the volume a magnetic separation was applied and Mn measured. The percentage of desorbed Mn, d, was calculated with the following expression:

$$d(\%) = \frac{C_d}{C_i}100 \tag{4}$$

where C_d represents the desorbed Mn concentration and C_i is the initial Mn concentration.

2.7. Reusability of magnetic particles

In order to evaluate the adsorption capacity of reused magnetic particles, an adsorption experiment was carried out using an initial Mn concentration of 100 mg L^{-1} at pH 9 and at room temperature. Two different sets of desorption experiments were later performed. In the first one, magnetic particles with adsorbed Mn were washed 2 times with 1 N NaOH and 3 times with 3 mM $NaHCO_3$. The magnetic particles were dried at 60 ºC for 24 h and then reused in a new adsorption experiment. In the second experiment, magnetic particles with adsorbed Mn were directly reused, that is, without washing the particles previously.

2.8. Statistical analysis

All experiments reported in this study were carried out in triplicates. Comparisons of means were performed using oneway ANOVA test and then Tukey's HSD test was applied, considering significant differences when $p < 0.05$. In order to verify ANOVA assumptions, data were transformed to logarithmic in some cases. ShapiroeWilk test to check normality and a Bartlett's test to check homogeneity of variances were performed. Statistical analysis was done using R software (R Development Core Team, 2012).

3. Results and discussion

3.1. Characterization

An SEM image of the bare magnetic particles used in this work is shown in Fig. 1a. The micrograph shows that magnetic particles used as adsorbents are quasi-spherical in shape and very polydisperse in size. The mean particle size is 3.8-5.5 mm as specified by the manufacturer (BASF SE). Particle size has important implications for adsorption capacity and magnetic separation techniques. Small particles have higher specific surface area, and thus, more adsorption capacity than larger particles. However, for such small particles it is necessary to apply a higher magnetic separation gradient. On the other hand, large particles tend to sediment faster under gravity. Recently, it has been reported in the literature that micron sized iron particles present a reasonably large P adsorption capacity and are easy to separate using conventional high gradient magnetic separation techniques (de Vicente et al., 2010).

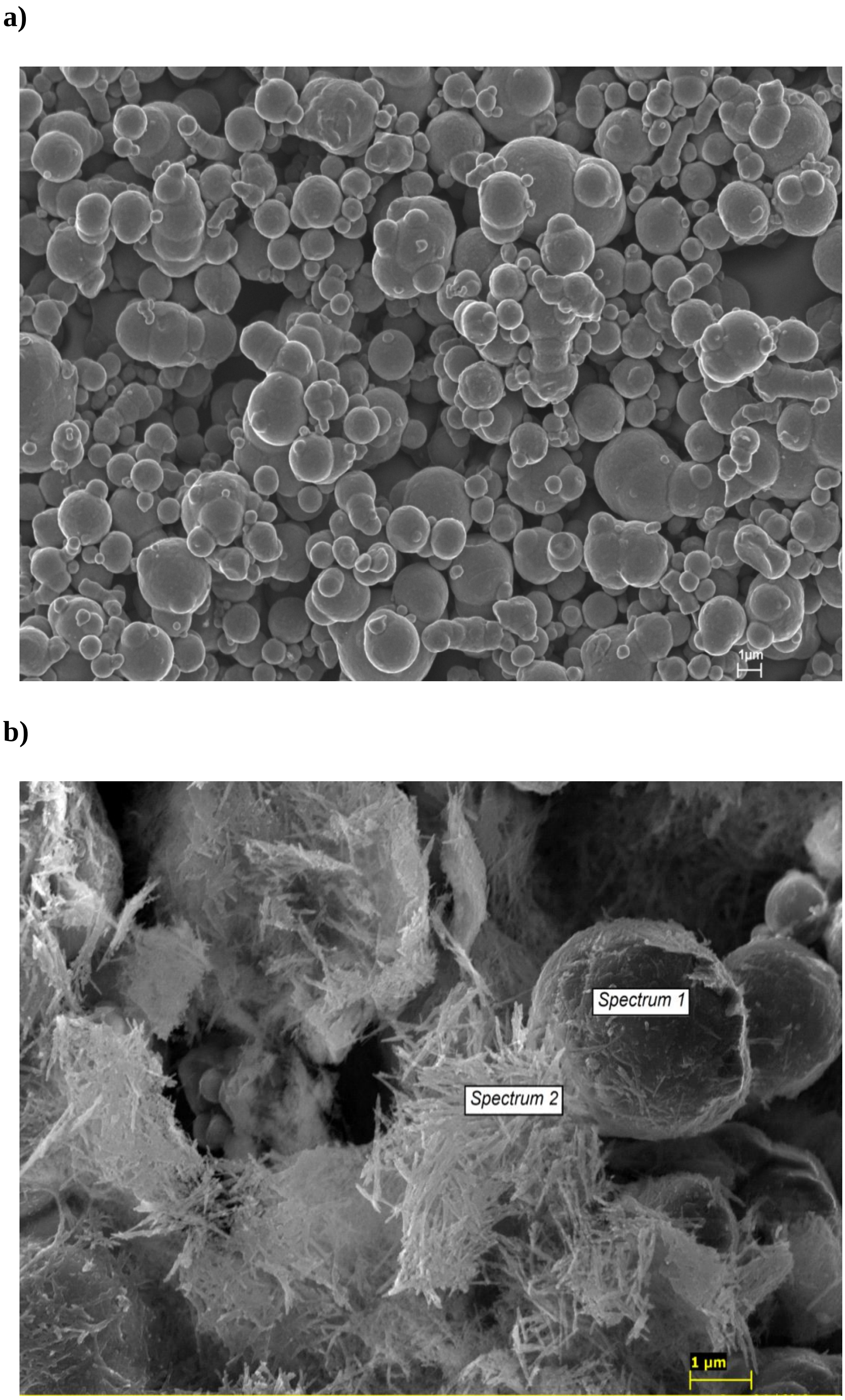


Fig. 1. HRSEM micrographs of magnetic particles. a) Magnetic particles before the adsorption experiment. b) Magnetic particles after Mn adsorption experiment. Initial

conditions: 100 mg Mn L^{-1}; pH = 9. Please note that EDX analysis of Spectrum 1 and 2 regions is shown in Fig. 7.

Bare magnetic particles (before adsorption test) are constituted of iron (97%), as it has been reflected by atomic absorption analysis of the bulk (data not shown). However, other chemical elements (carbon, oxygen and nitrogen) are also present in smaller amounts in the bulk (3%) as a result of the thermal decomposition process that was employed in the fabrication of the particles from pentacarbonyl iron. Table 1 shows the surface chemical composition of the particles as obtained by XPS. Both, before and after adsorption tests, more than 80% of the surface chemical composition is represented by C and O probably coming from surface oxidation and environmental "advent tious" contamination.

Table 1. Surface chemical composition of bare magnetic particles before and after adsorption tests by XPS technique. Initial conditions: 1 g L^{-1} of magnetic particles; 100 mg Mn L^{-1}; pH = 9.

Chemical element	**Atomic concentrations (%)**	
	Before adsorption test	**After adsorption test**
Fe	2.78	3.53
C	30.83	65.93
O	49.79	22.53
SiO_2 coating	6.70	1.27
Mn	0.00	6.74

The magnetization curve for bare magnetic particles used in this work is shown in Fig. 2. As observed in the figure, particles behave as soft magnetic materials with negligible coercitive field and remnant magnetization; magnetization becomes zero when removing the field. As a consequence, they are expected to be reutilized after adsorption and accordingly, particles should easily redisperse when removing the magnetic field.

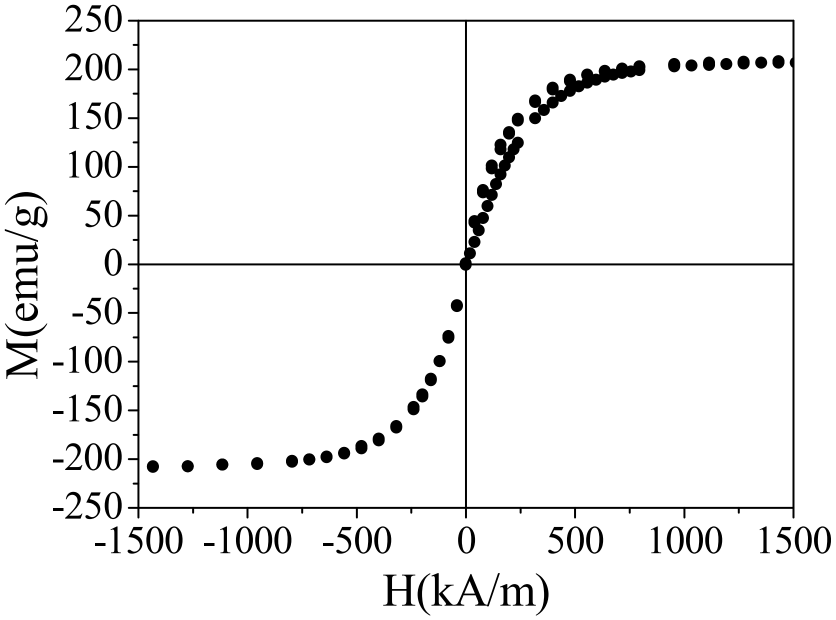


Fig. 2. Hysteresis curve of the magnetic particles. M: particle magnetization, H: magnetic field strength.

Table 2 shows the electrophoretic mobility of the magnetic particles as a function of pH. The results show that magnetic particles are negatively charged in the pH range studied (pH = 6-10). Data reveal that the magnitude of the surface charge increases when increasing pH value, as it is usual for inorganic oxides. Magnetic particles employed in the present study exhibit an oxidized surface layer with amphoteric functional groups (-OH) which determine the surface charge of magnetic particles depending on pH (Hunter, 1993). As a consequence, it is expected that the electrokinetic properties of the particles may play a role in the adsorption capacity.

Table 2. Electrophoretic mobility of bare iron particles as a function of pH value.

pH	**Electrophoretic mobility (μm cm $V^{-1}s^{-1}$)**	**Standard Deviation**
6	-2.446	0.010
6.5	-2.772	0.036
7	-3.009	0.046
8	-3.590	0.060
8.5	-3.730	0.077
9	-3.914	0.042
10	-4.217	0.066

3.2. Manganese oxidative removal by O_2

Mn is oxidized by O_2 in the range of pH 7-9 resulting in different Mn oxyhydroxides species which precipitate (Hem, 1981). The Mn oxidation reaction is slow (but not negligible) when the pH value of the solution is <9 (Sung and Morgan, 1981) and it becomes autocatalytic when pH is close to 9 (Kessick and Morgan, 1975). Thus, it is relevant to know the magnitude of Mn oxidation and precipitation as a function of pH in our experimental conditions. Our results demonstrate a strong dependence of Mn removal on pH values (see squares in Fig. 3). Remarkably, up to 100% of Mn removal is achieved for pH levels higher than 9, which is presumably due to the formation of large precipitates that become entrapped by the filters employed in the adsorption process as described in Section 2.5. By contrast, significantly lower Mn removal efficiencies (<20%) were obtained when pH was below 8.5.

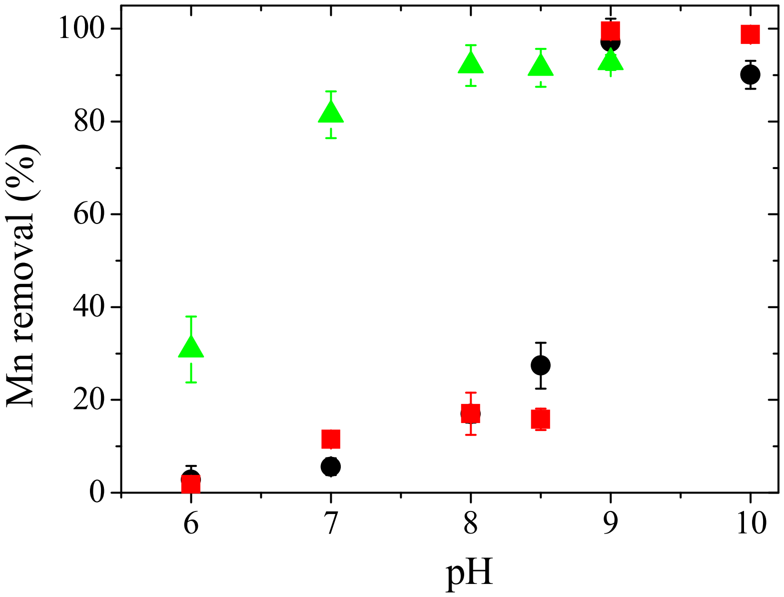
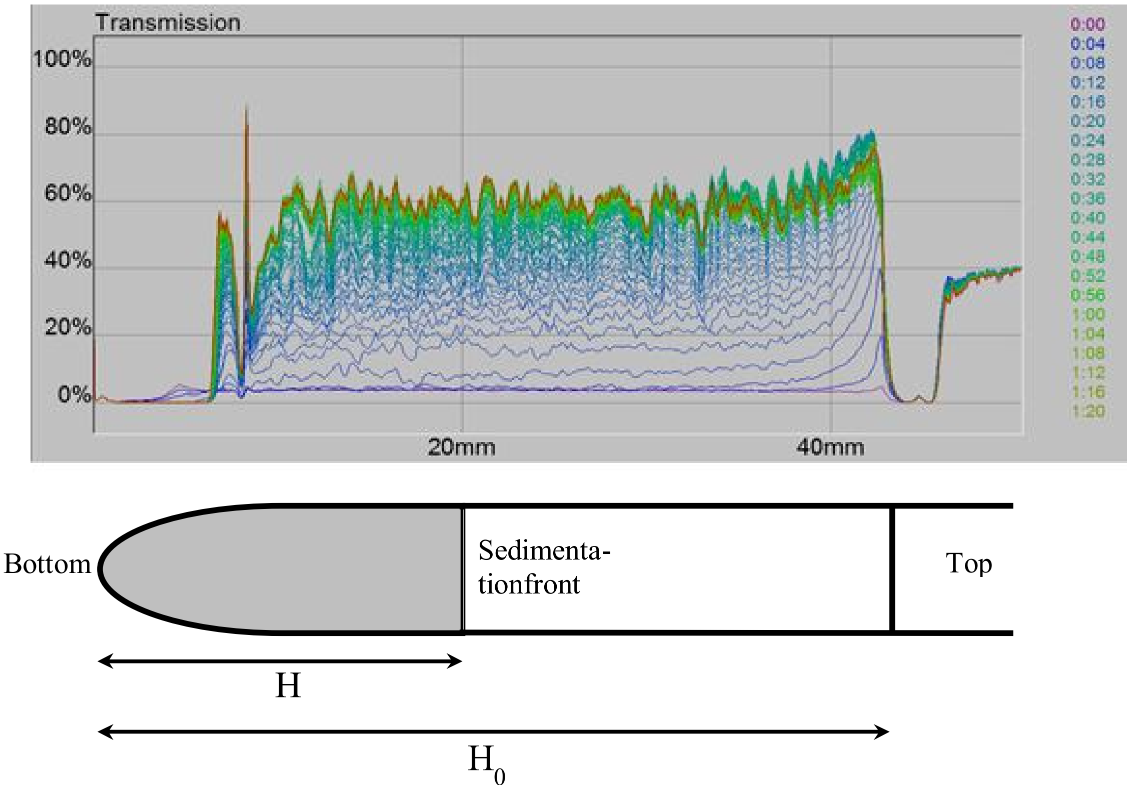


Fig. 3. Effect of pH on Mn removal efficiency, Mn_R. The standard deviation is represented by vertical bars. Squares: without magnetic particles in suspension; circles: with bare iron particles. Triangle: with MnOx(s) coated iron particles. Initial conditions: 1 g L^{-1} magnetic particles; 2 mg Mn L^{-1}.In both, with and without bare magnetic particles, Mn removal only takes place when Mn oxidizes, that occurs at pH higher than 9. By contrast, MnOx(s) coated iron particles clearly enhance Mn removal at all pH values.

Mn precipitation was also monitored by using turbidity measurements. In Fig. 4 we show the transmission profiles in reference mode (by subtracting the curve at time zero) along the tube for different times up to 80 min. Fig. 4a clearly shows that both sedimentation and flocculation processes are occurring in Mn solutions at pH 9. The sedimentation (gravity driven particle migration) process is observed as a result of the variations of the optical transmission profiles at the bottom and the top of the sample. In other words, it is evidenced by an increase in the transmission at the top of the sample due to a decrease of the concentration of the dispersed phase (clarification) and by a concomitant decrease in the transmission at the bottom of sample (sedimentation). Flocculation of the dispersed phase is manifested by an increase of optical transmission (decrease of the backscattering) over the whole height of the sample. As a way of illustration, Fig. 4b shows the temporal variation in the transmission data (in %), averaged in a middle height (between 15 mm and 25 mm from the bottom) of the sample tube, at two different pH values (7 and 9). As it can be observed, temporal changes in transmission were almost negligible at pH 7 in accordance with a stable solution that is not aggregating. On the contrary, transmission values at pH 9 experienced a marked temporal increase from 5% to 50% after 60 min as a result of the oxidation of dissolved Mn by O_2 and the eventual precipitation of the Mn oxides.

a)



b)

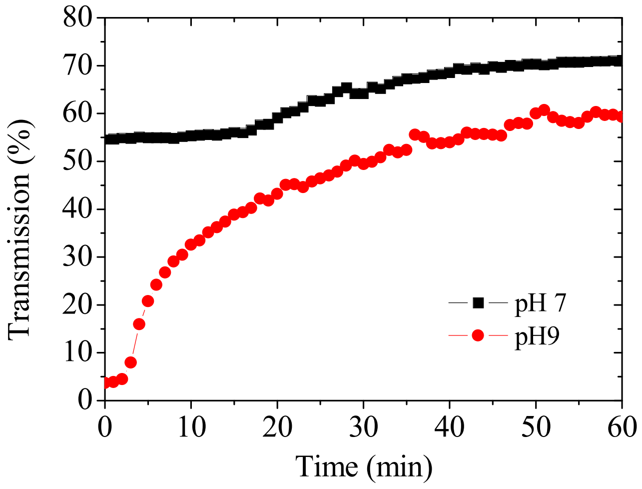


Fig. 4. Turbidity measurements of Mn solutions(without particles). a) Transmission (%) as a function of the height of measuring cell (mm) in reference mode (subtracting the curve at time zero). Each curve represents a transmission measure every 4 minutes. Initial conditions: 400 mg Mn L^{-1}; pH = 9. b) Averaged transmission (between 15mm and 25mm from the bottom) as a function of time at two different pH (pH =7 and pH = 9).

Initial conditions: 400 mg Mn L^{-1}; Changes in transmission over time come from the formation and sedimentation of MnOx(s) precipitates.

3.3. Mn removal by bare iron microparticles

The effect of pH on Mn removal, Mn_R, by bare magnetic particles is shown in Fig. 3 (circles). As it can be observed, Mn removal dramatically increased when increasing pH. No significant differences in Mn removal were observed ($p > 0.05$) neither between pH 6 and 8 nor between pH 9 and 10; while significant differences were found between pH 8 and 9. The same tendency in Mn uptake has been found by other authors when using adsorbents with similar surface composition (iron oxides) such as lepidocrocite (γ-FeOOH), akaganeite (β-FeOOH), hematite and goethite (α-FeOOH) (Davies and Morgan, 1989; Han et al., 2012; Kanungo, 1994; Stumm, 1993; Sung and Morgan, 1981). When comparing the Mn removal in the absence and in the presence of magnetic particles, we can observe that the particle effect was negligible, as Mn removal was close to 100% at pH 9 in both cases and it strongly decreased for pH lower than 9 (c.f. Fig. 3). Only at pH 8.5, Mn removal was significantly higher in the presence of magnetic particles than in the absence of these particles. An opposite tendency was observed at pH 10.

As a result of the striking differences in Mn removal efficiency depending on pH, next we analyzed in more detail if oxidation and precipitation mechanism take place in solution or on magnetic particles surface at pH 9 by using both turbidity measurements and SEM analysis. Then, we studied the adsorption mechanism at intermediate pH (between 6 and 9).

3.3.1. Oxidation at high pH (pH≥9)

As it was stated above, Mn ions precipitate in solution rather quickly at pH higher than 9. In fact, the kinetic isotherm has shown that Mn removal in the presence of bare magnetic particles was extremely fast (see Fig. 5). Hence, the precipitates do form in less than 1 h and so, the time period required for the system to reach equilibrium is very short. These results are similar to those obtained by Song et al. (2011) when using polyrhodanine-encapsulated magnetic nanoparticles for Mn adsorption at pH = 4. This fast removal may be due to the catalytic and autocatalytic mechanisms of Mn oxidation favored by suitable pH conditions (see section above) and it represents an important advantage for using magnetic particles as it makes the whole process less time consuming reducing economic costs.

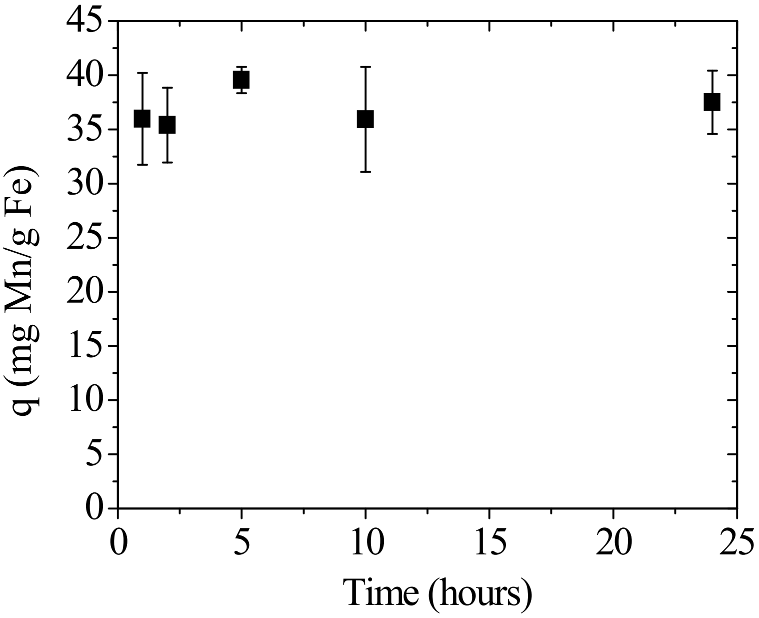


Fig. 5. Kinetics of Mn adsorption. Initial conditions: 40 mg Mn L^{-1}, pH = 9. The standard deviation is represented by vertical bars.

The effect of the presence of bare magnetic particles on MnOx(s) precipitation and sedimentation process is shown in Fig. 6. It represents the time evolution of the sedimentation front H at a constant value of transmission for three different systems: bare magnetic particles (squares); Mn solutions (circles) and magnetic particles dispersed in Mn solutions (triangles). In all cases the sedimentation front experienced a marked decrease over time reaching a constant value of 20% after 10 min that is in accordance with a sedimentation process. Importantly, remarkable differences among the systems studied came up. On the one hand, the sedimentation of bare magnetic particles occurred very fast. Actually, the iron particles started to sediment immediately after positioning the tube in the Turbiscan device (in less than 1 min). On the other hand, the sedimentation of MnOx(s) was the slowest probably due to the lower density of the MnOx(s) precipitates if compared to the iron particles. Results suggest that magnetic particles may affect MnOx(s) precipitation in two possible ways: Mn is precipitated on bare magnetic particles surface and sediment together, or bare magnetic particles drag along MnOx(s) formed in solution toward the bottom of the tube.

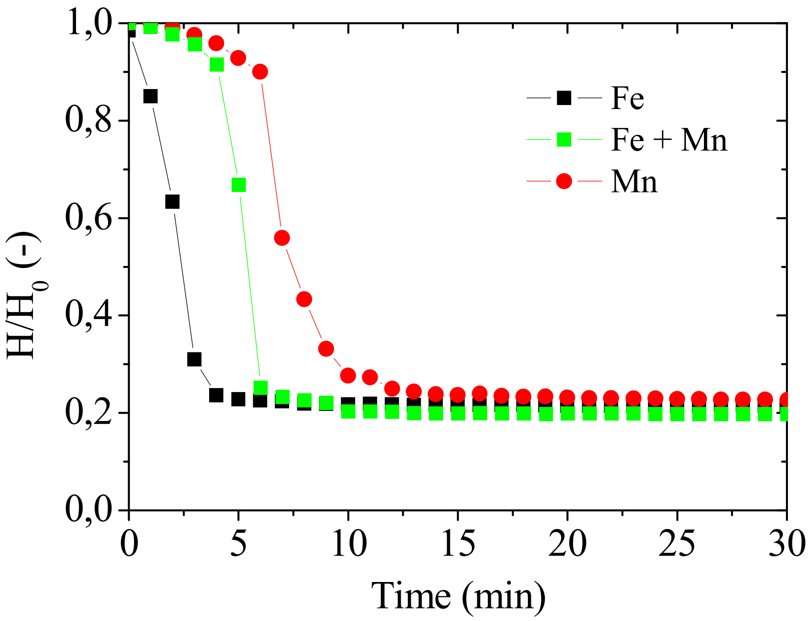


Fig. 6. Turbidity measurements of bare iron suspensions (Fe); Mn solution (Mn) and magnetic iron particles dispersed in Mn solutions (Fe + Mn). H= sediment front as determined at a constant value of transmission (around 30 %); H0= initial sample height (at time zero). Initial conditions: 1 g magnetic particles L^{-1}; pH=9; 400 mg Mn L^{-1}.

In Fig. 1b it can be observed an SEM image where magnetic particles are clearly covered by MnOx(s) precipitated on their surfaces. This finding is also reinforced by the results obtained by using EDX analysis (Fig. 7) which showed that large aggregates consisting only of overlapping MnOx(s) layers were precipitated on preferential sites of magnetic particle surfaces. Once MnOx(s) are formed on magnetic particle surface they become in the most reactive site for Mn adsorption (more than initial substrate), resulting in crystal growing (Junta and Hochella, 1994).

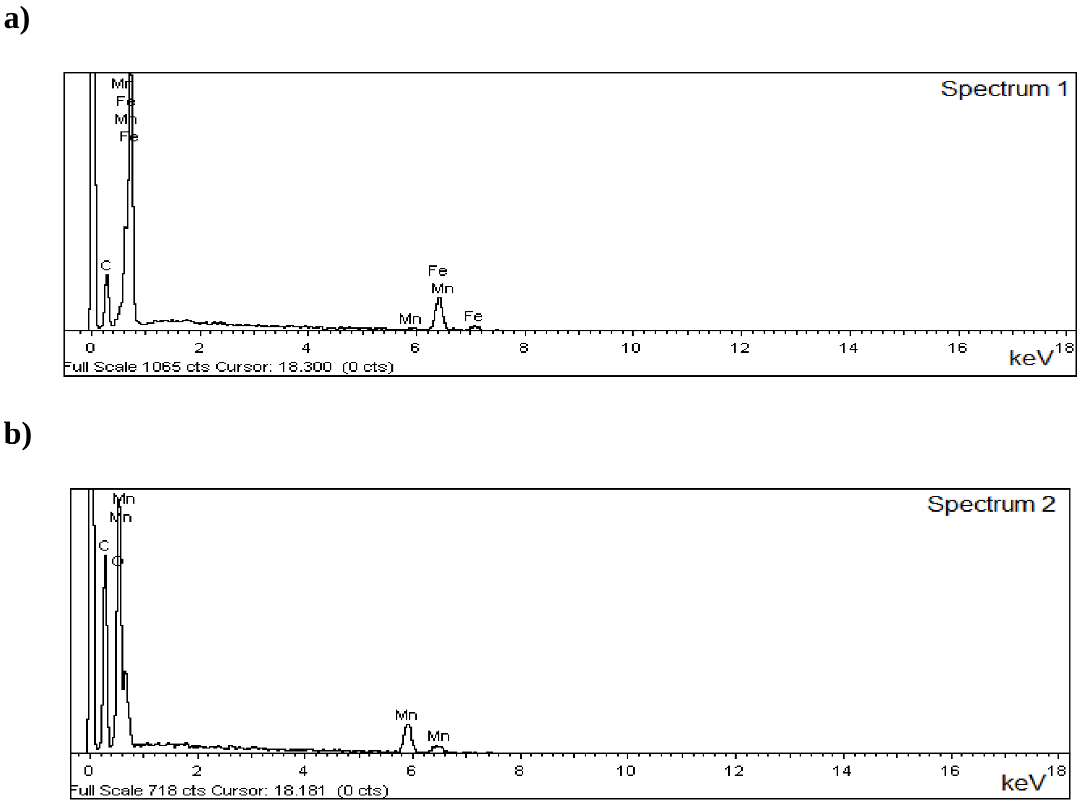


Fig. 7. EDX analysis of magnetic particles after a typical Mn adsorption experiment. a) and b) indicate elemental composition of two different substrates. a) Spectrum 1 belongs to a magnetic particle coated by precipitated Mn oxides. Note that in this spectrum, both Fe and Mn are present. b) Spectrum 2 belongs to a deposit of MnOx(s) clumps preferentially accumulated over a specific area of the magnetic particle. Note that in this case, only Mn and O are present. Initial conditions: 1 g L^{-1} magnetic particles; 100 mg Mn L^{-1}; pH = 9.

In addition, quantitative chemical composition of magnetic particles after an adsorption experiment, by using XPS technique, is presented in Table 1. The presence of high atomic concentrations of Mn and O suggests the presence of important amounts of MnOx(s) and support HRSEM-EDX results. We hypothesize that Mn firstly adsorb on binding sites (-OH) on oxides surfaces (magnetic particles) where it oxidizes and precipitates (Davies and Morgan, 1989). The fact that MnOx(s) surfaces accelerate Mn(II) oxidation process by O_2 (autocatalytic) has been reported by Morgan (2005). It must be pointed out that the pH at which Mn adsorption begins is strongly dependent on the type of adsorbate (Bleam and McBride, 1985).

A photograph of the experimental setup for a typical experiment at pH 9, is shown in Fig. 8, and demonstrates that the majority of MnOx(s) (represented as black-brown precipitates) were chemisorbed onto magnetic particles surfaces and retained by magnetic separation gradient, and only a minor proportion was precipitated as MnOx(s) in solution due solely to the oxidation by O_2. The reason behind this observation is that, under these conditions (pH = 9; 100 mg Mn L^{-1}), Mn oxidation and precipitation occur easier on surfaces oxides than in solution (Davies and Morgan, 1989).

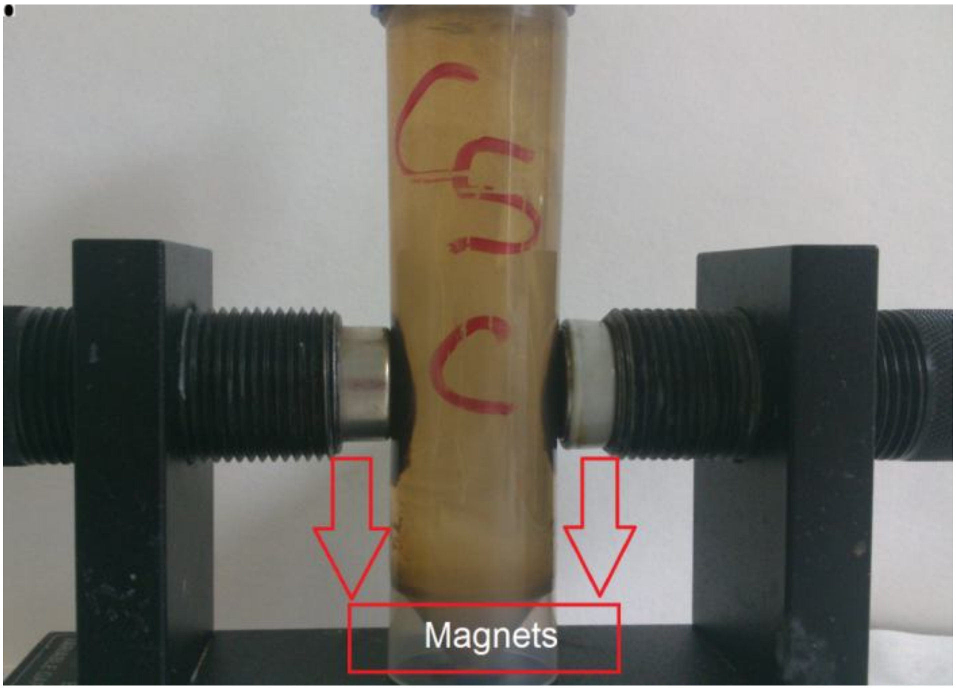


Fig. 8. Experimental set-up showing the magnetic field gradient separation technique.Most of Mn oxides are precipitated on magnetic particles and retained by magnetic field gradient. A non-negligible amount of Mn is precipitated in solution evidenced by brown coloration of solution. Initial conditions: 1 g L^{-1} magnetic particles; 100 mg Mn L^{-1}; pH = 9.

In summary, we can conclude that at high pH values: (i) Mn removal mechanism basically consisted in chemisorption in which adsorption capacity is continuously regenerated due to the formation of MnOx(s) layers by autocatalytic oxidation. (ii) There exist two types of possible adsorption surfaces, only if conditions of required pH for Mn oxidation are suitable: magnetic particles and MnOx(s) surfaces. MnOx(s) surfaces can be formed by adsorption and oxidation on magnetic particles surfaces or in solution due to Mn oxidative removal by O_2.

3.3.2. Adsorption at intermediate pH levels (6 ≤ pH ≤ 9)

For these intermediate pH values, Mn oxides form at a very low rate and they hardly precipitate onto the magnetic particles at the studied concentration levels (c.f. Figs. 3 and 4b). Nevertheless, the presence of magnetic particles resulted in a slight increase in Mn removal at pH = 8.5 (see circles in Fig. 3).

Therefore, in this section we aim to asses if increasing magnetic particles concentrations may enhance Mn removal from aqueous solutions. In Fig. 9 we show that Mn removal remained low for pH = 8 independently on iron concentrations. However, at pH = 8.5, Mn removal increased when increasing iron concentration. In particular, our results have shown that, at pH = 8.5, the percentages of Mn removal notably increased when adding 2 g L^{-1} (46%) instead of 1 g L^{-1} (27%), while no significant differences in Mn removal were observed between adding 2 and 4 g L^{-1}.

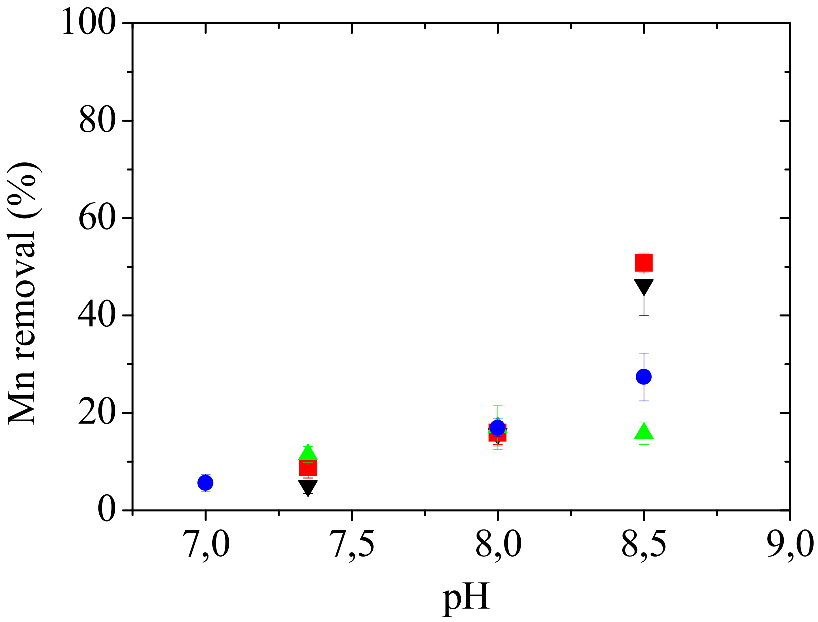


Fig. 9. Mn removal (%) as a function of magnetic particles concentration at different pH values, for an initial 2 mg Mn L^{-1} concentration. Triangles: no magnetic particles; Circles: 1 g L^{-1}; inverse triangles: 2 g L^{-1}; Squares: 4 g L^{-1}.

Also, we focused on identifying the optimum adsorbent doses, since it is an important factor to maximize the interactions between the adsorbent and adsorbate (Zhou et al., 2009). In Table 3, it is shown the effect of adsorbent concentration on Mn removal efficiency (%) and adsorption capacity, *q* (mg g^{-1}) at pH 8.5. As observed, when adsorbent concentration increased Mn removal efficiency (%) increased, but magnetic particles adsorption capacity (*q*) decreased. An increase in adsorbent doses results in higher removal efficiency due to an enhancement of available binding sites for Mn adsorption-oxidation, but further increase in doses does not imply a significant increase in Mn removal. The reduction in adsorption capacity can be due to the aggregation of magnetic particles, resulting in a reduction of surface area and thus, the availability of binding sites (Yong-Mei et al., 2010). Thus, our results may suggest that the product of these two parameters (Mn removal x *q*) could be used to find out the optimum concentration of adsorbent for achieving the highest efficiency. In our case, 2 g L^{-1} of magnetic particles is the most suitable concentration since it provides higher value for Mn removal efficiency and adsorption capacity than the other concentrations.

Table 3. Mn removal and adsorption capacity (q) as a function of magnetic particles concentration. 2 mg Mn L^{-1}; pH = 8.5.

	Without particles	**1g L^{-1}**	**2g L^{-1}**	**4g L^{-1}**
Mn removal (%)	15.81	27.37	46.28	50.78
q (mg Mn /g Fe)	-	0.60	0.50	0.27
Mn removal × q	-	16.48	23.19	13.96

3.4. Adsorption of Mn by MnOx(s) coated iron microparticles

In order to further increase Mn removal efficiency at pH values lower than 9, we tested the effect of coating iron particles with MnOx(s). The main reasons for selecting MnOx(s) as coating of iron particles were as follows: (i) MnOx(s) can enhance the affinity between Mn and magnetic particles since they are highly selective surfaces for Mn adsorption (Junta and Hochella, 1994) and (ii) MnOx(s) coatings remain as stable precipitates on surfaces magnetic particles over a wide pH range, as it was described in Section 3.3.

In Fig. 3, Mn removal by MnOx(s) coated magnetic particles is represented as a function of pH. Our results show a very remarkable increase in Mn removal when magnetic particles were coated with MnOx(s) compared to bare magnetic particles. As a way of example, Mn removal efficiency was larger than 80% when pH was ≥ 7 and the largest value (>90%) was achieved for pH 8 and 9. These results are in agreement with those obtained by Taffarel and Rubio (2010) when using Mn oxide coated zeolite. These authors found a Mn uptake close to 70% at pH 7 and 85% when pH was 8. In any case, for pHs higher than 7, final Mn concentration was always lower than 400 mg L^{-1} which has been suggested by the World Health Organization (WHO) as a health-based value (WHO, 2011).

In order to compare maximum Mn adsorption capacity of our adsorbent with others proposed in the literature (e.g. zeolites), data from adsorption isotherm were fitted to Freundlich model (Fig. 10). The selection of this model was based on the fact that it considers, among others assumptions (see Crini and Badot, 2008), the existence of a multilayer adsorption process which resembles our situation. Our experimental data were well fitted ($r^2 = 0.92$) to this isothermal model getting the following parameters: $K_F = 1.46 \pm 0.12$ and $N = 0.79 \pm 0.02$. It is essential to consider that no saturation conditions have been reached in this study, despite of using extremely high Mn initial concentration (up to 500 mg L^{-1}), as Mn adsorbed (q) continued increasing when increasing Ce. However, previous studies reported in the literature on other adsorbents (e.g. zeolites) exhibited a good fit of the experimental data to the Langmuir isotherm, suggesting the existence of a plateau region where no further increase in Mn adsorption exists (Doula, 2006, 2009; Erdem et al., 2004; Taffarel and Rubio, 2009, 2010).

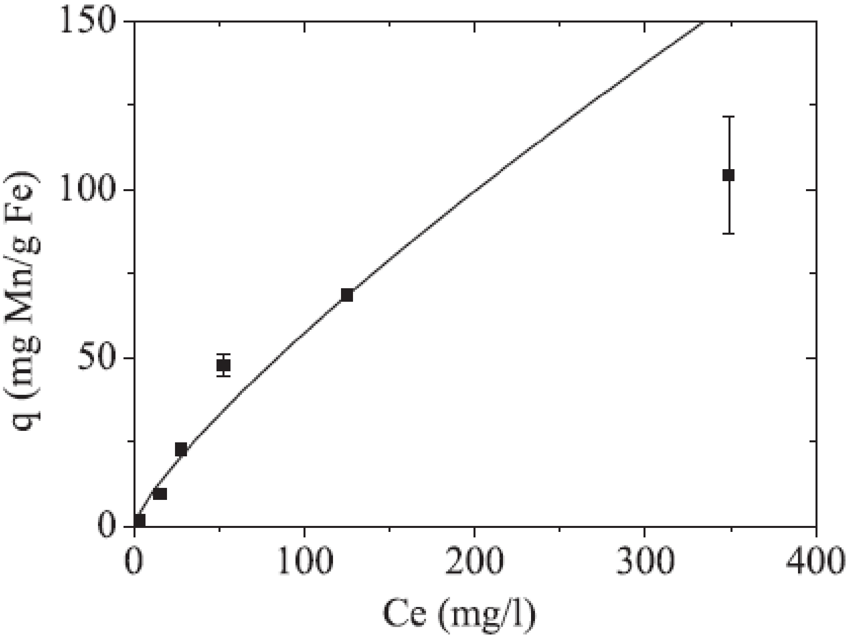


Fig. 10. Adsorption isotherm of MnOx(s)-coated magnetic particles including the fitting to Freundlich model. Initial conditions: pH = 7.

3.5. Desorption

Batch desorption experiments were carried out to evaluate the effect of pH on Mn desorption from Mn-loaded magnetic particles' surfaces. Such study provides an important insight into the reversibility of the adsorption process in a range of pH that covers those found in natural waters. As observed in Fig. 11, Mn desorption rate increased when reducing pH value from 9 to 6. Significant differences ($p < 0.05$) were observed in Mn desorption rate between pH 6 and 8 and pH 7 and 9. Nevertheless, it must be pointed out that Mn desorption rate was always relatively low (<15%) even when pH was near pH = 6. These results support the idea that MnOx(s) formed onto magnetic particles' surfaces at pH 9 are stable complexes that hardly go back to solution in a wide pH range. Similar results were found by Lloyd et al. (1983) when studying the desorption of Mn previously oxidized at pH 8.5 and 9 from iron oxides. This finding confirms the convenience of using magnetic particles for Mn removal from aqueous solutions.

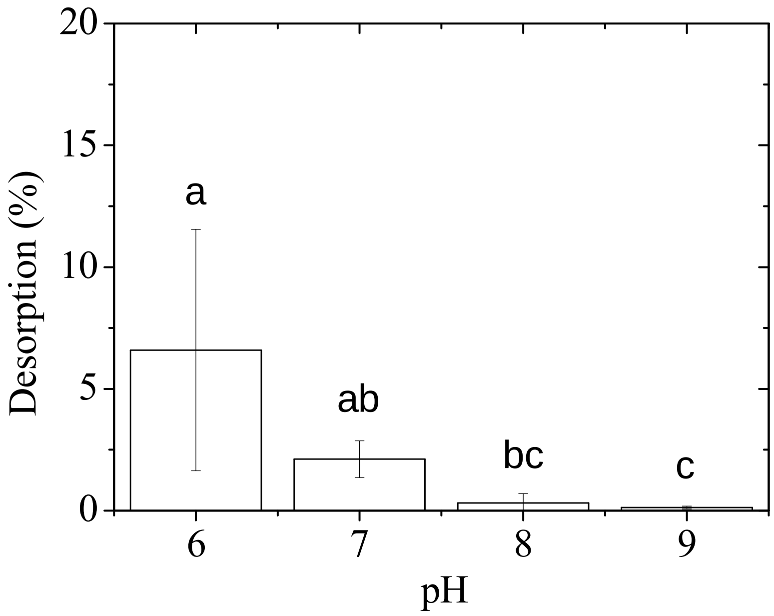


Fig. 11. Desorption of Mn (%) from Mn-loaded magnetic particles as a function of pH. The standard deviation is represented by vertical bars. Initial conditions:1 g $L^{-1}$of magnetic particles; 100 mg Mn L^{-1}; pH = 9.Significant differences at $p < 0.05$ between pH are indicated with letters.

3.6. Reutilization

Finally, we study the possibility of reusing magnetic particles for Mn removal. As it can be observed in Table 4, no significant differences ($p > 0.05$) in Mn removal efficiency between bare magnetic particles and reused magnetic particles neither with nor without previous washing were observed. In fact, Mn removal efficiency was in all cases higher than 98%. Therefore, we can conclude that there was not any reduction in Mn adsorption capacity of reused magnetic particles even if the adsorbent was not washed.

Table 4. Mn removal (Mn_R, %) by initial and reused particles (with and without washing). Initial conditions: 1 g $L^{-1}$of magnetic particles; 100 mg MnL^{-1}; pH = 9.

	Mn_R (%)	**Standard Deviation (%)**
InitialParticles	98.96	0.66
Reusedparticles (with washing)	99.50	0.32
Reusedparticles (without-washing)	99.38	1.04

Again, this result is consistent with the above mentioned continuous formation of MnOx(s) selective surfaces for Mn(II) adsorption, which was supported by isotherm results, and HRSEM and EDX analysis. However, it should be noted that the use of magnetic particles in successive removal processes without washing may affect to magnetic properties of magnetic particles and colloidal stability.

4. Conclusions

1. For pH values higher than 9, both in the presence and in the absence of bare magnetic particles, Mn was removed from aqueous solution by an oxidation and precipitation process with an efficiency of 98%. When particles are present, most of the MnOx(s) were precipitated on magnetic particles surface and therefore could be easily removed from solutions by magnetic field gradients. Only a minor proportion of Mn was precipitated as MnOx(s) in solution due to oxidation by O_2. At these pH values, Mn removal mechanism consists of two steps: First, Mn adsorbs and oxidizes onto magnetic particles surfaces. Second, MnOx(s) precipitated on adsorbent surface become the most reactive sites for Mn adsorption and its subsequent oxidation, causing a continuous regeneration of adsorption capacity. In addition, Mn can be adsorbed and precipitated onto MnOx(s) formed in solution due to the oxidation by O_2.

2. For intermediate pH values (7-8.5), no effect of increasing adsorbent concentration on Mn removal efficiency was observed, except for pH = 8.5, where an increase in adsorbent concentration from 1 to 2 g L^{-1} caused an increment in Mn removal from 27% to 46%. In fact, at this pH, our results suggest that the product of Mn removal percentage and Mn adsorption capacity (q) could be used as a proxy for finding out the optimum concentration of adsorbent for achieving the highest efficiency (2 g L^{-1} of magnetic particles in our case).

3. Apart from increasing magnetic particle concentration (which would cause more economic cost), the use of MnOx(s)-coated magnetic particles was the best alternative for improving Mn removal efficiency at intermediate pH values (7-9). In this sense, Mn removal efficiency was drastically improved at all pH values, being higher than 80% for pH between 7 and 9. Adsorption isotherm of MnOx(s)-coated magnetic particles has shown that Mn adsorption increased when increasing equilibrium Mn concentration and no saturation of adsorption sites was reached.

4. Desorption experiments of Mn-loaded magnetic particles, at different pH values (6-9), revealed that Mn adsorbed onto magnetic particles was a highly stable compound. Accordingly, very low desorption rates (always lower than 15%) were measured.

5. For economic reasons, the possibility of reusing magnetic particles for Mn removal was explored. Our experimental results suggest that Mn removal efficiency was very similar (and always higher than 98%) both when employing bare magnetic particles and reused ones. These results were consistent with the explanation provided for the Mn removal, that was based on the autocatalytic adsorption-oxidation of Mn on magnetic particles.

6. All in all, some of the advantages of using MnO(x) coated magnetic particles for Mn removal compared to other adsorbents are: (i) the absence of saturation up to initial concentrations of 500 mg Mn L^{-1}; (ii) the easy and efficient recovery of the ad-

sorbents from the solution due to magnetic properties, minimizing alterations of water quality and (iii) the reusability of the adsorbents contributing to economic cost savings.

Acknowledgments

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Chapter II: Magnetic microparticles as a new tool for lake restoration: a microcosm experiment for evaluating the impact on phosphorus fluxes and sedimentary phosphorus pools

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Abstract

In the last decades, magnetic particles (MPs) as adsorbents have gained special attention due to their high adsorption capacity and the possibility of recovering them by applying a magnetic separation gradient. For the first time MPs have been tested as P adsorbents in a microcosm experiment in a context of lake restoration. MPs were added to sediment cores from a hypertrophic lake, at Fe:P_{Mobile} molar ratio of 285:1 and 560:1 under both, oxic and anoxic conditions. We have found that, under anoxic conditions (anoxic), MPs are able to reduce P release rate from the sediment to the overlying water and to reduce sedimentary P_{Mobile} concentration (a 22-25% reduction within 0-4 cm depth compared to controls). Under oxic conditions, the addition of MPs do not affect P fluxes across the sediment and water interface since the lake sediment is naturally rich in iron oxides. However a measured reduction in sedimentary P_{Mobile} concentration (12-16 % reduction in 0-10 cm depth) contributes to a potential reduction in long-term P efflux.

1. Introduction

Eutrophication is subject of concern to managers since it is the main problem that impairs water quality of 30-40 % of water resources of all over the world (Hupfer and Hilt, 2008; Søndergaard et al., 2007). Since phosphorus (P) is the nutrient that often limits primary production in freshwater, it is a basic requirement to reduce its concentration in order to combat eutrophication and meet the requirements set by law (Schindler et al., 2008; Carpenter and Lathrop, 2008). Therefore, the first measure to consider in lake restoration should be the reduction of P external loading (Cooke et al., 2005; Reitzel et al., 2003). However, a successful reduction of external P loading is not always practical or economically feasible due to the difficulty in controlling external P inputs dominated by non point sources and due to the drastic reduction in external P loading required to accomplish the desired outcome (Marsden, 1989; Istvanovics and Somlyody, 2001; Deppe and Benndorf, 2002). Moreover, biological resilience and P release from lake sediments (P internal loading) usually delay ecosystem recovery after a reduction in external P loading (Jeppensen et al., 1991; Søndergaard et al., 2003). In such situations, it is recommended, apart from reducing P external loading, the application of in-lake techniques to decrease internal P loading and accelerate lake water improvement (Lürling and Oosterhout, 2013; Cooke et al., 2005). Some of these techniques are based on preventing P release from sediment by hypolimnetic aireation, nitrate (NO_3^-) addition or sediment capping (Hupfer and Hilt, 2008). Some other in-lake techniques are focused on enhancing P adsorption capacity of sediment by adding alum (Al), iron (Fe), calcium (Ca) salts, solid phase P-sorbing products (PSPs) coming from industrial waste and also Phoslock® (Cooke et al., 2005; Deppe and Benndorf, 2002; Lewandowski et al., 2003; Meis et al., 2012; Spears et al., 2013b; Yamada et al., 2012). In spite of its widespread application and success achieved, the main drawback about salt addition is that, although inactivated, P remains in sediment and may be released to water column under changing physicochemical 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). Although, in general, Al is preferred to Fe (Boers et al., 1992), since it keeps invulnerable to shifts in redox potential at sediment-water interface, Al application has also some limitations. For instance: (i) Al addition is limited to a pH range between 5.5 and 8 to assure the absence of toxicity and the efficiency in P removal (Lewandowski et al., 2003; Reitzel et al., 2013), (ii) Al-rich sediment layers reduce sediment consolidation degree, which hinders the establishment of macrophytes and favors resuspension events and temporal P release (Egemose et al., 2010) and (iii)the effectiveness of Al addition is time-limited since P maximum adsorption capacity of Al floc is notably reduced by ageing (76% of reduction after 3 months; de Vicente et al., 2008a).

To counteract the limitations of using Fe and Al salts, in the mid-1990s an alternative adsorbent, a lanthanum modified bentonite clay, Phoslock®, was invented for trapping P in aqueous solutions (Robb et al., 2003; Ross et al., 2008; Spears et al., 2013a). However, Phoslock® has some drawbacks since P binding capacity declines when pH is above 8.1 and when increasing alkalinity, having some limitations in hard-water systems (Ross et al., 2008; Reitzel et al., 2013). Moreover, chemical interferences with humic substances, similarly to the addition of Al salts (de Vicente et al., 2008b) and of MPs (de Vicente et al., 2011; Merino-Martos et al., 2015), which lastly affect P removal efficiency have been found (Lürling and Faassen, 2012; Reitzel et al., 2013; Lürling et al., 2014). In addition, the high Phoslock®: P mass ratio necessary for retaining P, which has been identified as higher than 100:1 (Reitzel et al., 2013), and its high price make this technique specially unviable from an economic point of view in some circumstances.

As a result, new methods may be useful for lake restoration in order to overcome drawbacks of current practices. A large background of laboratory experiments has shown that magnetic particles (MPs) are good and cost-effective pollutant adsorbents (Tang and Lo, 2013). Among the main advantages for using these particles are their high surface area, which is responsible for a high adsorption capacity; a fast adsorption kinetic and their magnetism properties (Huber, 2005; Tang and Lo, 2013). The last characteristic of MPs implies that contaminant-loaded MPs can be efficiently removed from solution by applying a magnetic separation gradient, minimizing alteration in water quality and enabling their reuse in subsequent adsorption processes (de Vicente et al., 2010b; Funes et al., 2014).

In the case of P removal from aqueous solutions, it has been previously tested the convenience of using MPs as P adsorbents in laboratory scale under both batch mode (de Vicente et al., 2010b) and flow conditions (Merino-Martos et al., 2011). Apart from being considered as efficient P adsorbents (P uptake efficiency is always higher than 80% whatever pH value from 6 to 9), MPs present a fast adsorption kinetic (less than 1 hour) and keep a high P removal efficiency when reusing them in subsequent removal processes (de Vicente et al., 2010b; Merino Martos et al., 2014).

In this context, the aim of this study was to assess the effect of adding MPs on P fluxes across the sediment-water interface and on the sedimentary mobile P concentration. To get these purposes, a microcosm experiment using sediment cores and lake water collected from a hypereutrophic wetland (Honda lake, Southeast of Spain) was run under both oxic and anoxic conditions. Similarly to Reitzel et al. (2003), we proposed to add MPs relative to sediment mobile P pool (P_{Mobile}). Microcosm experiments tries to mimic more realistic conditions than the previously performed laboratory experiments (de Vicente et al., 2010b) and although this type of design obviously lacks the complexity of the whole ecosystem (Schindler, 1998), it may constitute a previous and necessary step for a future whole-lake application.

2. Materials and Methods

2.1. Study site

Honda lake is a shallow (surface area = 9 ha, mean depth = 1.3 m and maximum depth = 3.2 m), hardwater (alkalinity 3.17–6.21 meq L^{-1}), brakish water (3.09 mS cm^{-1}) and hypertrophic coastal wetland located in Albuferas of Adra lacustrine complex (southeast Spain; Fig. 1).

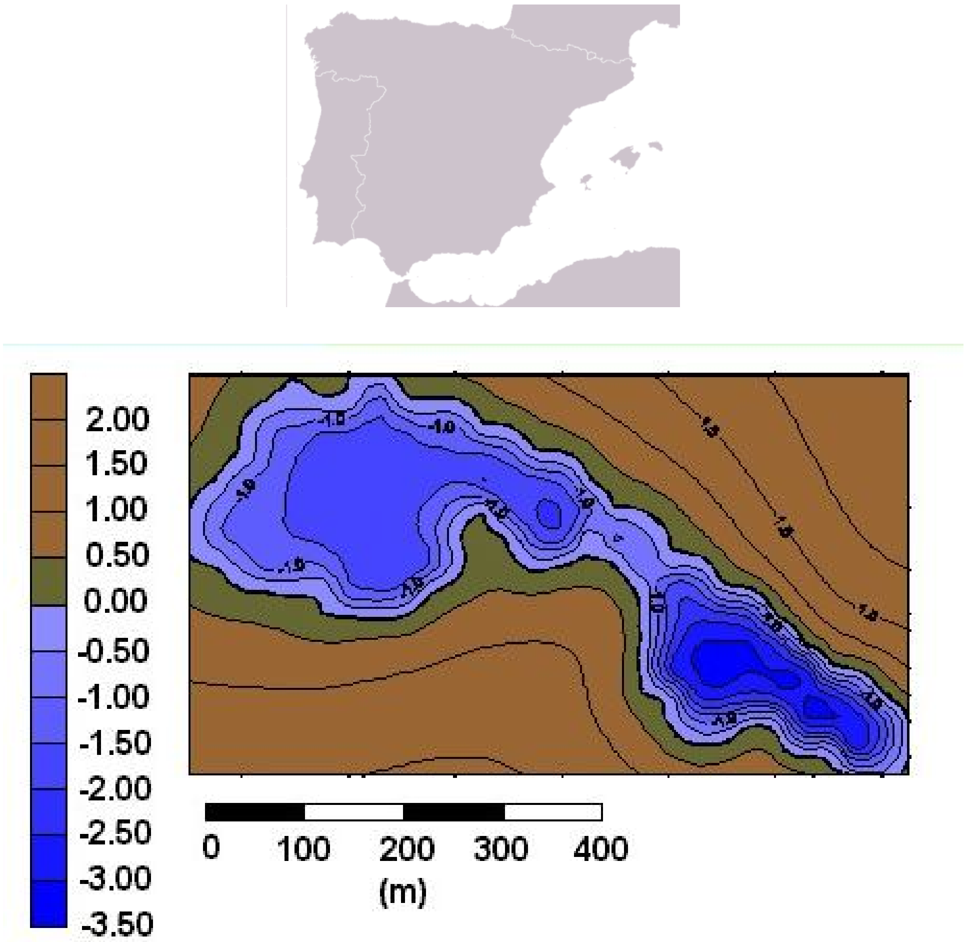


Fig. 1. Geographic location and morphometric map of the study lake. Black square indicates the sampling point at the maximum water depth.

Table 1 includes some information about the general limnological features of the study lake. Despite of being designated as Natural Reserve (1989) and Ramsar Site (1994), among others protection states, its high external and internal P loads have impaired water quality as a result of eutrophication processes (de Vicente et al., 2003). The external P loading (1.73 g P m^{-2} $year^{-1}$) is mostly constituted by diffuse inputs (about 90%; de Vicente and Cruz-Pizarro, 2003; de Vicente et al., 2003). In relation to the relevance of internal P load in this lake, its shallowness and the presence of intense winds cause frequent resuspension events which are lastly responsible for bringing back sediment associated particulate and dissolved nutrients into the water column where then can be released (de Vicente et al., 2010a). Apart from sediment resuspension, the release of nutrients across sediment-water interface may be favored by anoxic conditions at hypolimnion during the weak thermocline established during summer time period (de Vicente et al., 2003). As a result of the inherent wetland characteristics (shallowness, frequent resuspension events and hypolimnetic anoxia) the use of P binding salts commonly used for in-lake restoration techniques (Al or Fe salts) may be not recommended since P is likely to be released during resuspension or under shifting physicochemical conditions. Instead, new techniques intended to restore shallow and polymictic lakes may be focused on increasing P export from the system. In this context, the use of MPs as P adsorbents and its subsequent removal from the ecosystem seems to be a suitable restoration option.

Table 1. General limnological features of the study lake. All data correspond to July 2013 except for DOC which was measured on July 2015. Mean values have been estimated from surface, mid depth (1 m) and bottom samples (2 m). All analysis have been performed following standard methods (APHA, 1989).

	Mean ± SD
P-PO$_4^{-3}$ (µg L^{-1})	0.59 ± 0.09
TDP (µg L^{-1})	31.57 ± 2.77
TP (µg L^{-1})	493.54 ± 35.99
N-NO$_3^-$ (mg L^{-1})	0.108 ± 0.002
TDN (mg L^{-1})	4.95 ± 0.13
TN (mg L^{-1})	9.41 ± 0.45
DOC (mg L^{-1})	59.3 ± 1.49
Chl a (µg L^{-1})	57.2 ± 19.7

2.2 Materials

Micronsized Fe MPs were obtained from BASF (Germany) and used as P adsorbent. According to the manufacturer, MPs are chemically characterized as follows: 97.5% Fe, 0.9% carbon, 0.5% oxygen, and 0.9% nitrogen. Previous characterization has shown that MPs are spherical in shape and relatively polydisperse in size with a mean particle diameter of 800 ± 10 nm (de Vicente et al., 2010b). Previous data have revealed that MPs surface is negatively charge in the studied pH range (6-9) and the magnitude of charge increases as pH value increases (de Vicente et al., 2010b).

2.3 Sequential extraction procedure of sedimentary P pools

In July 2013, 3 large cores (Θ = 5.4 cm; h= 50 cm) were collected, at 2 m depth in Honda lake and sectioned at intervals of 2 cm up to 10 cm obtaining 5 different sediment slices. The three samples of each depth were pooled and homogenized before analysis. A sequential chemical extraction procedure according to (Paludan and Jensen, 1995) was used in order to quantify the concentration of different P pools in the in the upper 10 cm of sediment: $P_{\rightarrow Water}$ (pore water o-P extracted in distilled water), $P_{\rightarrow BD}$ (o-P bound to reducible metals such as Fe and Mn extracted in 0.11 M $NaHCO_3$, 0.11 M $Na_2S_2O_4$ bicarbonate dithionite (BD) solution reagent, $P_{\rightarrow NaOH}$ (o-P adsorbed to Al oxides, clay minerals and humic acids extracted in 0.1 M NaOH), $P_{\rightarrow NaOH,\ Humic}$ (P bound to humic acids precipitated in acidified solution), $P_{\rightarrow HCl}$ (magnesium and calcium bound o-P extracted with 0.5 M HCl) and $P_{\rightarrow HCl,\ Res}$ (refractory organic P extracted with 1 M HCl at 120 °C after combustion at 550ºC, 3.5h). Finally, organic P labile (Org-P_{Labile}) was calculated as the sum of non-reactive P (NRP) which stems from the difference between Tot-P (wet digestion with 0.18 M $K_2S_2O_4$) and o-P of the three first steps of extraction. Among all P pools that define total P in sediment, only P_{Mobile} is expected to contribute to internal P loading (Boström et al., 1988b; Rydin, 2000). P_{Mobile} was estimated as the sum of $P_{\rightarrow Water}$, $P_{\rightarrow BD}$ and Org-P_{Labile} in the upper 10 cm of sediment (Rydin, 2000; Hansen et al., 2003; Reitzel et al., 2003). Total sediment P (Tot-P_{sed}) and total sediment Fe (Tot-Fe_{sed}) were determined on parallel by ashing the sediment samples (520ºC, 5.5 h) followed by an acid hot digestion with 1 M HCl (120 °C, 1 h). P pools data were normalized according to Tot-P_{sed}, which is supposed to be more accurate.

Soluble reactive P (SRP) was determined spectrophotometrically using the molybdenum blue method proposed by Murphy and Riley (1962). In the same extractants for P measurements, Fe fractions ($Fe_{\rightarrow Water}$, $Fe_{\rightarrow BD}$, $Fe_{\rightarrow NaOH}$, and $Fe_{\rightarrow HCl)}$ were determined following the spectrophotometric ferrozine method proposed by Gibbs (1979). Finally, sediment was also analyzed for dry weight (DW, %) at 105 ºC for 24 h and loss on ignition (LOI, %) at 520 ºC for 5.5 h.

2.4 Microcosms experiment with sediment cores

In July 2013, 18 small cores (inner diameter = 5.4 cm, height = 25 cm) were taken at 2 m depth in Honda lake (at the same sampling site as for collecting the 3 large cores mentioned above). Sediment height was adjusted to give 10 cm of the overlying water in all cores. Before running the experiment, and similarly to Rydin and Welch (1999) and Reitzel et al. (2003), we first measured sedimentary P_{Mobile} in three large cores and the mass of adsorbent required was estimated as a function of P_{Mobile} pool. Considering that the maximum P adsorption capacity by MPs (under batch conditions) was 18.83 mg P g^{-1} Fe (de Vicente et al., 2010b) and that the mass of sedimentary P_{Mobile} in the upper 10 cm of the sediment was 19.51 mg, we obtained that 1.04 g MPs were needed to be added to the cores. As a result, we decided to consider two different treatments, one adding 10 g (T_1) and another adding 20 g (T_2) to check the performance of MPs in excess in order to counteract possible chemical interferences as previous studies have found (de Vicente et al., 2011; Merino-Martos et al., 2015), giving a final Fe: P_{Mobile} molar ratio of 285:1 and 560:1, respectively.

During the experiment, cores were kept in darkness and at room temperature (23 ± 2 ºC) for 24 h. In total, six different treatments, which were run in triplicates, were considered. On the one hand, three treatments were bubbled with oxygen (O_2) (oxic treatments): one of them was not amended with MPs, being considered as control (O-Control), while the other two treatments were enriched with 10 g (O-T_1) and 20 g (O-T_2) of MPs. On the other hand, the other three treatments were incubated under anoxic conditions and the same procedure as mentioned before was applied: no addition of MPs (A-Control), addition of 10 g (A-T_1) and addition of 20 g (A-T_2) of MPs. Sediment cores incubated under oxic conditions were continuously bubbled with O_2 during the whole experiment. Anoxic conditions were promoted by adding, at the beginning of the experiment, BD reagent to reach a final concentration of 4 mg L^{-1}, which was previously tested as the right concentration for assuring anoxic conditions. Thus, O_2 concentration was always higher than 4 mg L^{-1} in all oxic cores and lower than 1 mg L^{-1} in all suboxic ones during the whole experiment. When MPs were added (T_1 and T_2), they were homogeneously distributed over the entire surface of sediment cores. After 24 h of incubation, MPs were removed from sediment cores by applying a magnetic field gradient exerted by a permanent magnet (volume = 25.6 cm^3; NB032, Aiman GZ, Spain).

Removal process of MPs was carried out by immersing the magnet twice for 15 seconds in the cores. Water displaced by magnet immersion was considered in calculations. Initial and final (both before and after removing MPs) P concentrations were determined following the above mentioned method (Murphy and Riley, 1962). P efflux from sediment to water column was finally measured as the change in P concentration in overlying water during 24h of incubation time. Initial and final (after removing MPs) Fe concentrations were determined for the same period of incubation in order to see the effect of MPs on Fe concentrations in water column. In addition, pH and O_2 concentration (mg L^{-1}) were measured during the experiment using a VWR Symphony multiparametric sensor (VWR International Eurolab S.L.; Spain).

Finally, and in order to assess the effect of adding MPs on changes in sedimentary Fe and P pools (specifically on P_{Mobile} concentration) a sequential extraction procedure described in section 2.3 was carried out in sediment cores from all treatments at the end of the incubation time. In addition, P retention capacity of the sediment was estimated by the molar ratio between $Fe_{\rightarrow BD}$ and $P_{\rightarrow BD}$ in the upper 10 cm in the initial fractionation (Jensen et al., 1992).

2.5 Statistical analysis

All data reported in this work have been obtained in triplicates. In order to test significant differences in P fluxes and Fe concentrations in water column as a result of MPs addition, a comparison between control and treatments was carried out using one-way ANOVA and then Least Significant Difference (LSD) post-hoc test. Differences in initial and final Fe concentrations of each treatment and control were tested by applying paired t-test. Significant differences were accepted when $p<0.05$.

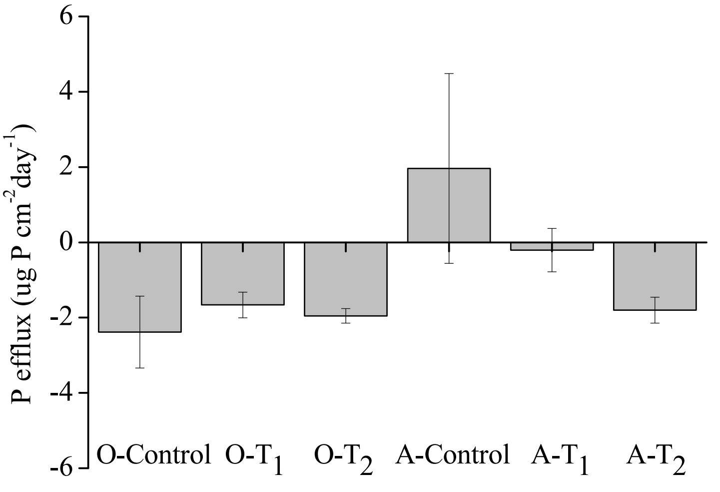
Shapiro-Wilk test and Levene´s test were performed to check normality and homogeneity of variances, respectively. In order to satisfy normality assumptions, data were log-transformed before performing statistical analysis. Statistical analysis was done using SPSS software.

3. Results

3.1 Effect of MPs addition on P fluxes across the sediment - water interface

P exchange rates across the sediment-water interface were estimated for two different situations after finishing the incubation period: a) when MPs were still in the sediment; b) after removing MPs from the cores by using magnetic separation. P fluxes across the sediment and water interface are shown in Fig. 2a and Fig. 2b, respectively. When MPs were still in sediment, all treatments amended with MPs evidenced a net P uptake by the sediment in both, oxic and anoxic conditions (Fig. 2a). In particular, P exchanges rates were -1.66 ± 0.34 and -1.95 ± 0.19 µg cm^{-2} day^{-1} in O-$T_1$and O-T_2 respectively; and -0.21 ± 0.58 and -1.80 ± 0.34 µg cm^{-2} day^{-1} in A-T_1 and A-T_2, respectively.

a)



b)

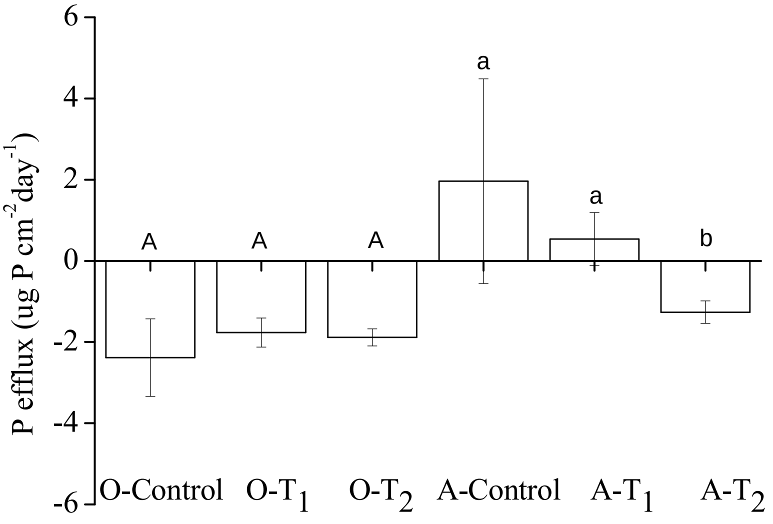


Fig. 2. Sediment P fluxes before (1a) and after removing MPs (1b). Significant differences ($p < 0.05$) between treatments are indicated with letters. Standard deviation is represented by vertical bars.

After removing MPs from sediment cores, control and treatments under oxic conditions kept exhibiting a net P uptake by the sediment and no significant differences among neither of them were noticed (Fig. 2b). In anoxic conditions, the highest P release rate (1.96 ± 2.52 µg P cm^{-2} day^{-1}) was observed when no MPs were added (A-Control) compared to treatments (A-T$_1$ and A-T$_2$). P efflux registered in A-T$_2$ (-1.27

±0.28 µg P cm^{-2} day^{-1}) was significantly lower than that found in A-T_1 (p= 0.043) and in A-Control (p= 0.011). By contrast, no significant differences were found between A-Control and A-T_1.

It is important to consider that P fluxes were measured before and after removing MPs from the cores in order to take into account possible P release during resuspension triggered by MPs removal with the magnet. However, statistical analyses have shown that P exchange rates were not significantly different between these two stages, neither in oxic nor in anoxic conditions in any treatment.

3.2. Effect of MPs addition on Fe concentrations in the water column

In order to assess the effect of MPs addition on Fe concentrations in lake water, Fe concentrations were measured in controls and treatments as initial Fe concentration (before adding MPs) and as final Fe concentration (at the end of the incubation period). While in oxic conditions (both in control and treatments) Fe concentration in the overlying water was always below the detection limit (1 µg Fe L^{-1}), in anoxic cores the trend was very different (Fig. 3). Fe concentration did not significantly change in A-Control during the experiment whereas in A-T_1 and A-T_2treatments there was a significant increase in Fe concentrations from 1.15 ± 0.40 to 8.63± 1.23 mg L^{-1}in the case of A-T_1 and from 0.88 ± 0.60 to 12.74 ± 3.43 mg L^{-1} in the case of A-T_2. As a consequence, at the end of the experiment, Fe concentration in A-T_1 was 10 times higher than in A-Control ($p<0.001$) whereas in A-T_2 was 15 times higher than in A-Control ($p<0.001$).

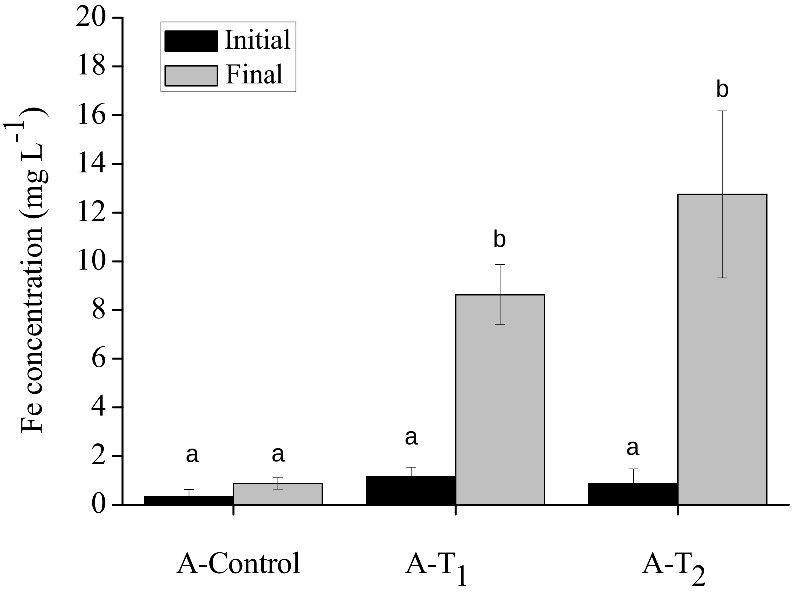


Fig. 3. Fe concentration in water column in control and treatments under anoxic conditions. Standard deviation is represented by vertical bars. Significant differences between initial and final concentrations are written with letters.

3.3 Sediment characterization

3.3.1 Initial sedimentary P pools

As it can be seen in Fig. 4, Tot-P_{sed} concentration decreased from 1022.26 µg P gDW^{-1} at sediment surface to 847.37 µg P gDW^{-1} at 10 cm depth. As an average of the first 10 cm, the percentage of each P fraction with respect to Tot-P_{sed} was: 1.6 ($P_{\rightarrow Water}$), 16.0 ($P_{\rightarrow BD}$), 5.7 ($P_{\rightarrow NaOH}$), 0.5 ($P_{\rightarrow NaOH,\ Humic}$), 54.2 ($P_{\rightarrow HCl}$), 9.3 (Org-$P_{Labile}$) and 12.8 % ($P_{\rightarrow HCl,\ Res}$). Org-$P_{Labile}$ concentration reduced with depth. $P_{\rightarrow NaOH,\ Humic}$ and $P_{\rightarrow HCl}$ increased slightly with depth whereas $P_{\rightarrow BD}$, $P_{\rightarrow NaOH}$ and $P_{\rightarrow HCl,\ Res}$ fractions kept practically constant at all depths.

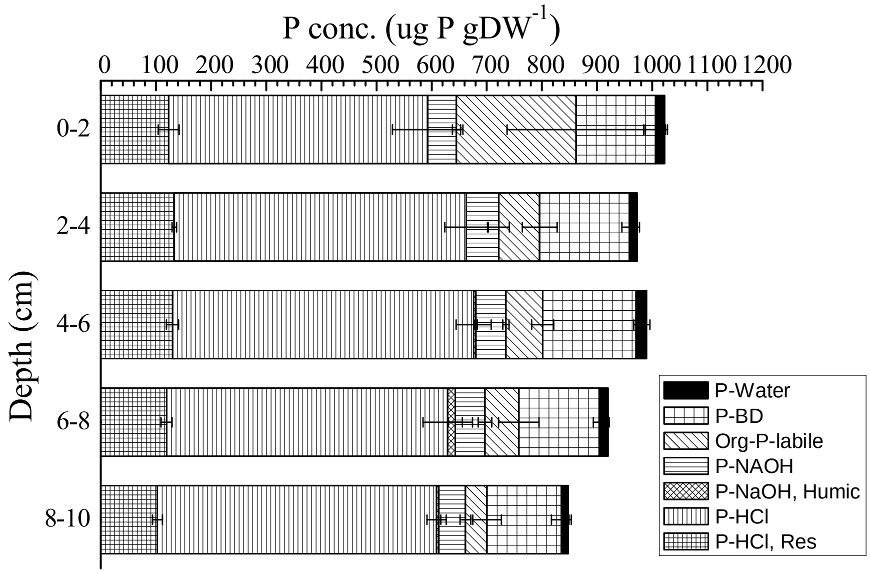


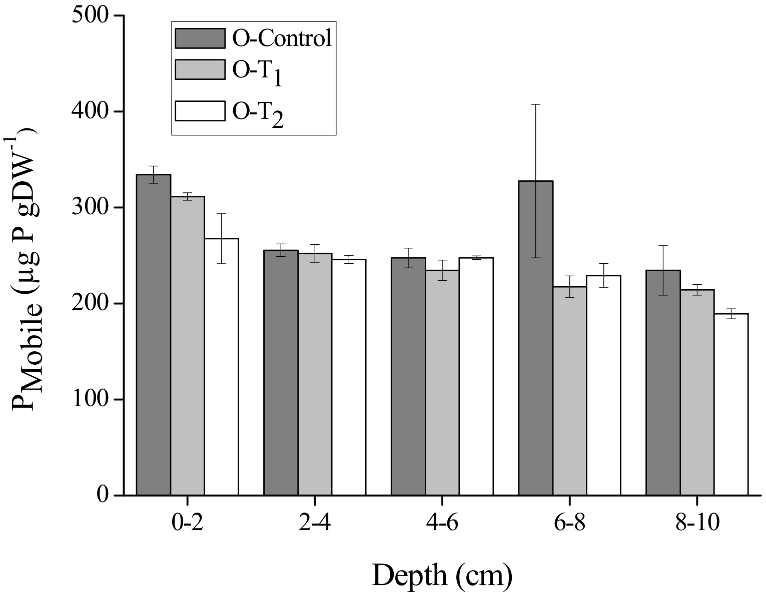
Fig. 4. Initial sedimentary P fractionation of untreated sediment. Standard deviation of each fraction is represented by horizontal bars.

3.3.2 Changes in sediment composition induced by MPs addition

The percentage distribution of the seven P pools with respect to Tot-P_{sed} was rather similar in the initial fractionation and the final fractionation in all treatments and controls. This similarity was in accordance to that obtained by Hansen et al. (2003) when applying different treatments (addition of Fe, Al and O_2 application) to sediment cores.

However, some quantitative differences in sedimentary P pools concentrations emerged when comparing treatments and controls in final fractionation. As a way of illustration, Fig. 5 shows P_{Mobile} concentrations at five depth intervals in oxic (Fig. 5a) and anoxic (Fig. 5b) conditions. Controls, both in oxic and anoxic conditions showed higher P_{Mobile} concentrations than treatments (T_1 and T_2) at all depths. Considering the 10 cm of the upper sediment, MPs addition caused a reduction in sediment P_{Mobile} concentration, ranging from 12 % in O-T_1 to 16 % in O-$T_2$with respect to O-Control, and 16 % in A-T_1 and the 17 % in A-T_2 compared to A-Control. By dividing the sediment in just two depths, the uppermost (0-4cm) and the deepest layer (4-10cm), we can see that the highest reduction in P_{Mobile} concentration occurred in the deepest sediment layer in oxic conditions (18 % both in O-T_1 and O-T_2). By contrast, an inverse tendency was observed for anoxic conditions where the highest reduction in P_{Mobile} concentration was measured in the upper sediment layer, which reached the 22% and 25% in A-T_1 and A-T_2, respectively.

a)



b)

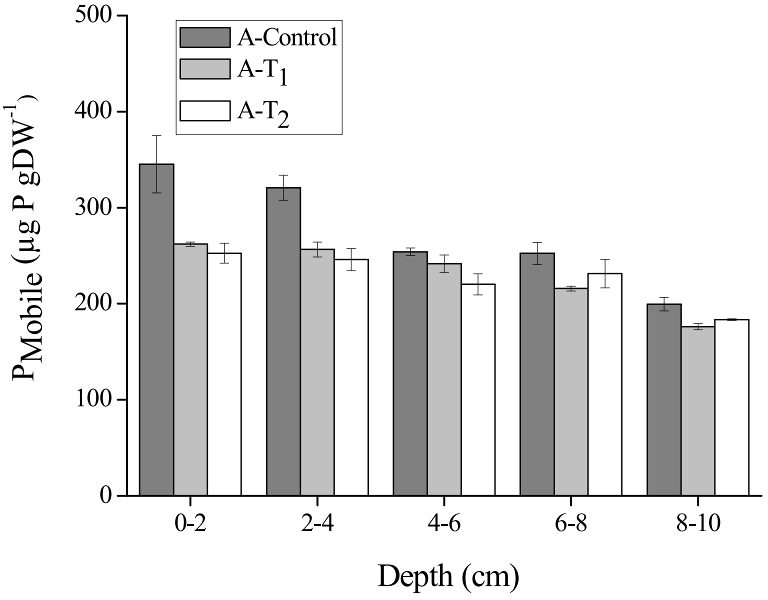


Fig. 5. Changes in P_{Mobile} concentration with depth after removing MPs. Fig. 5a corresponds to oxic treatments and Fig. 5b refers to anoxic treatments. Standard deviation is represented by vertical bars.

It is worth to remark that changes in P_{Mobile} pool between treatment and controls were mostly due to changes in Org-P_{Labile} fraction, since no differences were noticed for

$P_{\rightarrow Water}$ and $P_{\rightarrow BD}$ after MPs addition. Because of that, next we focused on Org-P_{Labile} fraction. Specifically, considering the upper 10 cm, Org-P_{Labile} reduction was 34 % in O-$T_1$and 44% in O-T_2 compared to O-Control and 32 % in A-T_1 and 45 % in A-T_2 compared to A-Control. Similarly to what occurred with P_{Mobile} under oxic conditions the higher reduction in Org-P_{Labile}took place within the deepest layer accounting for 45% in O-T_1 and 53% O-T_2 compared to O-Control. In anoxic conditions, Org-P_{Labile} concentration was reduced in a higher proportion in the upper sediment layer than in the deepest sediment, reaching 37 % of Org-P_{Labile} reduction in A-T_1 and 54 % of reduction in A-T_2.The rest of P fractions does not seem to have suffered any significant change when comparing control and treatments.

Considering sedimentary P_{Mobile} mass that MPs addition (T_1 and T_2) was able to reduce compared to control; we have estimated that, in the whole lake (94000 m^2 of surface area and referred to the upper 10 cm of the lake sediment), a total mass of 130, 195, 62 and 141 kg P in O-T_1, O-T_2, A-T_1 and A-T_2, respectively, would be removed after MPs application.

Changes in $Fe_{\rightarrow BD}$ and Tot-Fe_{sed} pools as a consequence of MPs performance can be seen in Fig. 6a. By analyzing $Fe_{\rightarrow BD}$ and Tot-Fe_{sed}, no remarkable differences in Fe concentration were observed between controls and treatments, neither in oxic nor in anoxic conditions over the depth intervals. The same trend was found for the other different Fe fractions (data not shown). Fig. 6b also shows that similar LOI contents were present at the end of incubation period in treatments and controls.

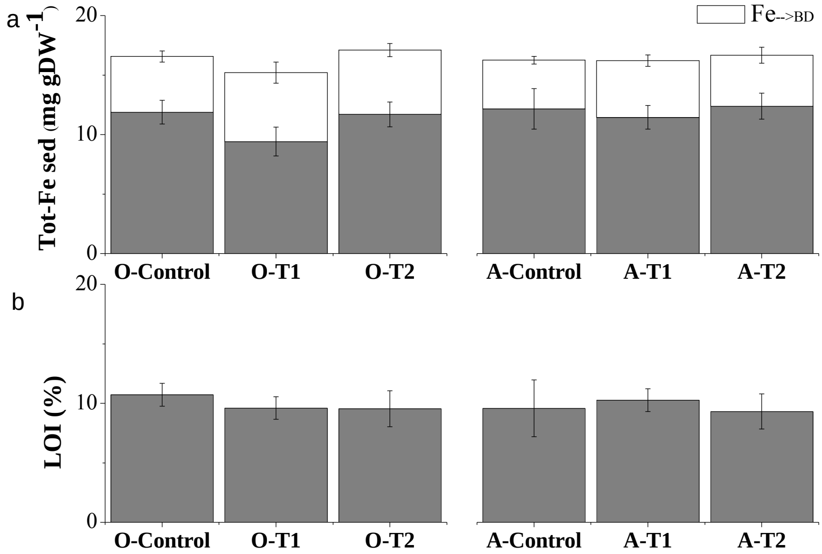


Fig. 6. a) $Fe_{\rightarrow BD}$, Tot-Fe_{sed} and b) LOI (%) in treatments and controls within 0-10 cm depth at the end of the incubation period. Standard deviation (SD).

4. Discussion

4.1. Effect of anoxic conditions on P exchange across the sediment and water interface

Comparison of controls (A-Control and O-Control) in which the only different factor was redox potential, highlights the importance of the classical model proposed by Einsele (1936) and Mortimer (1941) which pointed out the close coupling between redox-sensitive Fe compounds and P exchange between sediment-water interface. This classical model has been later confirmed by many other studies such as Boström et al. (1988a), Gomez et al. (1999), Burley et al. (2001) and Hansen et al. (2003).

Under oxic conditions, sediment of Honda lake exhibited a net P uptake. It is very well known that in these conditions, there exists an oxidized microzone in the uppermost sediment layer in which precipitated Fe naturally present in sediment is able to retain P (Mortimer, 1971). It is important to remark that under oxic conditions, sediment P adsorption capacity and thus, P transport across sediment-water interface is mainly controlled by $Fe_{\rightarrow BD}$: $P_{\rightarrow BD}$ molar ratio (Jensen, 1992). In Honda lake, this molar ratio was 15:1 which is considerably higher than the molar ratio (8:1) required for trapping P in the sediment (Jensen, 1992).

By contrast, in anoxic conditions, P exchange rate across the sediment and water interface was 1.96 µg P cm^{-2} day^{-1} (A-Control) which is in the range of those values reported for hypertrophic systems (Carter and Dzialowski, 2012; Nürnberg, 1988; 1997). This positive P efflux and the increase of Fe concentration in water column along the incubation period evidenced the existence of Fe reduction and the subsequent P mobilization from lake sediment to the overlying water (Marsden, 1989; Hupfer and Lewandowski, 2008). In such conditions, P transport from sediment to water column is mediated by the concentration gradient between dissolved P in pore water sediment and overlying water (Eckert et al., 1997; Sondergaard et al., 2001).

As a way of illustration of the relevant effect of internal P loading on the P concentration of the water column, next we present some calculations. Since the lake presents an average of 204 anoxic days per year (de Vicente et al., 2003) and in this study we have measured a P release flux of 1.96 µg P cm^{-2} day^{-1} under anoxic condition, the internal P loading is calculated to be responsible for 376 kg P yr^{-1}. However under oxic conditions, natural Fe oxides present in this sediment would retain 360 kg P during this period (161 days yr^{-1}). The net result of both processes would give rise to a P concentration in the water column of 135.6 µg L^{-1}, contributing to almost half of the annual mean TP concentration (312 µg L^{-1}; de Vicente et al., 2003). However it is important to consider that anoxic conditions promoted by BD addition are likely to be a severe treatment which it may cause an overestimation of these calculations.

4.2. Effect of adding MPs on P exchange fluxes across the sediment-water interface and on Fe concentrations in water column.

4.2.1 Oxic treatments

Sediment from Honda lake experienced a net P uptake from the overlying water after MPs amendment (O-T_1 and O-T_2), both before and after removing them from the cores. This behavior highlights the scarce relevance of P release due to the resuspension when removing MPs under oxic conditions. On the other hand, the rather similar P fluxes values in the control and treatments evidenced the negligible effect of adding MPs on P efflux. The reason behind this finding is that in the sediment of the study lake, as it has been already stated, Fe oxides concentration was high enough to trap P under oxic conditions. Regarding Fe concentrations in water column, it was observed that initial and final Fe concentrations kept similar in control and treatments. These data along with the fact that Tot-Fe_{sed} was not significantly increased in treatments compared to control indicates that MPs do not release Fe in oxic conditions.

4.2.2 Anoxic treatments

In anoxic conditions, the addition of MPs caused an important reduction in P fluxes compared to the control (A-Control). While in the case of A-Control P was released from the sediment to the overlying water, in the case of A-T_2 treatment, P mobilized by the reduction of Fe oxides did not go to the water column but was adsorbed on MPs, as it is reflected by a net P uptake by the sediment. Hence, our results have shown that the addition of MPs in a molar ratio of 560:1 respect to P_{Mobile} concentration was enough to inactivate P internal loading. A similar P efflux reduction (100 % reduction compared to control) under anoxic conditions has been reported by some authors such as Hansen et al. (2003) (P efflux= -0.025 μg P cm^{-2} day^{-1}) when using Al: P_{Mobile} molar ratio of 10:1; Reitzel et al. (2003) (P efflux= -0.097 μg P cm^{-2} day^{-1}) when using Al: P_{Mobile} molar ratio of 4:1 and Gibbs et al. (2011) (-0.7 μg P cm^{-2} day^{-1} and -0.8 μg P cm^{-2} day^{-1}) when adding Phoslock® at a 50:1 and 100:1 Phoslock®: P weight ratio, respectively. It is worth to note that P uptake rate by sediment was a little bit lower in these studies compared to our value. By contrast, P efflux after removing MPs reflected that the 285:1 Fe P_{Mobile} molar ratio was not enough to suppress short-term P efflux (95 % of reduction). It is important to remark that P concentration in water column was measured right after removing MPs, and thus higher concentration of P due to resuspension could be measured. Likewise, the 10:1 Fe:P molar ratio proposed by Hansen et al. (2003) as well as the 8:1 Al: P_{Mobile} molar ratio proposed by Reitzel et al. (2003) were not enough to retain P by the sediment (P efflux = 0.053 μg P cm^{-2} day^{-1} and 0.16 μg P cm^{-2} day^{-1}, respectively) giving a 95 % of reduction in both cases.

Final Fe concentrations in water column were remarkably higher in treatments (A-T_1 and A-T_2) compared to A-Control, reflecting that under anoxic conditions MPs were releasing Fe to the water column, probably due to the dissolution of MPs in contact to BD reagent used to guarantee anoxic conditions. Hunt et al. (1995) and Fine et al. (1992) reported that fine grain magnetite (with a similar surface of our MPs) undergo dissolution in the presence of Citrate-BD solution. Fe concentration in freshwater is naturally less than 1 mg L^{-1}, whereas in anaerobic groundwater where the Fe^{+2} species predominates it usually ranges 0.5-10 mg L^{-1} (World Health Organization, 2003; Xing and Liu, 2011). According to some studies, there is no evidence of negative effects of increasing Fe concentrations on the maximum achievable ecological quality for fish, macrophyte and diatom communities although it has been reported a decrease of macroinvertebrate populations in contact with high total Fe concentrations (WFD-UKTAG, 2012). Therefore, some thresholds have been proposed to guarantee the good state of these communities such as 1 mg L^{-1} total Fe (USEPA, 1976), and 0.21 mg L^{-1} or 1.74 mg L^{-1} for no changes or slight to moderate changes in community structure, respectively (Linton et al., 2007). At this point, it is of great importance to consider that the use of BD in this study for achieving anoxic conditions could have increased Fe concentrations more than natural anoxic conditions occurring in lakes, so lower Fe concentrations are likely to be measured when adding MPs in natural anoxic conditions. In addition, it is essential to work in synthesizing new MPs that do not release Fe in anaerobic conditions in order to reduce undesirable effects on biota.

4.3 Effect of adding MPs on sedimentary P pools

From initial sediment fractionation analysis we can conclude that $P_{\rightarrow HCl}$ pool which is not expected to contribute to internal P loading, is by fast the most important P form in our sediment. In ecosystems with high alkalinity (as in our case) P can be immobilized in sediment by coprecipitation or adsorption with carbonates at high pH values contributing to the burial of P in sediment (Otsuki and Wetzel, 1972; Eckert et al., 1997). Since in these wetlands the amount of P bound to carbonates is remarkable, potential internal P loading is likely to be closely related to reductions in pH values (Rydin and Welch, 1998). P_{Mobile} pool, which accounted for 8.52 g m^{-2} within the first 10 cm, has an outstanding importance as it can be used for predicting future internal P loading. The three P pools contributing to P_{Mobile} fraction ($P_{\rightarrow Water}$, $P_{\rightarrow BD}$, Org-P_{Labile}) consist of 37 % of Tot-P_{sed} at surface sediment and decrease with depth up to 21% at 10 cm depth due to the well-known upward transport of P to upper sediment layers (Rydin, 2000), suggesting that these three pools do not contribute to burial flux of P in sediments (Reitzel et al., 2003).

On the other hand, the addition of MPs has caused a reduction in P_{Mobile} concentration both in oxic and anoxic condition even in the deeper sediment layer (10 cm). In fact, the presence of MPs is not restricted to surface sediment as they are characterized by a much higher density (7.8 g cm^{-3}) than sediment (1.1 g cm^{-3}). Moreover, the high ionic strength present in the lake (conductivity of 3.09 mS cm^{-1}, de Vicente, 2004) implies a reduction in the zeta potential value which is involved in electrostatic repulsion interactions between MPs, leading to their aggregation and rapid sedimentation (de Vicente et al., 2011; Gunnars et al., 2002). The high density of MPs compared to the low density of other P-capping agents as Al salts (Lewandowski et al., 2003) entails a different behavior in sediment, causing P inactivation in deeper sediment layers. Phosphorus adsorption mechanisms onto MPs has been previously reported to take place by ligand exchange, in which hydroxyl groups are replaced by P species forming inner-sphere surface complexes at water-oxide interface (de Vicente et al., 2011; Daou et al., 2007). Despite being negatively charged under these experimental conditions of pH (pH=8), MPs are able to adsorb considerable amounts of P due to specific adsorption (de Vicente et al., 2011; Daou et al., 2007). Org-P_{Labile} was the only P sedimentary pool comprising P_{Mobile} which experienced a reduction in treatments compared to controls both in oxic and anoxic conditions. However, no changes in LOI % content between control and treatments have been observed which could be explained by the lower sensibility of the loss on ignition calculation method. The higher affinity of MPs for Org-P_{Labile} is likely to be the result of the well-known strong affinity of organic compounds by Fe oxides. In particular, Illés and Tombácz (2003) reported a notable adsorption of organic substances such as fulvic and humic acids (HA) by magnetite at low pH (4.5) but even at higher pH similar to those registered in our experiments (anoxic conditions, pH 6.2 ± 0.18; oxic conditions, pH= 8.15 ± 0.08). Similarly, Merino-Martos et al., (2015) reported a remarkable decrease in P adsorption efficiency, when using the same carbonyl Fe particles as in this experiment, in the presence of high HA concentrations (from 94% when HA = 17 mg C L^{-1} to 12 % when HA= 35.5 mg C L^{-1}).

4.4 Some considerations for lake restoration

In the framework of the whole available methods for lake restoration, MPs addition could be considered as a method focused on increasing P export for reducing lake P concentration. The use of MPs has several advantages compared with other drastic techniques that aim at increasing P export, such as sediment dredging. High turbidity due to resuspension of finely divided sediment, liberation of nutrients and toxic metals to water column, destruction of benthic fauna and high costs are some of the negative aspects about using sediment dredging (Petersen, 1982). In this sense, MPs minimizes impacts of resuspension and water column since they are more selective with sediment (they have more affinity with sedimentary P forms). Next, we will summarize some advantages of using MPs. First, P can be recovered from the aquatic ecosystem, which allows the later potential use of P as P-containing fertilizers. In this sense, it is nowadays considered that P is a non-renewable resource and could be exhausted in the next 50-100 years (Cordell et al., 2009). This situation has become alarming due to the increasing population density, above all in developing and emerging countries, which demands a high food production. So, it is required new challenges to find solutions where the equilibrium P-cycle is reached. Second, MPs can be recovered from the solution reducing both economic costs and toxic effects on the biota. In relation to the economic cost, it is important to consider that before making any whole lake application it is essential to gain also some knowledge about the economic balance. For making a economic balance, we have compared some crucial characteristics (price and P removal efficiency) of MPs and some of the most commonly used P adsorbent for lake restoration such as $AlCl_3 \cdot 6H_2O$ (i.e. Rydin, 2000; Reitzel et al., 2005; Egemose et al., 2011) and Phoslock® (i.e. Robb et al., 2003; Lürling and Tolman, 2010). In brief, we can conclude that when re-using MPs more than 2 times it is economically worth to use this adsorbent instead of $AlCl_3 \cdot 6H_2O$ or Phoslock®. In relation to the toxic effect on the biota, recent studies have found that MPs concentration have to be added in high concentration to cause negative effect on planktonic and benthonic organisms. In particular, Álvarez-Manzaneda et al., (unpublished results) have measured that the concentration of MPs that produces the immobilization of 50% of the individuals during exposition period (CE_{50}; OCDE 2004) was 0.9 and 0.4 g L^{-1} for *Daphnia magna* and *Chironomus* sp., respectively. At this point, it is relevant to note that, considering the MPs: P ratio reported in previous studies (de Vicente et al., 2010; Merino-Martos et al., 2011), the addition of 0.4 g L^{-1}would not be required as it corresponds to extremely high P concentrations. As a result, it is expected that in a whole lake application, MPs will not cause any toxic effect on the biota.

5. Conclusions

Despite the broad use of P-binding compounds for restoring eutrophicated waters, up to date, there is not a universal adsorbent for applying in whatever physico-chemical conditions (i.e. pH, redox potential) and therefore more research in new adsorbents is required. This study has evidenced that internal P loading during the anoxic period contributes to half of the annual mean P concentration in the water column of the study lake. In this sense, the use of MPs seems to be a suitable technique to apply since it is able of completely suppressing the short-term P efflux under the most undesirable conditions (anoxic) at the 560:1 MPs: P_{Mobile} ratio. However, when Fe oxides naturally present in sediment are enough to uptake P from water column, MPs do not show any effect on P fluxes. Apart from the effect of MPs on P efflux under anoxic conditions, MPs also reduces sedimentary P_{Mobile} concentration in both oxic and anoxic conditions, leading to a reduction in long-term P efflux. The best results have been found when adding MPs at the Fe: P_{Mobile} molar ratio of 560:1, which conducted to the removal of 141 kg of sedimentary P in anoxic and to 191 Kg P under oxic conditions for the whole lake area.

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Chapter III: An enclosure experiment for testing the consequences of using magnetic microparticles on water quality and sediment phosphorus pools

Funes, A., del Arco, A., Álvarez-Manzaneda Salcedo, I., de Vicente, J., de Vicente, I., 2016. An enclosure experiment for testing consequences of using magnetic microparticles on water quality and sediment phosphorus pools. Water Res. (in preparation to be submitted).

Abstract

By using microcosm enclosures containing brackish water (6000 µS cm^{-1}) and natural surface sediment from an hypertrophic aquatic ecosystem (Honda lake, in the southeast of Spain), this study assessed the effects of adding iron (Fe) magnetic microparticles (MPs) on water quality, with special focus on P concentrations in water column and in the sediment. In the experiments, two different treatments were considered for a constant 85:1 MPs:P_{Mobile} molar ratio; T-W -MPs applied above the surface layer of water-, and T-S -MPs added directly above the sediment-. Our study demonstrates the convenience of adding MPs for reducing both P concentrations in lake water and lake sediment. T-W and T-S exhibited an average reduction of 68% in dissolved inorganic P (DIP) concentrations in the water column compared to control for a 70-days oxic period, and a reduction of 80% for a 5-days anoxic period. Reactive silicate (Si) concentrations also decreased on average more than 50% for both periods and treatments compared to control, whereas dissolved organic carbon (DOC) concentrations were only reduced to 15% after 24 h of MPs addition. An interference of Si and DOC in P removal efficiency by MPs was detected. Despite the drastic reduction in DIP concentration, chlorophyll *a* (Chl *a*) was not reduced after MPs addition. A likely explanation is that total P (TP) concentrations were still very high after treatments (> 200 µg L^{-1}) and previous studies have reported that much lower TP concentration (0.05–0.1 mg P L^{-1}) are required for significant and sustained changes in the biological community. Sedimentary P fractions such as P bound to humic substances ($P_{NaOH, Humic}$) or easily degradable organic P (Org-P_{Labile}) were significantly reduced to 22% and 12%, respectively in T-S compared to control. Recovery of MPs by the magnetic device was much lower for T-S (31%) than for T-W (90%). Finally, from the comparison of both treatments (T-W and T-S) and in order to recommend a future whole-lake application, we conclude that T-W is the most suitable treatment to ensure the effectiveness of P export (in water and sediment) and the recovery of MPs.

1. Introduction

For combating the worldwide spread problem of eutrophication, large investments in improving wastewater treatment combined with the implementation of other measures to reduce, in particular, the phosphorus (P) input to aquatic ecosystems have been accomplished (Jeppesen et al., 1999; Gulati and Van Donk, 2002; Søndergaard et al., 2005). Although some lakes may respond fast to a reduction in external P loading (Sas, 1989), many other lakes are still eutrophic and exhibit an unsatisfactory water quality (Marsden, 1989; Jeppesen et al., 1991; van der Molen and Boers, 1994). The delayed recovery could be caused by a fish community dominated by zooplanktivorous species which make weaker the top-down control on algal biomass (Benndorf, 1990; Hansson et al., 1998), but also by a continued P release from the sediment (Marsden, 1989; Jeppesen et al., 1991; Søndergaard et al., 2001). As the internal P loading may have a very significant impact on lake water P concentrations and subsequently on lake water quality (Phillips et al., 1994), many restoration focus on increasing P retention by the sediment. Among these methods, we highlight the next: hypolimnetic aeration (Beutel, 2003; Liboriussen et al., 2009), calcium addition (Otsuki and Wetzel, 1972; Prepas et al., 2001; Dittrich et al., 2011), nitrate (NO_3^-) addition (Ripl, 1976; Foy, 1986), iron (Fe) addition (Boers et al., 1992; Hansen et al., 2003), aluminum (Al) addition (Cooke et al., 1993; Rydin and Welch, 1999; Egemose et al., 2011) and Phoslock® addition (Robb et al., 2003; Meis et al., 2012, 2013; Reitzel et al., 2013a; Spears et al., 2013, 2015).

Among all of the above mentioned methods, those most frequently used today are based on increasing sedimentary P binding capacity (P inactivation methods) by the addition of different compounds. However, the P removal efficiency of most P sorbing materials is typically related to pH, potential redox and the presence of other dissolved ions (Westholm, 2006; de Vicente et al., 2008; Vohla et al., 2011; Lürling et al., 2014). In particular most frequently used adsorbents (Al and Phoslock®) do not efficiently remove P at high pH values (Ross et al., 2008; Reitzel et al., 2013b) which are typically encountered in typical hypereutrophic aquatic ecosystems. Moreover, after P inactivation, adsorbents remain within the sediment, and this implies that they could be eventually released to the water column under changing physicochemical 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).

In this context, several studies have recently proposed the convenience of using magnetic particles (MPs) for removing P from aqueous solutions by first adsorbing P and by the later application of a magnetic separation gradient, minimizing alteration in water quality and enabling their reuse in subsequent adsorption processes (de Vicente et al., 2010). Some of the advantages of using MPs for lake restoration are as follows: (i) MPs can efficiently adsorb P under both batch and flow conditions (de Vicente et al., 2010; Merino-Martos et al., 2011) so they can be used for trapping P in rainfall ponds and hence for controlling P input through run-off. Also, they can be applied in water treatment plants and/or point sources under continuous flow conditions; (ii) MPs are able to adsorb considerable amounts of P for pH > 7, despite being negatively charged, due to specific adsorption (Daou et al., 2007; de Vicente et al., 2010) (iii) P adsorption by MPs does not depend on redox conditions (Funes et al., 2016); (iv) MPs addition causes a reduction in sedimentary P_{Mobile} concentration (under both oxic and anoxic conditions) hence contributing to a potential reduction in long-term P efflux; (v) Based on a preliminary economic balance, Funes et al. (2016) found that when re-using MPs more than 2 times, it is economically worth to use this adsorbent instead of $AlCl_3•6H_2O$ or Phoslock® and (vi) No toxic effect on the planktonic and benthonic organisms are expected to be caused after MPs addition (Álvarez-Manzaneda et al., unpublished results).

Before using MPs in a whole-lake restoration project, it is also important to remark that some of the major ions present in natural waters, in particular humic acid (HA) and reactive silicate (Si), cause a reduction in the effectiveness of P adsorption by MPs. In the context of the MPs used in this work, this has been observed both in complex natural matrices (de Vicente et al. 2011) and single-ion laboratory experiments (Merino-Martos et al. 2015). With this in mind, it would not be surprising that the addition of MPs may influence not only P cycling but also the cycling of other nutrients.

The goal of this study was to assess the effectiveness of MPs application on improving water quality of a hypertrophic Mediterranean lake. For this aim, a large enclosure, containing both lake water and lake sediment, was undertaken. During the experiment, which lasted for 84 days, oxic and anoxic conditions were simulated and physicochemical and biological variables were monitored in order to evaluate the long-term effect of MPs addition on nutrient cycling; especially on P cycle. The specific objectives of the study include: (i) to test the effect of MPs addition on lake water P concentration; (ii) to evaluate the effect of MPs application on sedimentary P pool; (iii) to assess the effects of MPs addition on nutrient cycling (carbon C, nitrogen N, Si) and (iv) to establish the best working conditions for achieving both the highest MPs removal and the highest improvement in water quality.

2. Materials and Methods

2.1 Sampling site

Honda lake is a shallow (surface area = 9 ha, Z_{mean} = 1.3 m; Z_{max} = 3.2 m), hypertrophic and brackishwater (6.0 mS cm^{-1}) wetland situated in the coast of Almería (Southeast of Spain; de Vicente et al., 2003; Funes et al., 2016). The catchment area is principally dominated by intensive agricultural areas although the lake was designated as Natural Reserve (1989) and Ramsar Site (1994). Apart from the high external P inputs, internal P loading has also special importance due to frequent resuspension events and the anoxic periods during summer in which a large amount of sedimentary P is continuously released to the overlying water (de Vicente et al., 2006).

2.2 Materials

MPs used as P adsorbents (HQ grade, BASF, Germany) were micronsized soft magnetic spherical particles with negligible coercitive field and remnant magnetization (i.e. magnetization becomes zero when removing the magnetic field). More details on the particles used can be found in de Vicente et al. (2010) and Merino-Martos et al. (2011).

A specially designed device was constructed to remove MPs from the sediment in the enclosures (Fig. 1). This device essentially consists in a magnetic rake (Fig. 1a). It has a maximum diameter of 20 cm and contains a platform (with a handle) provided with an array of 89 cylindrical Neodimium magnets individually inserted on its base. A one-piece hard-plastic shell (1 mm thickness) protected the magnets from the direct contact with MPs in order to facilitate MPs recovery and cleaning. A detail of the base and hard –plastic shell is provided in Fig. 1b. In Fig. 1c we show a surface plot of the magnetic field density on the hard –plastic shell. Fig. 1d shows the magnetic field density profiles in the x- and diagonal (45º) directions. Figs. 1c and 1d demonstrate that the interference between the magnets is minimal. The size of the magnets (diameter 4 mm, height 25 mm), arrangement (cubic array) and separation between them (14 mm) were modeled with Comsol Multiphysics software (COMSOL Inc., Burlington, MA) in order to ensure an optimum magnetic flux density gradient of approximately 20 T/m to remove the maximum number of MPs.

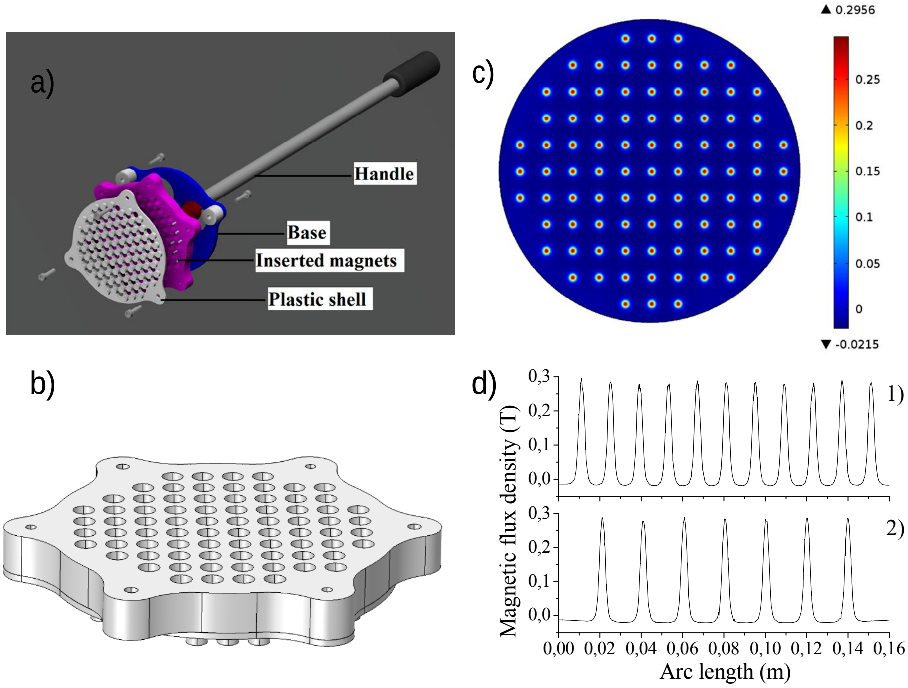

Fig. 1. Design of the magnetic rake for MPs removal. a) Schematics of the magnetic rake. b) Detail of the base and hard-plastic shell protecting the magnets. c) Surface plot of the magnetic field density in perpendicular direction to the platform on the surface of the shell (1 mm distance from the top of the magnets). d) Magnetic field density profiles in the x- (1) and diagonal (45º) directions (2).

2.3 Microcosm enclosures experiment

On July 2015, 100 dm^3 of surface sediment were collected with an Ekman dredge at the deepest site of the lake. Surface water was also collected near the shore with a peristaltic pump and kept at 14 ºC until use. Sediment was thoroughly homogenized in a big tank before dividing it up among fifteen PVC enclosures (Ø = 38 cm; h = 58 cm). Once in the laboratory, 6600 cm^3 of surface sediment and 40 L of surface lake water were added to each enclosure. These enclosures were randomly distributed through space in an outdoor roofed area to avoid any bias.

After one-week of stabilization period (at day 0), the physico-chemical parameters were measured and the enclosures were subjected to their corresponding treatment. This physico-chemical analysis reflects the initial state prior to the treatments application. Three different treatments (n = 5 replicates per treatment) were considered: (1) MPs addition over the surface water (T-W); (2) MPs addition over the surface sediment (T-S); and (3) No addition of MPs. The later treatment (#3) served as the control. MPs were added to the enclosures in aqueous dispersion (distilled water, 120 g L^{-1}) by using a peristaltic pump and a grid to guarantee a homogeneous distribution of the MPs in the replicates. The MPs:P_{Mobile} molar ratio applied was 85:1. This is three times higher than that proposed by de Vicente et al. (2010) when testing P adsorption efficiency by MPs in batch experiments. We decided to overdose the proposed ratio to counteract possible chemical interferences effects (de Vicente et al., 2011).

After a contact time of 24 h (at day 1), P loaded MPs were removed by fully immersing the magnetic rake into the enclosures just over the sediment surface. For consistency, the rake was passed only once as this already resulted in a MPs removal above 90 %. Of course, passing the rake more than one would result in a removal enhancement.

In the following days the enclosures were subjected to an intensive study by monitoring the time evolution of physico-chemical parameters. The whole subsequent experiment comprised two stages: an initial 70 days-oxic period (from day 0 to day 70) in which the enclosures were continuously air bubbled, and a 5 days-anoxic period (from day 79 to day 84) where the enclosures were continuously N_2 bubbled. It is worthy to highlight that from day 70 enclosures were N_2 bubbled until stable anoxic conditions were reached at day 79.1.5 L of water was sampled from each enclosure on days: 2 (i.e. 24 h after MPs removal), 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 79, 81 and 84 to analyze physico-chemical parameters. Water removed by sampling and evaporation was weekly replaced by previously filtered (30 µm) lake water. Temperature, pH, dissolved oxygen (DO) concentration, conductivity and total dissolved solids (TDS) were measured with a multi-parameter probe (Hanna Instrument, HI 9829). Total suspended solids (TSS) were determined by filtering 50 mL of water sample through pre-weighed filters (Whatman GF/F). The residue retained in the filters was dried at 105 ºC for 24 h and the TSS was estimated. Determination of chlorophyll *a* (Chl *a*) was achieved by filtering 50 mL of water sample (Whatmann GF/F). The filters were placed in a glass vial with 5 mL of 90 % acetone at 4 ºC in darkness for 24 h. Extract was filtered and measured in a spectrophotometer (Biochrom-Libra S50) at 630, 645, 665 y 750 nm. Chl *a* was calculated according to Jeffrey and Humphrey (1975):

$$Chl\ a = \frac{(11.85\ E_{665\ o} - 1.54\ E_{645\ o} - 0.08\ E_{630\ o})v}{V Z} \qquad (5)$$

here $E_{665\ o}$, $E_{645\ o}$ and $E_{630\ o}$ correspond to the absorbances (corrected for turbidity at 750 nm) at 665, 645 and 630 nm, v is the extracted volume (5 mL), V is the volume of filtered water (0.05 L) and Z is the cuvette pathlength (1 cm). Color samples were filtered (Whatman GF/F) and measured in a spectrophotometer at 440 nm in 1-cm cuvettes. Water color was expressed as absorption coefficient (a_{440}) in units of inverse meters (m^{-1}) according to the following equation (Reche and Pace, 2002):

$$a_{440} = \frac{2.303\, A_{440}}{Z} \qquad (6)$$

where A_{440} is the measured absorbance at 440 nm and Z is the optical pathlength in m. Dissolved organic C (DOC) was only measured at days 0, 2 and 7, by filtering the samples through precombusted Whatman filters GF/F, acidified to pH < 2 and measured with a total organic C analyzer (Shimadzu TOC-V, CSH, Japan). Total dissolved Fe (Tot-Fe_{dis}) was measured by the ferrozine method (Gibbs, 1979). Dissolved inorganic P (DIP) was analyzed by molybdenum blue method (Murphy and Riley, 1962). Total P (TP) and total dissolved P (TDP) were determined after the digestion of unfiltered/filtered aliquots, respectively, with potassium persulphate (APHA, 1995). Dissolved organic P (DOP) was calculated by subtracting DIP to TDP. Total particulate P (TPP) was calculated as the difference between TP and TDP. In relation to the dissolved inorganic N (DIN) pool, NH_4^+-N was determined by phenate method (Rodier, 1989), NO_3^--N was determined by ultraviolet spectrophotometric screening method (APHA, 1995) and NO_2^--N was analyzed by sulfanilamide method (Rodier, 1989). Total N (TN) and total dissolved N (TDN) were determined by ultraviolet method after digestion of unfiltered/filtered aliquots, respectively (APHA, 1995). Dissolved organic N (DON) was determined by subtracting DIN to TDN. Total particulate N (TPN) was calculated as the difference between TN and TDN. Major cations [sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+})] and anions [sulphate (SO_4^{2-}) and chloride (Cl^-)] were measured by ion cromatography (IC; 940 Professional IC Vario, Metrohm). Finally, reactive Si was determined by using a spectrophotometric method described by Koroleff (1983), after a reaction with ascorbic acid and molybdate.

Sedimentary P fractions were also determined within the first 6 cm of sediment, at the beginning (initial fractionation) and at the end of the experiment, following Paludan and Jensen (1995). In particular, the following fractions were obtained: orthophosphate (o-P) extracted from porewater in distilled water ($P_{\rightarrow Water}$), o-P bound to reducible metals such as Fe and Mn ($P_{\rightarrow BD}$) extracted in 0.22 M bicarbonate dithionite solution (BD), P adsorbed to Al oxides, clay minerals and humic acids (HA) extracted in 0.1 N NaOH ($P_{\rightarrow NaOH}$), P bound to HA precipitated in acidified solution ($P_{\rightarrow NaOH,\ Humic}$), o-P bound to magnesium and calcium extracted in 0.5 N HCl ($P_{\rightarrow HCl}$), and refractory organic P extracted with 1M HCl at 120 ºC after combusting the pellet at 550 ºC for 3.5 h ($P_{\rightarrow HCl,\ Res}$). Finally, organic P labile (Org-P_{Labile}) was calculated as the sum of non-reactive P (NRP) coming from the three first steps of extraction which resulted from substracting o-P to Total P (wet digestion with 0.18 M $K_2S_2O_4$). P_{Mobile}, the only fraction that is expected to contribute to internal P loading, was calculated as the sum of $P_{\rightarrow Water}$, $P_{\rightarrow BD}$ and Org-P_{Labile} within 6 cm depth. Additionally, sedimentary Fe fractions were similarly determined in the sequential fractionation. Sedimentary total P (Tot-P_{sed}) and sedimentary total Fe (Tot-Fe_{sed}) were measured on parallel extractions by combusting the sediment samples (520 ºC, 5.5 h) followed by acid hot digestion with 1 M HCl (120 ºC, 1 h). P and Fe data were normalized with Tot-P_{sed} and Tot-Fe_{sed} data.

2.4 Data analysis

Statistical analyses were done with Statistica 7.1 software (Stat Soft Inc., Tulsa, USA). Differences in water chemical parameters due to treatments application over time (split plot design: one between-subjects factor and one within-subjects factor) were tested by using repeated measures ANOVA (RM ANOVA) with Fisher´s Least Significance Difference (LSD) post hoc test. The effect of a specific treatment on a dependent variable was assumed significant when inter-treatments significant differences were found in more than 2 consecutive sampling days. Since sphericity assumption was usually violated, the Greenhouse-Geissler correction of the degrees of freedom was used (when epsilon < 0.75). Changes in sedimentary P and Fe pools were explored with one-way ANOVA and LSD post hoc test and considered significant when $p < 0.05$.

3. Results

3.1 Changes in water column

Table 1 shows the averaged values of all physico-chemical and biological parameters (Chl *a* concentration) measured in the overlying water during the oxic and the anoxic period. For completeness, the associated statistical results are contained in Table 2.

	Control		**T-W**		**T-S**	
	Oxic	**Anoxic**	**Oxic**	**Anoxic**	**Oxic**	**Anoxic**
T (ºC)	21.2 ± 3.9	14.6 ± 1.3	21.2 ± 4.0	14.5 ± 1.3	21.2 ± 3.9	14.4 ± 1.4
pH	9.0 ± 0.3	9.0 ± 0.0	9.0 ± 0.3	9.1 ± 0.0	9.0 ± 0.3	8.7 ± 0.7
O_2 (mg L^{-1})	7.5 ± 0.8	0.9 ± 0.1	7.5 ± 0.8	0.8 ± 0.1	7.4 ± 0.9	0.9 ± 0.2
Conductivity (µS cm^{-1})	6625 ± 469	6750 ± 485	6516 ± 423	6958 ± 89	6437 ± 392	6838 ± 81
TDS (mg L^{-1})	3369.0 ± 357.4	3255.1 ± 460.4	3306.5 ± 276.7	3483.6 ± 48.7	3226.5 ± 194.9	3427.3 ± 52.8
TSS (mg L^{-1})	83.1 ± 38.3	45.6 ± 2.0	79.8 ± 28.1	47.2 ± 2.9	84.5 ± 37.6	46.1 ± 6.9
Chl *a* (µg L^{-1})	39.2 ± 11.3	46.8 ± 7.6	32.8 ± 13.4	35.9 ± 8.4	34.1 ± 14.9	25.8 ± 7.1
Color (m^{-1})	4.4 ± 0.8	4.2 ± 0.4	3.9 ± 0.9	4.0 ± 0.1	4.1 ± 0.7	3.1 ± 0.5
DOC (mg L^{-1})	67.0 ± 15.0	-	58.9 ± 9.0	-	61.5 ± 4.8	-
Tot-Fe_{dis} (mg L^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DIP (µg L^{-1})	289.5 ± 69.5	402.5 ± 18.2	91.7 ± 30.7	84.3 ± 29.2	91.2 ± 25.5	78.9 ± 15.3
TDP (µg L^{-1})	315.6 ± 69.3	406.8 ± 18.5	119.0 ± 30.3	124.1 ± 38.7	116.9 ± 27.7	93.4 ± 17.0
TP (µg L^{-1})	506.5 ± 121.0	500.5 ± 17.2	311.6 ± 99.3	221.6 ± 16.5	300.5 ± 110.2	214.9 ± 25.2
DIN (mg L^{-1})	1.6 ± 0.2	1.2 ± 0.2	1.8 ± 0.4	1.0 ± 0.4	2.0 ± 0.5	1.2 ± 0.4
TDN (mg L^{-1})	2.5 ± 0.3	2.2 ± 0.0	2.8 ± 0.4	2.3 ± 0.0	2.8 ± 0.5	1.9 ± 0.0
TN (mg L^{-1})	3.3 ± 0.4	3.2 ± 0.0	3.6 ± 0.6	3.6 ± 0.0	3.8 ± 0.5	3.6 ± 0.0
Si (mg L^{-1})	7.3 ± 1.0	8.3 ± 1.1	4.1 ± 0.6	3.2 ± 0.5	4.0 ± 0.8	2.7 ± 0.4
Na^+ (mg L^{-1})	838.35 ± 17.5	958.75 ± 0.0	831.72 ± 76.7	981.46 ± 0.0	846.89 ± 1.3	894.8 ± 0.0
K^+ (mg L^{-1})	115.1 ± 6.6	126.8 ± 0.0	111.0 ± 6.4	130.1 ± 0.0	115.3 ± 8.1	114.1 ± 0.0
Mg^{2+} (mg L^{-1})	30.7 ± 3.3	22.0 ± 0.0	26.7 ± 2.4	10.2 ± 0.0	26.3 ± 8.3	7.2 ± 0.0
Ca^{2+} (mg L^{-1})	215.73 ± 121.1	352.10 ± 0.0	195.48 ± 4.9	347.21 ± 0.0	276.14 ± 30.0	308.31 ± 0.0
Cl^- (mg L^{-1})	1451.6 ± 45.7	1676.0 ± 0.0	1448.7 ± 130.3	1708.6 ± 0.0	1434.6 ± 0.3	1569.2 ± 0.0
SO_4^{2-} (mg L^{-1})	712.3 ± 10.6	853.9 ± 0.0	696.7 ± 59.5	771.8 ± 0.0	666.6 ± 45.8	687.9 ± 0.0

Table 2. Statistical results of the RM ANOVA model. df = degrees of freedom and p value= * (<0.05); ** (<0.005); *** (<0.0005).

	TREATMENT				TIME				TREATMENT x TIME			
	df 1	**df 2**	**F**	**p value**	**df 1**	**df 2**	**F**	**p value**	**df 1**	**df 2**	**F**	**p value**
T (°C)	-	-	-	-	-	-	-	-	-	-	-	-
pH	2	12	0.6	no sig	1.3	15.9	7,0	*	2.6	15.9	1.1	no sig
O_2 (mg L^{-1})	-	-	-	-	-	-	-	-	-	-	-	-
Conductivity (µS cm^{-1})	2	10	0.7	no sig	1.4	13.9	27.5	***	2.8	13.9	0.9	no sig
TDS (mg L^{-1})	2	12	0.8	no sig	2.2	26.7	3.7	*	4.4	26.7	0.8	no sig
TSS (mg L^{-1})	2	9	0.4	no sig	2.0	18.3	6.7	*	4.1	18.3	0.6	no sig
Chl *a* (µg L^{-1})	2	8	1.5	no sig	3.2	25.9	3.2	*	6.5	25.9	0.9	no sig
Color (m^{-1})	2	11	4.3	*	2.5	27.9	15.0	***	5.1	27.8	0.7	no sig
DOC (mg L^{-1})	2	12	5.1	*	2.0	24.0	14.6	***	4.0	24.0	1.6	no sig
Tot-Fe$_{dis}$ (mg L^{-1})	-	-	-	no sig	-	-	-	no sig	-	-	-	no sig
DIP (µg L^{-1})	2	12	40.2	***	3.5	42.3	15.7	***	7.0	42.3	6.6	***
TDP (µg L^{-1})	2	12	39.0	***	4.4	52.9	13.1	***	8.8	52.9	5.6	***
TP (µg L^{-1})	2	12	44.3	***	5.0	59.5	139.8	***	9.9	59.5	5.7	***
DIN (mg L^{-1})	2	12	7.0	*	2.6	31.6	41.8	***	7.1	42.8	2.4	*
TDN (mg L^{-1})	2	11	2.9	no sig	6.0	66.0	16.8	***	12.0	66.0	1.3	no sig
TN (mg L^{-1})	2	12	7.6	*	6.0	72.0	16.4	***	12.0	72.0	1.4	no sig
Si (mg L^{-1})	2	12	61.0	***	2.8	33.2	13.0	***	5.5	33.2	7.4	***
Na^+ (mg L^{-1})	2	12	0.2	no sig	3.0	36.0	11.1	***	6.0	36.0	0.9	no sig
K^+ (mg L^{-1})	2	9	0.1	no sig	3.0	27.0	6.0	**	6.0	27.0	1.0	no sig
Mg^{2+} (mg L^{-1})	2	6	3.4	no sig	1.2	7.3	11.6	*	2.4	7.3	0.9	no sig
Ca^{2+} (mg L^{-1})	2	12	0.6	no sig	1.4	16.6	7.6	*	2.8	16.6	1.5	no sig
Cl^- (mg L^{-1})	2	12	0.6	no sig	1.6	18.8	11.9	**	3.1	18.2	1.1	no sig
SO_4^{2-} (mg L^{-1})	2	12	4.9	*	1.9	22.2	8.1	**	3.7	22.2	2.4	no sig

With regards to P, the time-evolution of DIP, TDP and TP for control and treatments is shown in Fig. 2. A significant effect of treatments on DIP concentrations and also a significant variation of DIP concentrations over time were observed (c.f. Table 2). DIP concentration was substantially reduced in T-W and T-S as compared to control during several sampling days (see Fig. 2). It is noteworthy to highlight that DIP concentrations were similar in all enclosures before treatments application (day 0), however a notable reduction took place in T-S and T-W (65 % of reduction) compared to control just 24 h after MPs removal (day 2). On average, DIP was reduced 68 % in both MPs treatments compared to control for the 70-days oxic period whereas a reduction even greater was found for the 5-days anoxic period in which DIP was reduced 80 %. Treatment x time interaction was also significant (c.f. Table 2) suggesting that the magnitude of the differences between treatments and control are influenced by time, being the magnitude of the differences higher over time. Data suggest that although differences in DIP between control and MPs treatments existed within the first two weeks, the most remarkable differences took place from day 56 onwards. No significant differences in DIP were noted between amended MPs treatments (T-W and T-S). In terms of accumulated DIP fluxes (data not shown), values changed from 14.0 µg cm^{-2} in control to 4.6 µg cm^{-2} in T-W and to 2.9 µg cm^{-2} in T-S, (67 % and 79 % of P flux reduction, respectively) in the oxic period, and from to 5.2 µg cm^{-2} in control to -2.2 in T-W and -0.9 in

T-S (100 % of P efflux reduction) in the anoxic period. Inter-treatments differences in TDP and TP were mostly due to changes in DIP concentrations since neither differences between treatments nor treatment x time interaction were found for TPP and DOP (data not shown). On average, TDP and TP were reduced 62 % in both T-W and T-S compared to control for the oxic period whereas for the anoxic period a reduction of 70 % and 77 % was found for T-W and T-S, respectively.

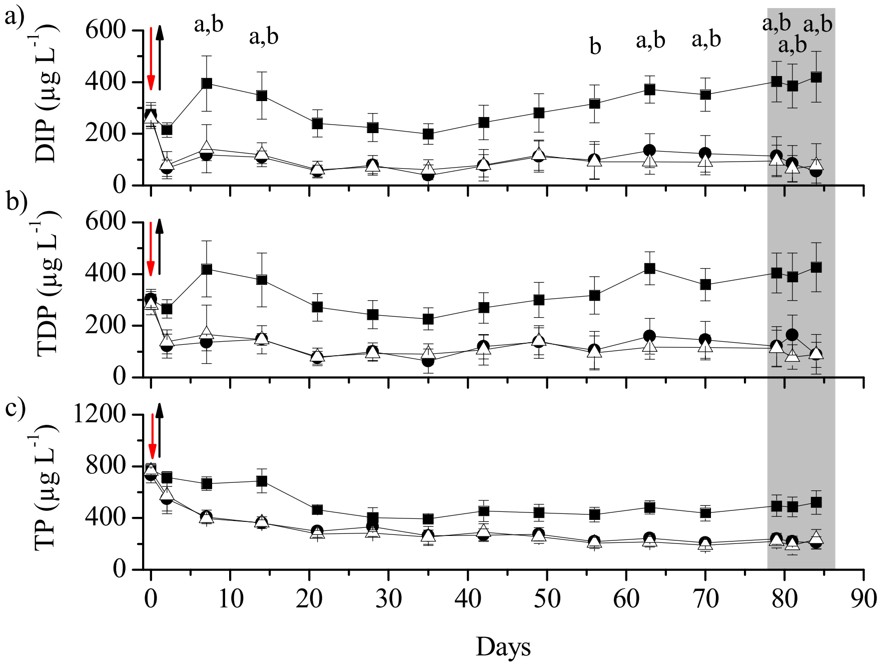

Fig. 2. Temporal evolution of DIP (a), TDP (b) and TP (c) in lake water for control (closed squares), T-W (closed circles) and T-S (open triangles). Red-downwards arrows indicate the moment (day 0) when MPs were added, while black-upwards arrows reflect MPs removal (after 24 h). Significant differences ($p < 0.05$) between treatments and control, for DIP fraction, are indicated by letters: a (control and T-W) and b (control and T-S). Not-shadowed area represents the oxic period (70 days; n = 12); shadowed area represents the anoxic period (5 days; n = 3).

Changes in the concentration of the different N fractions (DIN, TDN and TN) over time are shown in Fig. 3. No significant effect of treatments were found for any N pool as significant inter-treatment differences were only found at 2 sampling days for DIN and TN (see Table 2). Specifically, at day 7 T-W and T-S showed higher DIN and TN than control, and on day 21 DIN was higher in T-S than in control. Changes in TN between treatments were attributed mostly due to changes in DIN since treatments did not have any effect on DON and TPN (data not shown). It is noteworthy that all N forms varied synchronically over time in all the enclosures. In particular, the average DIN, TDN and TN concentrations narrowly ranged between 1.5-1.8 mg L^{-1}, 2.5-2.7 mg L^{-1} and 3.3-3.8 mg L^{-1}, respectively, in all enclosures.

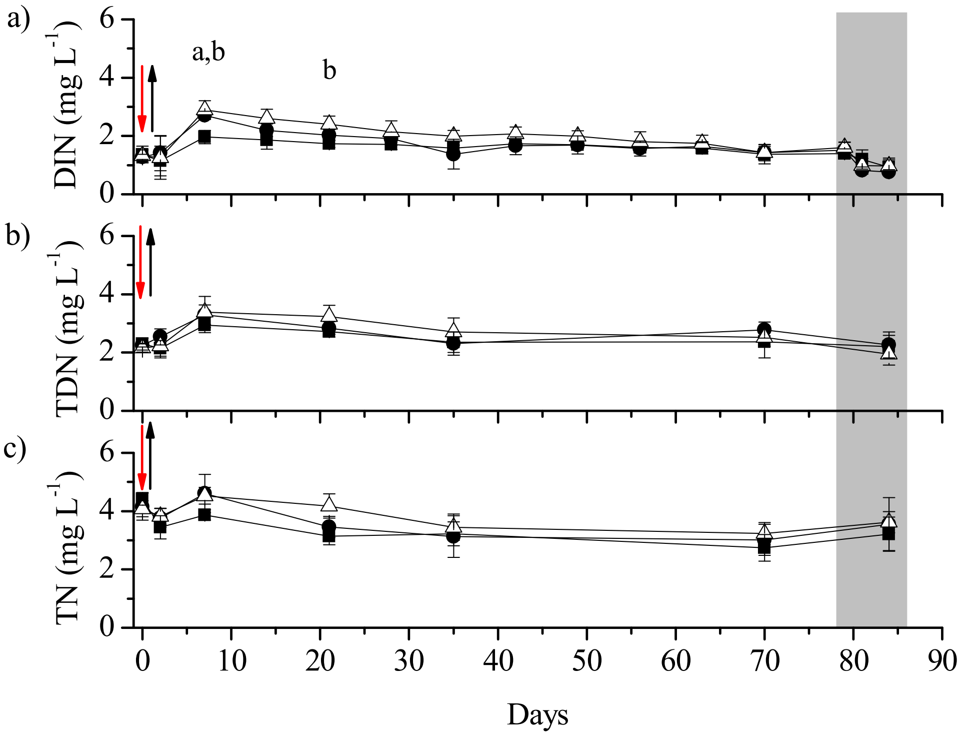

Fig. 3. Temporal evolution of DIN (a), TDN (b) and TN (c) in lake water for each treatment and control. Symbols and arrows are the same as described in Fig. 2. Significant differences ($p < 0.05$) between treatments and control, for DIN fraction, are indicated by letters: a (control and T-W) and b (control and T-S). Not-shadowed/shadowed areas illustrate the same as in Fig. 2

Si concentration was significantly affected by treatments in almost all sampling days (Fig. 4a) and it also changed over time (Table 2). On average, Si was reduced 45 % in both MPs treatments compared to control for the oxic period. In the anoxic period, Si concentration was 61 % lower in T-W and 68 % in T-S compared to control. However, no differences in Si between MPs treatments were noticed. A significant effect of treatment x time interaction was observed, indicating a significant effect of time on the magnitude of the differences between treatments which tend to increase over time.

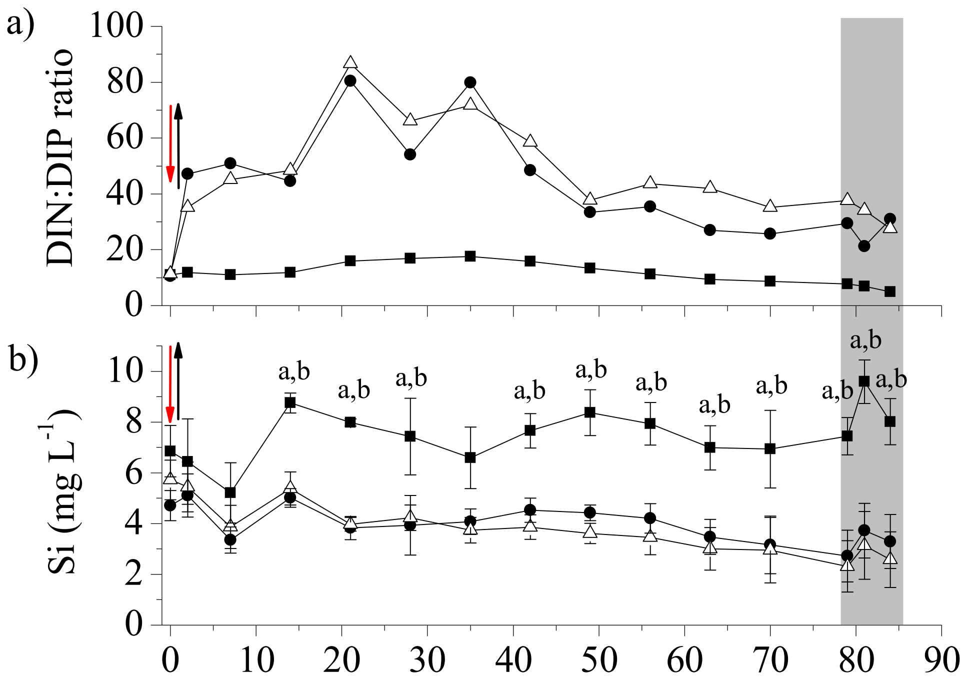

Fig. 4. Changes of DIN: DIP molar ratio (a) and Si concentration (b) in the water column over time. Symbols, letters for significant differences in Si concentration, arrows and not-shadowed/shadowed are the same as described in Fig. 2.

In relation to DIN:DIP molar ratio, a sharp increase of 4-fold in T-W and 3-fold in T-S compared to control took place 24 h right after MPs removal and it was maintained at a high level in both MPs treatments through all the oxic period with an average of 48:1 and 52:1 respectively (Fig. 4b). In the anoxic period the DIN:DIP molar ratio changed to 27:1 and 33:1 in T-W and T-S, respectively. In the control, DIN:DIP molar ratio was much lower than in treatments with an average of 13:1 for the oxic period and 6:1 for the anoxic period.

Temporal changes in Chl *a* concentration are represented in Fig. 5. Chl *a* was not significantly affected when adding MPs but it synchronically changed over time in all enclosures (Table 2). No significant effect of treatment x time interaction has been found.

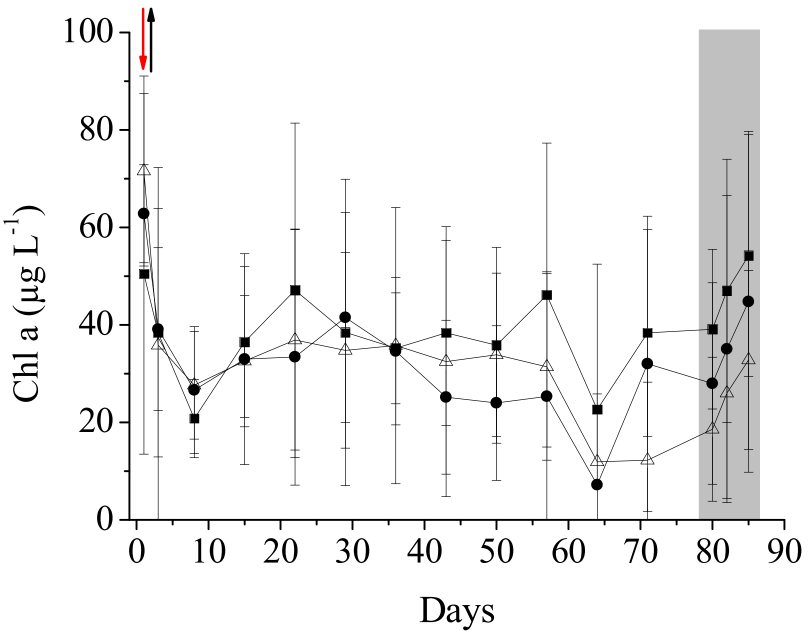

Fig. 5. Temporal changes in Chl *a* concentration. The meaning of vertical arrows and not-shadowed/shadowed areas is the same as described in Fig. 2

In relation to the physico-chemical variables, a gradual reduction in temperature over time was registered in all the enclosures due to seasons change from summer to autumn. pH was not significantly affected by treatments (Table 2) being in all enclosures very close to 9 (Table 1). Dissolved O_2 concentrations kept constant at 7.5 mg L^{-1} and 1 mg L^{-1} during the oxic and anoxic conditions, respectively. Conductivity, TDS, TSS, color and major cations and anions concentration significantly changed over time but no significant differences were found between treatments (Table 2). A significant effect of MPs addition on DOC concentration was also observed (Table 2). DOC significantly changed from 77.7 mg L^{-1} (control) to 65.0 mg L^{-1} in T-W (LSD $p = 0.0039$) and T-S (LSD $p = 0.0033$) at day 2 (24 h after MPs removal), giving a 15 % of DOC reduction in both MPs treatments. A visible change of DOC over time was also noticed but no effect of treatment x time interaction on DOC was observed. Tot-Fe_{dis} concentration was negligible (below the detection limit) not only in the oxic but also in the anoxic period in treatments and control (see Table 1). Inter-treatment differences in SO_4^{2-} and water color were only noticed on day 84 (SO_4^{2-}: LSD $p = 0.0010$; Water color: LSD $p = 0.0181$). Specifically, SO_4^{2-} was higher in control (853 mg L^{-1}) than in T-S (687.89 mg L^{-1}). Similarly, water color was high in control (4.2 m^{-1}) compared to T-S (3.1 m^{-1}). However, no significant effect of treatment on these two variables was considered because they were isolated cases.

3.2 Changes in sedimentary pools

Initial fractionation of the sediment (data not shown) revealed a Tot-P_{sed} of 810.42 µg P gDW^{-1} within 6 cm depth. P_{Mobile} pool accounted for 23.7 % of the Tot-P_{sed}. The higher amount of P was contained in $P_{\rightarrow HCl}$ (52.2 % of Tot-P_{sed}) and in $P_{\rightarrow BD}$ fraction (19.8 % of Tot-P_{sed}) followed by $P_{\rightarrow HCl,\ Res}$ (13.4 % of Tot-P_{sed}), $P_{\rightarrow NaOH,\ Humic}$ (6.4 % of Tot-P_{sed}), $P_{\rightarrow NaOH}$ (4.4 % of Tot-P_{sed}), Org-P_{Labile} (3.4 % of Tot-P_{sed}) and $P_{\rightarrow Water}$ (0.6 % of Tot-P_{sed}). Tot-Fe_{sed} was 15.78 mg gDW^{-1} with most of the Fe in $Fe_{\rightarrow HCl,\ Res}$

(40.5 % Tot-Fe_{sed}), in $Fe_{\rightarrow BD}$ (36.1 % of Tot-Fe_{sed}) and in $Fe_{\rightarrow HCl}$ (18.1 % of Tot-Fe_{sed}). The rest of Fe pools accounted for less than 3% of Tot-Fe_{sed}.

Sequential fractionation of P pools at the end of the experiment showed a similar P fractions distribution between treatments (Fig. 6a). No significant differences were found in Tot-P_{sed} concentration between control (1150.5 µg P cm^{-2}) and T-S (1152.3 µg P cm^{-2}). Although not statistically significant, a slightly less Tot-P_{sed} concentration was observed in T-W (1072.3 µg P cm^{-2}) compared to control and T-S. $P_{\rightarrow NaOH,\ Humic}$, $P_{\rightarrow NaOH}$, and Org-P_{Labile} fractions were significantly lower in T-S (21 %, 15 % and 12 %, respectively) than in control. $P_{\rightarrow NaOH,\ Humic}$ fraction was also decreased in T-W (11 %) compared to control.

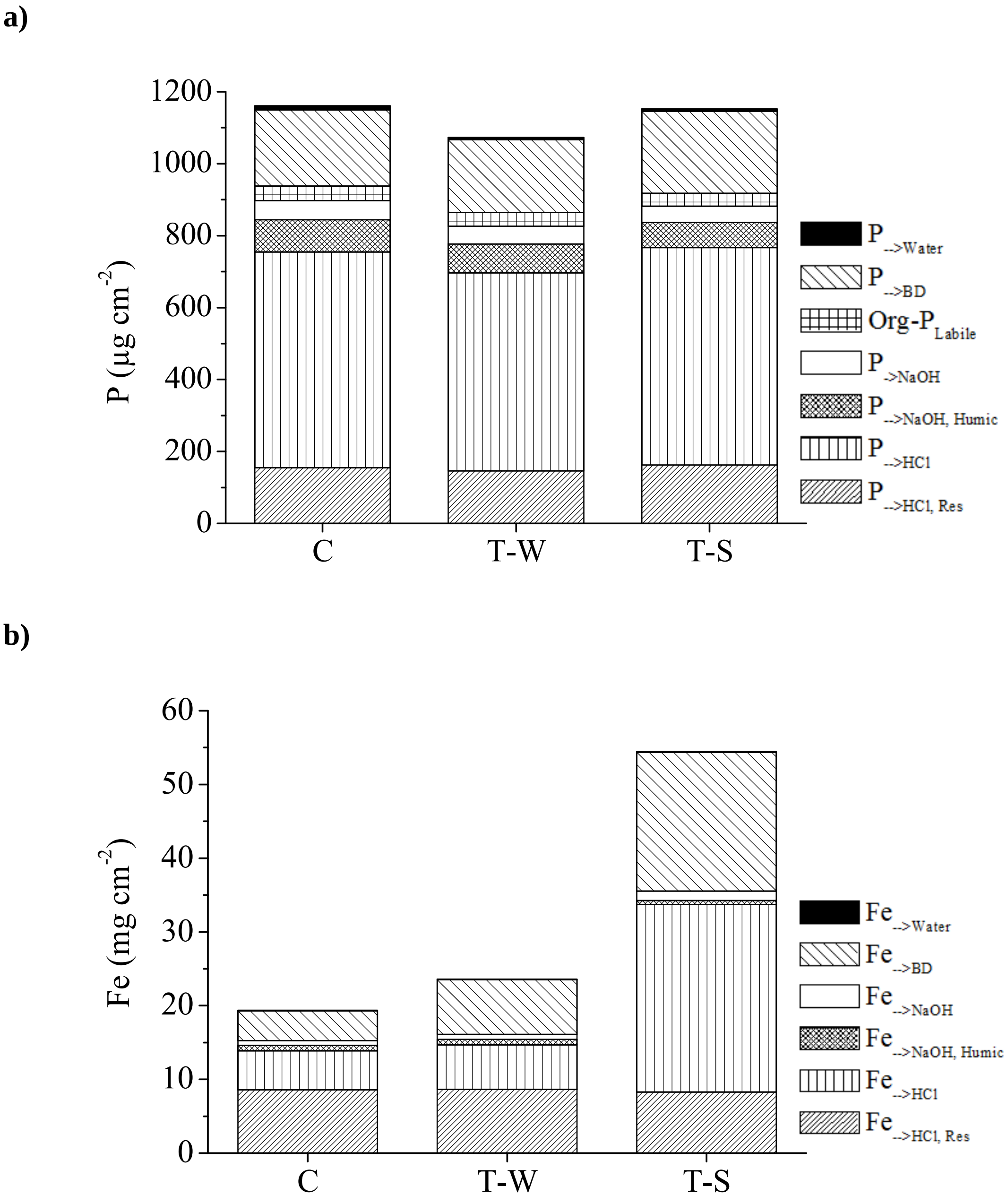

Fig.6. P (a) and Fe (b) sedimentary pools in treatments and control after the experiment.

In contrast, important changes in the final fractionation were measured for Fe pools (Fig. 6b). Tot-Fe_{sed} concentration was not significantly different between T-W (23.6 mg cm^{-2}) and control (19.4 mg cm^{-2}) whereas Tot-Fe_{sed} in T-S (54.5 mg cm^{-2}) was 3-fold higher than in control and twice higher than in T-W. This outstanding increase of Fe concentration in T-S was specifically found in $Fe_{\rightarrow BD}$ and $Fe_{\rightarrow HCl}$ fractions. In particular, an increase of 20.2 mg Fe cm^{-2} in Fe_{HCl} was measured for T-S compared to control and T-W. Although $Fe_{\rightarrow BD}$ in T-W was also significantly higher than in control (increase of 3.4 mg cm^{-2}), T-S showed the highest increase in $Fe_{\rightarrow BD}$ compared to control (increase of 14.4 mg cm^{-2}).

Finally, and in relation to the recovery of MPs in T-W and T-S by using the magnetic rake, 91 % of the MPs were recovered in T-W while, as expected, a much lower MPs recovery efficiency was noted for T-S (32 %).

4. Discussion

Up to date, there exists a wide variety of methods for restoring eutrophic aquatic ecosystems but no universal and efficient method has been already proposed, so more knowledge about novel restoration tools is required. In addition, only very few studies (Egemose et al., 2011; Lürling and van Oosterhout, 2013) have monitored the effects of adding P sorbing materials on nutrient cycling apart from P cycle during lake restoration. Therefore, and previous to a whole-lake restoration strategy, it is imperative to assess the final effect of any P adsorbent on water quality.

4.1 Changes in water column

This study confirmed the convenience of adding MPs for reducing P concentration in lake water and lake sediment. Similarly to what occurs with most P adsorbents, after the addition of MPs, a sharp decrease in DIP concentrations has been observed in both treatments but no effect on other P fractions, such as DOP or TPP, have been found. However, other studies have reported that adsorbents such as Al hydroxide [$Al(OH)_3$] adsorbs and precipitates both DIP and dissolved organic-P compounds (Reitzel et al., 2009).

It is important to consider that although sedimentary $Fe_{\rightarrow BD}$:$P_{\rightarrow BD}$ molar ratio was 19:1 which according to Jensen et al. (1992) reflect that internal P-loading may be controlled by P adsorption onto oxidized surface sediments, lake water DIP concentrations were quite high (> 200 µg L^{-1}) in control during oxic conditions. The addition of MPs drastically reduced P fluxes (67-79 % in T-W and T-S, respectively) during the oxic period and a net P sedimentary uptake was recorded during anoxic conditions. These results are in agreement with those found by Funes et al. (2016) who observed that when adding MPs, in anoxic conditions, sediment act as a sink for P. If we keep in mind that typical hypertrophic aquatic ecosystem, the most imperious systems to be restored, are characterized apart from the high pHs, as mentioned before, by prevailing anoxic periods, it is decisive to assess the ability of the selected P adsorbent to retain P under such adverse conditions. In this sense, it is fair to recognize that both Al (Hansen et al., 2003) and Phoslock® (Ross et al., 2008) have been also verified to be able to trap P in anoxic conditions.

Changes in water quality may be the result of external forcing instead of the MPs addition. In fact the synchrony of a given parameter among spatially distant ecosystems is considered indicative of a significant (external) climatic control in the region (Baines et al., 2000). In our case, the circumstance that a certain parameter is significant and positively related in treatment and control may suggest the existence of an external forcing. In particular, we found synchronous dynamics in Chl *a* concentration for all treatments and control ($p < 0.05$) and the slope of the regression line was for all cases close to 1 (0.84 ± 0.11). A similar pattern was observed for NO_3^-, DIN and TN fractions, where a significant and positively relationship was detected between treatments and controls. However, no effect of MPs addition on N forms and Chl *a* concentration have been found.

The absence of any effect of MPs addition on Chl *a* is coherent with the lack of effect on TPP concentrations. It is very well-known the strong relationship between TP concentrations and Chl *a* in lake water, so reducing the P concentrations in the lake water is the most important strategy for eutrophication control (Hupfer and Hilt, 2008). Despite this worldwide accepted tendency, there exists a high variance between one specific P concentration and the resulting Chl *a* concentration illustrating that other environmental factors and different feedbacks can also affect the primary production (Hupfer and Hilt, 2008). In addition, MPs addition caused an important and significant reduction in DIP and consequently on TP concentrations, but TP concentrations were still very high in treatments (> 200 $\mu g\ L^{-1}$). Previous studies have reported that significant and sustained changes in the biological community and water transparency of shallow, temperate, freshwater lakes may not appear unless TP is reduced below 0.05–0.1 $mg\ P\ L^{-1}$ (Jeppesen et al., 2000) or, for deep lakes, 0.02–0.03 $mg\ P\ L^{-1}$ (Sas, 1989). As a result, in order to get a significant reduction in Chl *a* concentration it would be necessary to: (i) continue long-term monitoring of changes in water quality, (ii) repeat MPs addition or (iii) add a higher MPs dose in the first application.

As DIN concentrations were rather similar in control and treatments but DIP concentrations were lower in treatments than in control, DIN:DIP ratio was notably higher in treatments than in control. According to the Redfield 16:1 N:P molar ratio to meet the phytoplankton´s metabolic requirements, the average DIN:DIP molar ratio in control for the whole period (oxic and anoxic) suggested N limitation. By contrast, the average DIN:DIP molar ratio in both treatments (T-W and T-S) exceeded well above the Redfield ratio, pointing out a potential change in limiting nutrients from N (in control treatment) to P in microcosms treated with MPs. However, in terms of absolute concentration, there is no nutrient deficiency in enclosures treated with MPs since the average DIP and DIN concentrations for the whole period surpassed the minimum concentrations needed for phytoplankton growth proposed by Reynolds (1992, 1999), being those 3 $\mu g\ P\ L^{-1}$ for DIP and 80 $\mu g\ N\ L^{-1}$ for DIN concentrations.

Nutrient-loading ratios can exert a strong selective effect on natural communities of phytoplankton and, in turn, can affect the biomass yield for the nutrient limiting most species (Smith, 1982; Hecky and Kilham, 1988). Del Arco et al. (unpublished results) have found that during this particular study, microcosms were dominated by cyanobacteria, even thought there was a significant decrease of P in the treatments (around 70 % with respect to the controls in DIP), it did not yield a phytoplankton abundance decrease neither a community change. Nevertheless, there were marginal (p-value ranged between 0.05 and 0.1) decrease of cyanobacteria on T-S on specific sampling days. It may suggest that cyanobacteria were sensitive to the decrease of nutrients availability but need longer period to show relevant community changes.

MPs addition led to significant changes not only in lake water P concentrations but also in Si concentrations. This result is not surprising. In fact, similar observations were described by de Vicente et al. (2011) and Merino-Martos et al. (2015) when using the same MPs in natural waters (complex matrix) and in single-ion experiments, respectively. These authors observed that P removal efficiency was reduced from 81 % to 22 % when the Si concentration increased from 5 to 10 mg L^{-1}. In the literature it is very well documented that Si and P have the same specific adsorption behavior, and competition for adsorption sites should occur (Sigg and Stumm, 1981; Cornell and Schwertmann, 1996; Hartikainen et al., 1996). For example, de Vicente et al. (2008) concluded that Si significantly decreased the effectiveness of P adsorption by $Al(OH)_3$ in single-ion experiments. In a whole-lake experiment, Egemose et al. (2011) observed that after Al treatment to a eutrophic lake (Lake Nordborg, Denmark), the concentration of Si was significantly reduced from 1.5-4.4 mg L^{-1} to 0.2-0.5 mg L^{-1}. Despite of the strong influence of MPs addition on Si cycle, Si concentration was always higher than 2 mg L^{-1} during our microcosm experiment in both T-S and T-W, so no limitation for diatom was likely to occur. Although different diatom species have different Si requirements, Pearson et al. (2016) assumed values of 0.04-0.1 mg Si L^{-1} as the likely range of diatom Si limitation. Moreover, concentrations of Si must be lower than 0.23 mg L^{-1} (0.5 mg L^{-1} SiO_2) to promote the abrupt decline of diatom dominance during the phytoplankton spring maximum (Wetzel, 2001).

Apart from Si, MPs addition also caused changes in DOC concentration while no significant changes in water color have been detected. DOC initial concentration in the study lake (67.0 mg C L^{-1}) was much higher than other used in previous single-ion experiment (17 and 35.5 mg C L^{-1}; Merino-Martos et al. (2015). In spite of it, MPs addition promoted a reduction in P concentration, as it is mentioned before, of 68 %. In relation to changes in DOC, 24 h after MPs removal, a 15 % reduction of DOC concentration was detected. The adsorption of HA on Fe oxides (Zhang et al., 2009), $Al(OH)_3$ (de Vicente et al., 2008) and Phoslock® (Lürling et al., 2014) has been widely reported in literature and it is known that HA can bind metals over the entire pH range. However, it is fair to recognize that there are also publications that demonstrated negligible interactions between HA and P adsorption (Borggaard et al., 1990, 2005). In this sense, a very recent study has evidenced that P adsorption by $Fe_3O_4@SiO_2$ coated magnetite nanoparticles functionalized with hydrous lanthanum oxide is scarcely dependent on HA concentration (Lai et al., 2016). In fact, P removal efficiency was reduced only 7 % in the presence of 50 mg C L^{-1}.

4.2 Changes in sedimentary pools

Probably, the major advantage of using MPs particles instead of other restoration strategies is that P is eventually removed from the ecosystem so no further sediment P release can occur. Following the addition of MPs, no significant changes in Tot-P_{sed} concentration was observed in treatments compared to control. However, a significant reduction in some sedimentary P pools was noticed. As a rough estimation, we have calculated that MPs addition in T-W compared to control, considering 94,000 m^2 as lake area (de Vicente et al., 2003) and referring to the upper 6 cm of the lake sediment, would contribute to a removal of a total mass of 73.4 kg P. By contrast, from the comparison of Tot-P_{sed} concentration in T-S and control, we can infer that no changes in the total mass of sedimentary P occur. The explanation for this observation is that in T-S, and due to the much lower percentage of MPs recovery (31%), most of MPs are still retained in the sediment together with the adsorbed P. For a complete removal of the MPs, the magnetic rake had to be used at least twice. At this point, it is necessary to consider that Funes et al. (2016) found, in a laboratory experiment with sediment cores collected from the same study lake, a higher reduction in the total mass of P (ranging from 62 to 195 kg P referred just to the P_{Mobile} and to the first 10 cm sediment layer) but a much higher MPs dose was applied (MPs:P_{Mobile} molar ratio of 285:1 and 560:1 compared to 85:1 of the present study).

It is also important to note that the addition of MPs also affected Tot-Fe_{sed} concentration. In particular, a significant increase in T-S compared to the control was observed due to the low percentage of MPs recovery (31%). In view of our results we can conclude that P bound to MPs are likely to be extracted in BD and in HCl as significant differences were observed in these Fe fractions. Despite of these results, no effect of MPs addition on dissolved Fe concentration in the water column was noticed either in oxic or anoxic condition in any of the amended treatments. So, no toxic effects linked to the presence of dissolved Fe are expected to be caused when adding MPs.

5. Conclusions

This study confirms the convenience of adding MPs for reducing, in a long-term, P concentration in lake water and lake sediment in oxic and anoxic conditions, even at a lower dose than those used in previous studies (Funes et al., 2016).

From the comparison of both treatments (T-W and T-S) and in order to recommend a future whole-lake application, we can conclude that T-W have more advantages than T-S. In particular, the main advantages of adding MPs directly to the overlying water (T-W) are: i) a high percentage of MPs recovery (91%); ii) an important reduction in lake water DIP concentration during both oxic and anoxic conditions that is persistent in time; iii) a significant reduction in the concentration of sedimentary $P_{NaOH,\ Humic}$ fraction (11%) and iv) an easier operation in a whole-lake application compared to adding the MPs directly on the upper sediment (see below). In relation to the T-S treatment, where MPs are added directly on the top of the sediment, the only advantage compared to T-W is that we get a significant reduction in P concentration in some more sedimentary P fractions, apart from $P_{\rightarrow NaOH\text{-}Humic}$ (21%), such as $P_{\rightarrow NaOH}$ (15%) and Org-P_{Labile} (12%). However, the application of the particles is more involved and a lower MPs recovery results. As a conclusion, we state that the best option for adding MPs is T-W as it causes, on a short-term, a reduction in lake water DIP concentration but also on a long-term, a sedimentary P uptake under both oxic and anoxic conditions. In addition, we get a high effectiveness of MPs recovery therefore reducing economic cost and potential toxicity on aquatic biota.

Apart from the P cycle, and similarly to other P adsorbents (Phoslock® and $Al(OH)_3$), MPs addition caused an important effect on Si and C cycles. Despite the drastic reduction in DIP concentrations, Chl *a* was not reduced after MPs addition. A likely explanation is that TP concentrations were still very high in treatments (> 200 µg

L^{-1}) and previous studies have reported that significant and sustained changes in the biological community and water transparency of shallow, temperate, freshwater lakes may not appear unless TP is reduced below 0.05–0.1 mg P L^{-1} (Jeppesen et al., 2000). As a result, in order to get a significant reduction in Chl *a* concentration it would be necessary to: (i) continue long-term monitoring of changes in water quality, (ii) repeat MPs addition or (iii) add a higher MPs dose in the first application.

Acknowledgments

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Chapter IV: Hybrid magnetic chitosan microspheres: synthesis, characterization and applications in lake restoration

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Abstract

Magnetic chitosan microparticles were prepared using a reverse-phase suspension cross-linking technique. The resulting particles and suspensions were characterized using scanning electron microscopy (SEM), X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), magnetometry, thermogravimetric analysis (TGA), electrophoretic mobility and turbidity measurements. Results demonstrate that the hybrids are multicore particles consisting of well dispersed magnetite nanoparticles (approx. 10 % w/w) homogeneously distributed within the biopolymer matrix. These microparticles can be easily separated from the water column and sediment using magnetic field gradients. Their Phosphorus (P) adsorption capacity is evaluated in batch conditions resulting in a maximum P adsorption capacity of M_L = 4.84 mg g^{-1} at pH = 7. These particles are demonstrated to be excellent candidates to extract P mobile from the upper sediment layers because they sediment slower than traditional carbonyl iron microparticles.

1. Introduction

Enrichment of phosphorus (P) is considered the main cause of eutrophication of inland waters. [1]. In situations in which P release from sediment (internal P load) impedes lake recovery once external P load has been reduced, in-lake addition of P-binding adsorbents appears as an advisable alternative for lake management [2] and [3]. Z2G1 or Aqual-P® (Al- modified zeolite), Phoslock® (La-modified bentonite), calcium (Ca), iron (Fe), or alum (Al) salts have been demonstrated to increase sediment P-sorption capacity [2], [4], [5], [6] and. Although some of these techniques could represent a long-lasting sink for P, changes in physicochemical conditions or resuspension events can lead to undesirable P release to water column along with toxic substances stemming from the adsorbents composition [7] and [8].

In the last decades, magnetic nano- and microparticles, mostly based on nano zero-valent Fe (nZVI), magnetite (Fe_3O_4), and maghemite (γ-Fe_2O_3), have attracted special attention to adsorb contaminants from waters and soils due to their easy separation and recovery from the medium for further reuse. This obviously leads to cost savings and to the reduction of the contact time with biota [9], [10], [11], [12] and [13]. With this in mind, more recently, these magnetic particles have been successfully used for P removal in the context of lake restoration and improvement of water quality [14], [15], [16], [17], [18], [19], [20] and [21]. In brief, the protocol consists in the addition of the magnetic particles to the system and their subsequent removal, once P is adsorbed on their surface, by applying a magnetic separation gradient. In a final stage, P recovered from the magnetic particles can be separated and potentially used as a fertilizer [22]. Experimental studies in laboratory have reported a very satisfactory P adsorption capacity by commercial carbonyl Fe particles (18.83 mg P g^{-1}) and synthesized Fe_3O_4 nanospheres (27.15 mg P g^{-1}) under batch conditions [14]. A large adsorption capacity (13.65 mg P g^{-1}) has also been reported for a Fe-Zr binary oxide [20]. As a preliminary step for *in situ* application, magnetic particles have also been tested in the recent literature as P sorbents on sediment cores from a hypertrophic brackish-water lagoon, resulting in a complete reduction of short-term P efflux as well as of some sedimentary P fractions under anoxic conditions [16]. In a more complex experiment using microcosms enclosures, the application of carbonyl Fe particles reduced more than 80 % water column DIP concentrations under anoxic conditions with a high efficiency of magnetic particles recovery from the systems (90 % of recovery) (Funes et al., unpublished).

Apart from high adsorption P capacity and reusability, magnetic particles must meet other stringent requirements for lake restoration applications. Particle size has very important implications not only in adsorption capacity but also in biological toxicity. Small (nano) particles are preferred to enhance adsorption capacity (because of their large surface area) [23]. However, there are evidences of toxicological effects on aquatic organisms [24]; because cladocerans such as *Daphnia sp.* filter large volumes of water, considerable amounts of nanoparticles can be ingested by them, having negative consequences in their physiology and also in upper steps of the food chain [25]. Unfortunately, nanoparticles can also negatively affect the aquatic biota by adsorption on their carapaces and filtrations apparatus [26]. Finally, Fe dissolution is another indirect effect of using Fe based nanoparticles which can led to phytocitoxicity [27].

Of outstanding importance in this work is particle density. Actually, particle density is a key factor driving adsorbent distribution through the sediment profile and determines its availability for P adsorption. It is worth to stress here that P mobile, which is the target sedimentary P pool, mostly concentrates within the first 10 cm of sediment and decreases rapidly with depth [28]. From this perspective, very large density particles are not desirable because they sink deeper in sediment being not available for P adsorption. Moreover, sinking of magnetic particles into deeper layers may contribute to intense sediment resuspension when removing the magnetic particles with the magnet and this is not desirable because it may entail an increase of internal P load [29]. Moreover, large density particles may sink into the deepest sediment layers. Here, magnetophoretic forces exerted by the magnets could not be large enough to overcome opposing surface (cohesive) forces within the sediment making the recovery process inefficient [23]. Thus, a balance between high P removal efficiency (high affinity and large surface area), low bio-toxicity (biocompatible components and large particle size for not to be ingested by biota) and low density (to remain in the first 10 cm of sediment) must be pursued.

In this work we fabricate hybrid magnetic particles that accomplish the three requirements discussed in paragraph above. This kind of particles have been proved to be successful in reducing toxic effects of the particles [13], [11], [30] and [31]. The particular approach to be followed in this work consists in the encapsulation of magnetic multicores within a biopolymer spherical matrix that is not toxic and therefore should minimize Fe dissolution. In particular, we aim to synthesize magnetic chitosan microspheres (MCMs). With this, the particle density is obviously reduced (especially if compared to magnetic carbonyl Fe microparticles previously proposed to adsorb P [14]). Interestingly, the adsorption capacity is still large to make these hybrid particles of interest in lake restoration applications.

2. Materials and Methods

All reagents were of analytical grade and used without further purification. $FeCl_3 \cdot 6H_2O$, ethylene glycol (EG) and sodium acetate (NaAc) were supplied from VWR (AnalaR Normapur). Trisodium citrate (Na_3Cit) was obtained from Carlo Erba. Chitosan (low molecular weight; 75-80 % deacetylated), glutaraldehyde (8 %), mineral oil, Span 80 and petroleum ether were acquired from Sigma Aldrich. Acetic acid (5 %) and acetone were obtained from Panreac. Carbonyl Fe microparticles were obtained from BASF (grade HQ, average diameter = 800 nm, Germany).

2.1 Synthesis of magnetite (Fe_3O_4) nanoparticles

Undoubtedly, the most convenient magnetic material to be used in biological and ecological applications is magnetite (Fe_3O_4). There are many routes, reported in the literature, for the synthesis of Fe_3O_4 depending on the particle size to be obtained. For instance, Fe_3O_4 nanoparticles of typical sizes in the range ~ 10 nm can be easily prepared by the celebrated Massart's method [32]. In this case, Fe^{+2} and Fe^{+3} are coprecipitated in an alkaline solution. Another option to obtain Fe_3O_4 particles in the micronsized range, ~ 1000 nm, is to follow Sugimoto and Matijevic [33]. In this case, the oxidation of the Fe^{+2} cation is slowed down by a nitrate salt allowing the particles to grow larger. In this particular work, we are interested in particles having an intermediate particle size

(~ 100 nm): large enough to respond to magnetophoretic forces and small enough for a large surface activity and hence adsorption capacity.

Fe_3O_4 nanoparticles, having typical sizes in the range ~ 100 nm, were synthesized using a solvothermal method following the recommendations by Liu et al., [34] with slight modifications. Briefly, 3.38 g of $FeCl_3{\cdot}6H_2O$ (0.25 mol L^{-1}) and 0.5 g of Na_3Cit (34 mmol L^{-1}) were dissolved in 50 mL of ethylene glycol by using a centrifugal mixer (3 min at 1000 rpm). NaAc (3 g) was then added and the suspension was mixed again (15 min at 1000 rpm). Next, the mixture was sealed in a Teflon-lined stainless-steel autoclave (125 mL capacity) and heated at 215 ºC for 10 h. The obtained magnetic nanoparticles were washed several times with ethanol and dried at 50 ºC for 4 hours.

2.2 Preparation of magnetic chitosan microspheres MCMs

Magnetic chitosan microspheres MCMs where fabricated by a reverse-phase suspension cross-linking technique [31] following Jiang et al., [35] with slight modifications. First, previously synthesized Fe_3O_4 powder (0.082 g) was dispersed via ultrasonication in a solution of chitosan (0.25 g) in acetic acid (10 mL) for 10 min. The Fe_3O_4:chitosan ratio (by weight) was 1:3. Next, 10 mL of this suspension were added drop-wise into a conical flask containing a mixture of mineral oil (37.5 mL) and Span 80 (2.5 g) as a surfactant. During all the process, the formed water-in-oil (w/o) emulsion was stirred with a mechanical stirrer at 2500 rpm for 30 min. Then, 5 mL of glutaraldehyde were added dropwise and the dispersion was stirred for another 1 h at 40 ºC to promote the glutaraldehyde cross-linking. Finally, the magnetic microspheres were separated from the oil phase by applying a magnet for 30 min and washed several times with petroleum ether and acetone. MCMs were then dried at 50º C for 4 h and stored until use. Chitosan microspheres (CMs) were prepared following the same procedure as MCMs without addition of Fe_3O_4. CMs were separated from oil phase by centrifuging the samples at 3000 rpm for 5 min, washed and stored as in MCMs procedure.

2.3 Characterization of the hybrid particles

Size and surface morphology of Fe_3O_4, MCMs and CMs particles were elucidated with high-resolution field-emission scanning electron microscopy (FIB-FESEM, AURIGA, Carl Zeiss SMT Inc.). Average particle size was investigated by counting at least 300 particles. Environmental scanning electron microscopy (ESEM, FEI Quanta 400) was used to determine internal distribution of Fe_3O_4 particles within MCMs. Samples were embedded within an Epofix resin and cross-sectioned. Crystal structure of Fe_3O_4 and MCMs was analyzed with a Bruker D8 Advance X-Ray difractometer (XRD) with scattering angle (2θ) of 0-90º using Cu-Kα as incident radiation (λ = 0.1540 Å) at 40 kV. Fourier transform infrared spectroscopy (FT-IR) was recorded between 4000-400 cm^{-1} on a JASCO 6200 spectrophotometer in attenuated total reflection (ATR) mode to observe interactions between functional groups of the different synthesized systems. Samples were prepared with KBr disks. Thermal stability of the different composites was analyzed by a thermogravimetric analyzer (METTLER TOLEDO mod.DCS1). Samples were heated from 37 to 950 ºC with a heating rate of 10 ºC min^{-1} in N_2 atmosphere. The magnetization measurements of the composites were performed with a superconducting quantum interference device (SQUID) magnetometer (MPMS XL, Quantum Desing) at room temperature. The external field was swept from -4000 to 4000 kA m^{-1}. Electrophoretic mobility of the composites was determined using a zetameter (Zetasizer Nano Z, Malvern instruments, Germany) at 25 ºC using the Laser Doppler micro-electrophoresis technique. To minimize sedimentation of magnetic particles under gravity, prior to the test, the samples were sonicated for 5 min. Each mobility data point reported in this work is the average of three measurements in the course of 10-40 "runs". Samples for measuring electrophoretic mobility were prepared as follows: a suspension of Fe_3O_4 (0.1 g L^{-1}) or MCMs (0.72 g L^{-1}) or CMs (0.62 g L^{-1}) with 3mM $NaHCO_3$ was agitated for 24 h in a horizontal shaker (150 rpm) at different pH values. Afterwards, pH was readjusted and the suspensions were made up to 25 mL of volume. Turbidity measurements were performed using a Turbiscan MA 2000 (Formulaction, Toulouse, France) in order to compare sedimentation rates of MCMs and carbonyl Fe microparticles used by de Vicente et al., [14]. Briefly, dispersions were placed in a cylindrical plastic cell which was scanned from the bottom to the top with an incident near infrared light source (λ_{air} = 850 nm). Transmission (180 º from the incident radiation) and backscattering (45 º from the incident radiation) sensors detected the light transmitted or backscattered by the sample, respectively, supplying information every 40 µm [36]. Measurements reported here only consider transmission data -as a function of sample height and time-. Samples were prepared by mixing a suspension of carbonyl Fe microparticles (0.1 g L^{-1}) or MCMs (0.72 g L^{-1}) with 3 mM $NaHCO_3$ under agitation for 24 h at pH 7 in a final volume of 25 mL.

2.4 Batch adsorption experiments

Fe_3O_4, MCMs and CMs stock suspensions were prepared by mixing either 100 mg or 720 mg or 620 mg, respectively, with 100 mL of distilled water in a polyethylene container. Suspensions were sonicated for 5 min prior to their use for adsorption experiments to ensure homogeneity of the dispersion. As 2.5 mL of the stock solution (1 g Fe_3O_4 L^{-1}; 7.2 g MCMs L^{-1}; 6.2 g CMs L^{-1}) were added to a final volume of 25 mL, the final concentration for each suspension was 0.1 g Fe_3O_4 L^{-1}, 0.72 g MCMs L^{-1} and 0.62 g CMs L^{-1}.

In order to investigate the effect of pH on P removal efficiency by MCMs, 2.5 mL of a MCMs stock suspension (7.2 g L^{-1}) were mixed with 20 mL of 3 mM $NaHCO_3$ (acting as a buffer) and shaked for 24 h in a horizontal shaker (150 rpm). Then, 2 mL of a 0.125 mM P stock solution were added to each tube and pH was adjusted to 7, 8 and 9. Suspensions were shaked (150 rpm) for 24 h. After this time, pH was readjusted and the volume was made up to 25 mL with 3 mM $NaHCO_3$. Initial P concentration accounted for 0.01 mM P. A magnetic separation gradient (PASCO scientific; EM-8641) was applied for 5 min and the supernatant was filtered (Whatman, GFC) to measure P by spectrophotometric procedure according to Murphy and Riley [37].

Maximum P adsorption capacity of Fe_3O_4, MCMs and CMs was tested by adding 2.5 mL of the corresponding stock suspension to 20 mL 3mM $NaHCO_3$ in 25 mL centrifuge tubes. Final adsorbent concentrations of Fe_3O_4 and CMs isotherms were fixed according to Fe_3O_4 and chitosan content in MCMs as obtained from magnetometry tests. Suspensions were shaked (150 rpm) for 24 h and then, 2 mL of KH_2PO_4 solutions with P concentrations ranging from 0.125 to 2 mM P were added. The pH value was adjusted to 7 and suspensions were shaked again (150 rpm) for 24 h. Afterwards, pH was readjusted and the volume was made up to 25 mL with 3 mM $NaHCO_3$. The magnetic separation gradient was applied for 5 min and P determination was carried out as previously mentioned. The equilibrium adsorption capacity of P, q, was calculated as follows:

$$q = \frac{C_0 - C_e}{M_a} V \qquad [1]$$

where C_0 and C_e are the initial and equilibrium P concentration (mg L^{-1}), respectively, M_a is the mass of adsorbent in grams and V is the total volume of the suspension (L).

3. Results and discussion

3.1 Characterization of composites

Synthesized Fe_3O_4 particles were dark-brownish black, suggesting the dominance of Fe_3O_4 with possible ancillary γ-Fe_2O_3. Fig. 1a shows a typical SEM image of the particles.

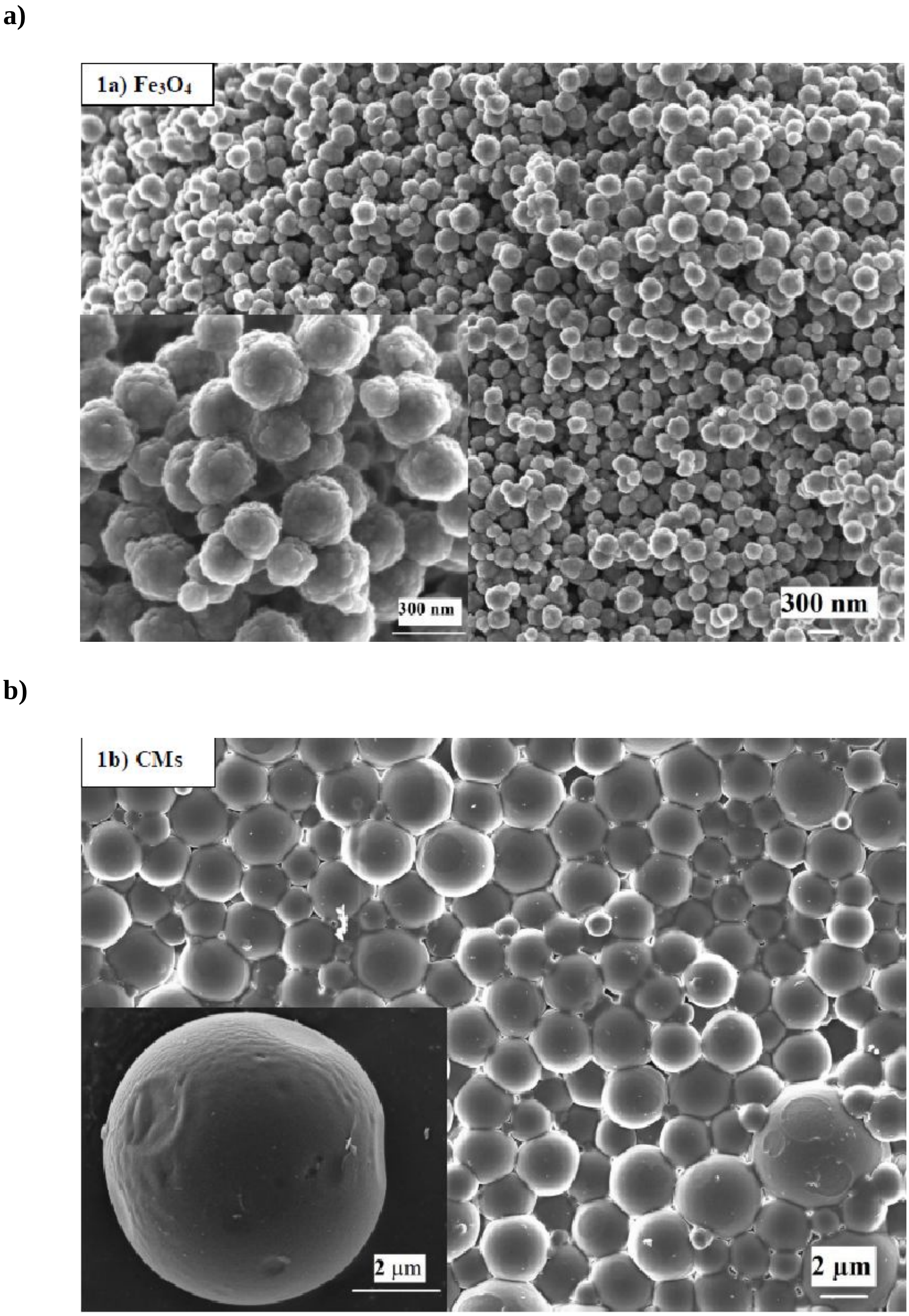


Fig. 1. SEM micrographs of (a) Fe_3O_4 (b) and CMs

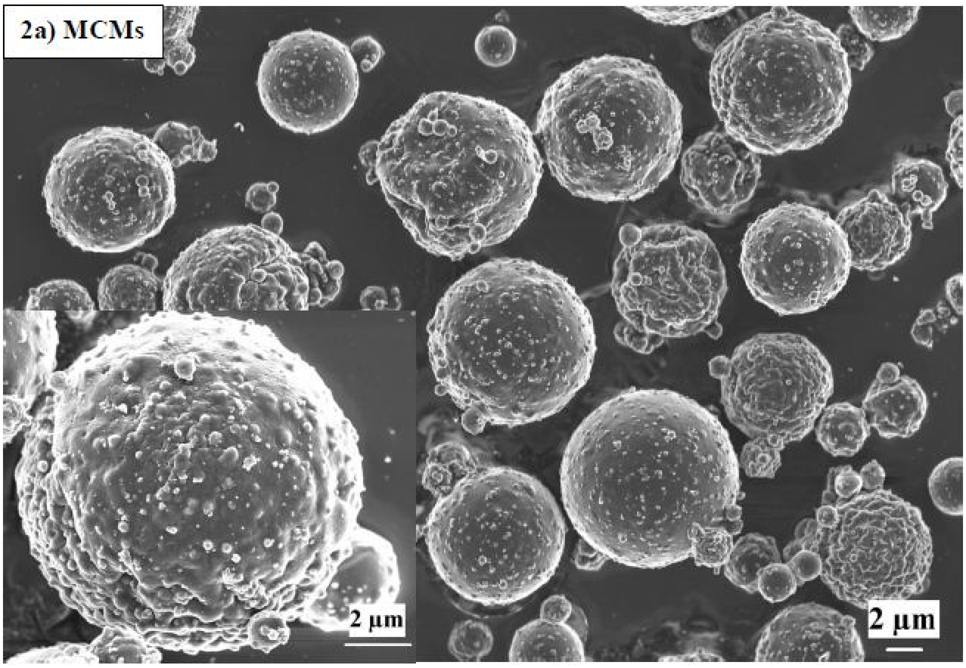
As observed, they are quasi-spherical in shape and uniform in size with a mean diameter of about 280 nm. A detailed description of their morphological characteristics is provided in Table 1.

Table 1. Size parameters and polidispersity indexes of Fe_3O_4, MCMs and CMs.

	Fe_3O_4	MCMs	CMs
Number of particles, N	316	422	298
Number average diameter, Dn (nm)	270	4820	3200
Weight average diameter, Dw (nm)	310	8300	5330
Volume average diameter, Dv (nm)	290	6020	3870
Area average diameter, Ds (nm)	290	5450	3540
Zeta average diameter, Dz (nm)	320	9080	5930
Polydispersity index, PDI	1.1	1.7	1.7
Standard deviation, SD	65	2552	1515
Geometric standard deviation, GSD	1.3	1.8	1.6
Variation coefficient, VC (%)	24	53	47

Higher magnification images indicate that Fe_3O_4 consisted of clusters of small nanocrystals (Fig. 1a inset). Chitosan microspheres (CMs) were also visualized under the microscope. As demonstrated in Fig. 1b, they were spherical in shape but significantly larger, and more polydisperse, than Fe_3O_4 nanoparticles (~ 3200 nm average number diameter with a polydispersity index of 1.7). Fig. 2a reveals that MCMs were also polydisperse and spherical in shape with slightly larger diameter than CMs (average number diameter ~ 4800 nm).

a)



b)

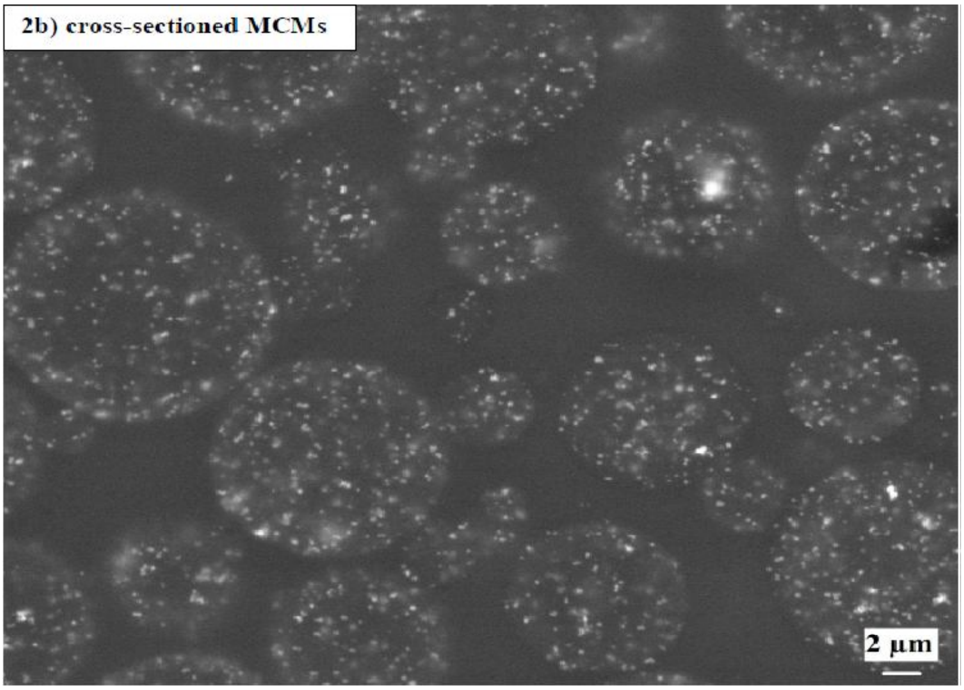


Fig. 2. SEM micrographs of (a) MCMs and (b) cross-sectioned MCMs

More details on the morphological characteristics of the particles can be found in Table 1. In contrast to CMs, MCMs showed a grainy surface (Fig. 2a inset) whereas CMs surface was smooth (Fig. 1b inset). The cross-sectioned MCMs depicted in Fig. 2b revealed a homogeneous distribution of Fe_3O_4 nanoparticles (which correspond to the white spots in the image) within the chitosan microspheres indicating that the embedding process resulted in little Fe_3O_4 aggregation.

Fig. 3 shows the XRD patterns of Fe_3O_4 and MCMs particles. Six diffraction peaks at 2θ = 30.18°, 35.5°, 43.16°, 53.37°, 56.86° and 62.71° were clearly identified in both composites. These peaks fit to Fe_3O_4 according to the Joint Committee on Powder Diffraction Standards (JPCS) database but it is difficult to differentiate between Fe_3O_4 and γ-Fe_2O_3 since both have similar spinel structures and therefore similar XRD patterns [38]. The broad peak at 2θ = 20.53° is ascribed to the presence of chitosan [39]. Results corroborate that Fe_3O_4 is embedded within the chitosan matrix and the lower intensity found in MCMs compared to bare Fe_3O_4 comes from the amorphous chitosan structure.

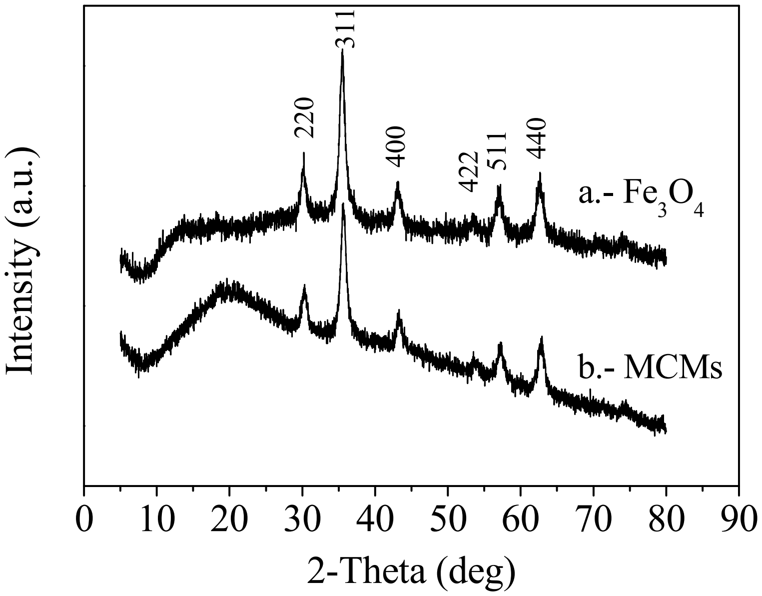


Fig. 3. XRD patterns of (a) Fe_3O_4 and (b) MCMs

FT-IR spectra of the synthesized Fe_3O_4, MCMs and CMs particles are shown in Fig. 4. For comparison, this figure also includes the spectra corresponding to chitosan powder. The sharp peak at 587 cm^{-1} is assigned to vibrations of Fe-O bonds in Fe_3O_4 [40]. This characteristic peak is also present in MCMs but shifts to 621 cm^{-1}. The absorptions at 1607 and 1385 cm^{-1} in Fe_3O_4 are attributed to stretching vibrations of C=O bonds indicating the presence of carboxyl groups coming from a thin layer of Na_3Cit stabilizer on the surface of Fe_3O_4 particles in agreement with Liu et al., [34]. For chitosan powder the N-H band vibration is found at around 1639 cm^{-1} [41]. In MCMs and CMs a new peak appears at around 1563 cm^{-1} which is ascribed to ethylenic bond (C=N) indicating the reaction of carboxyl groups of glutaraldehyde and amine groups of chitosan to form the *Schiff base* [42], [43] and [44]. The absorption band at 1720 cm^{-1} indicate the presence of free aldehydic group in CMs whereas no evidence was found for MCMs [45]. A peak around 2923 cm^{-1} is related to streching vibration of C-H bonds [46] and [47]. The broad peak at 3437 cm^{-1} assigned to O-H bond stretching or N-H bond stretching increased in intensity in the case of MCMs possibly indicating high proportion of unreacted free amine and/or hydroxyl groups compared to CMs [48] and [45].

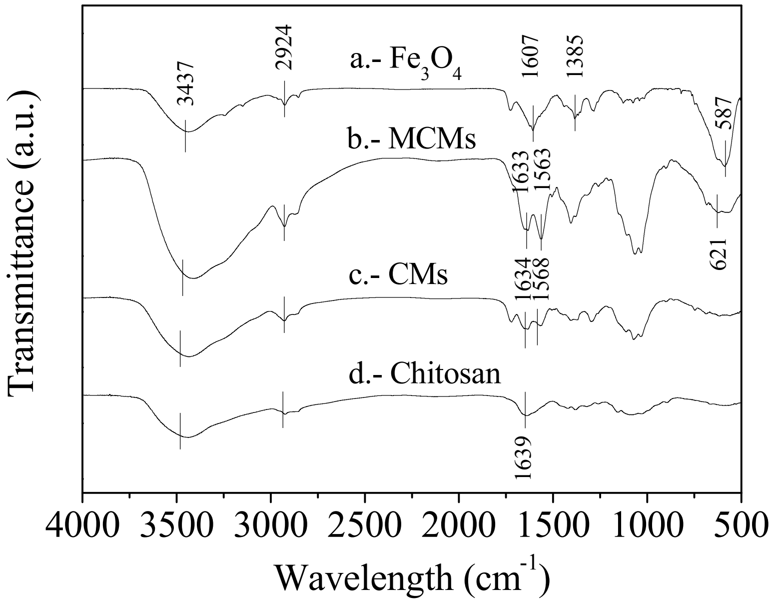


Fig. 4. FT-IR spectra of (a) Fe_3O_4, (b) MCMs, (c) CMs and (d) chitosan powder

Magnetization curves of the Fe_3O_4 particles and MCMs at 293 K are represented in Fig. 5. As observed, Fe_3O_4 and MCMs behave as soft magnetic materials with no coercivity (the field required to reduce magnetization to zero) and remanence (residual magnetization) pointing out that the single-domain magnetic nanoparticles persisted in MCMs [49]. This soft magnetic character implies that magnetic particles should be easily redispersed when withdrawing magnetic field: a key property required for further reutilization [11]. The saturation magnetization (*Ms*) of the synthesized Fe_3O_4 (64 emu g^{-1}) is well below the theoretical value for bulk Fe_3O_4 (84 emu g^{-1}) [31] but close to that obtained by Liu et al., [34] (73 emu g^{-1}). This can be explained by a reduction in crystallinity due to the presence of Na_3Cit during synthesis process [34]. Embedding magnetic particles within a biopolymer results in reduction of *Ms* value since this property is defined as magnetic moment per unit mass [13] and [50]. Also, biopolymer ligands can modify the magnetic moment of the surface atoms of the magnetic particles hence further reducing their magnetization [11]. Accordingly, *Ms* of MCMs (8.78 emu g^{-1}) was far below the value obtained for Fe_3O_4. This is essentially due to the low magnetic content in the chitosan matrix. Using the reference value for the synthesized Fe_3O_4, the magnetic content of the hybrid particles is approx. 14 % w/w. Anyhow, MCMs retained a magnetic character that is sufficient enough to ensure separation from the medium when applying an external magnetic field (see below).

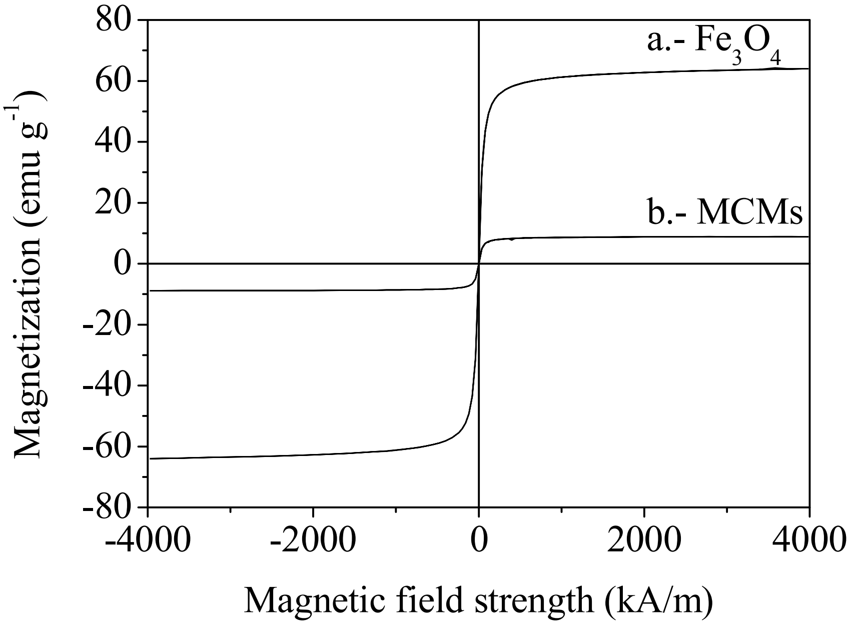


Fig. 5. Magnetic hysteresis curve of (a) Fe_3O_4 and (b) MCMs

TGA measurements for the different particles are summarized in Fig. 6. Fe_3O_4 showed an important weight loss (16 %) in the range of 37-678 °C which is slightly higher than that reported by Liu et al., [34]. A weight loss of about 3% occurred from 37 °C to 180 °C. This corresponds to the evaporation of water molecules adsorbed on the surface of Fe_3O_4 [51]. The most significant weight loss (13%) occurred in the interval 180-678 °C. This is attributed to the removal of the Na_3Cit organic compound used during Fe_3O_4 synthesis [34]. Finally, a slight weight gain of approximately 2 % was observed in the range 678-950 °C. This is attributed to the oxidation of Fe_3O_4 to γ-Fe_2O_3 [40]. MCMs showed a 11% of weight loss below 180 °C due to the removal of adsorbed water. However, the most significant degradation was at 180-550 °C corresponding to breakage of chitosan chains [52] and this continues until 950 °C. CMs started to degrade at lower temperature than pure chitosan indicating than cross-linking reduces thermal stability [53] and [43]. Also, CMs showed less thermal stability than MCMs due to the absence of Fe_3O_4. Chitosan powder showed an overall 76% of weight loss. A slight weight loss of 9 % is observed below 100 ° C and ascribed to the release of adsorbed water molecules [54]. However, it is mostly degraded between 300-500 °C [52]. The magnetic content of the hybrid particles as estimated from TGA measurements is approx. 9 % w/w. This result is in reasonably good agreement with magnetometry measurements reported in the paragraph above.

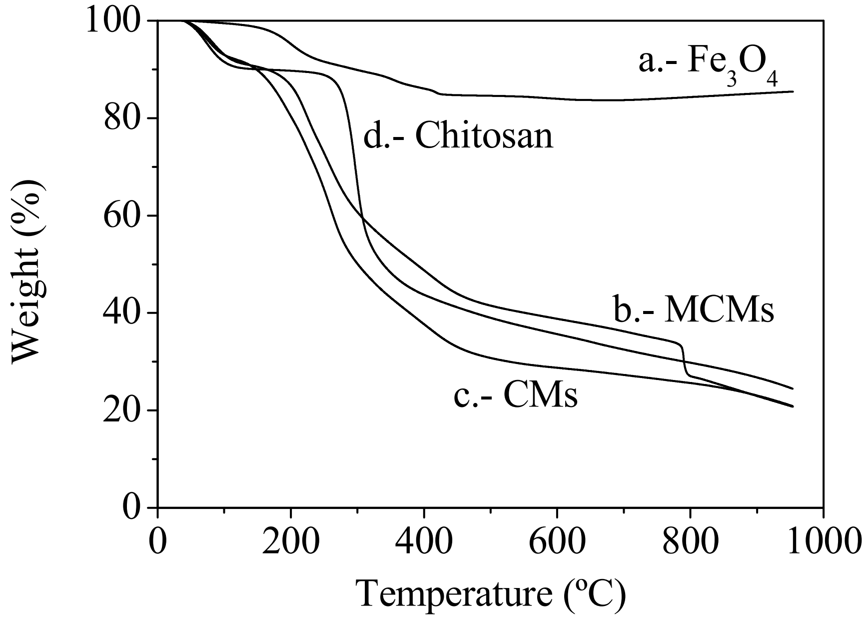


Fig. 6. TGA curves of (a) Fe_3O_4, (b) MCMs, and (c) CMs

3.2 Turbidity measurements: sedimentation

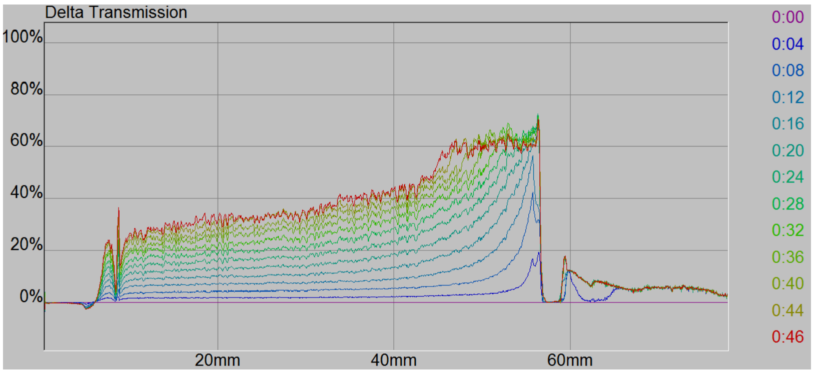
The penetration of particles within the sediment layer depends on the so-called sedimentation rate. Generally speaking, the sedimentation rate V of a colloidal suspension is governed by the density mismatch between the particles ρ_p and the carrier fluid ρ_c, particle diameter d, gravity constant g, continuous phase dynamic viscosity ν and particle volume fraction ϕ [55]:

$$V = \frac{|\rho_p - \rho_c| g d^2}{18 \nu \rho_c} \frac{1-\phi}{1+\frac{4.6\phi}{1-\phi^3}} \qquad [2]$$

In the case of MCMs particles, the $|\rho_p - \rho_c|$ term clearly approaches to zero (if compared to classical carbonyl Fe particles) and hence the sedimentation rate is expected to be reduced if the other parameters remain fixed in Equation [2]. However, the diameter of the MCMs particles is approximately 5 times larger than carbonyl Fe (see Table 1) and this contributes, as well, to increase the sedimentation rate.

As a result, turbidity measurements were also performed to interrogate the sedimentation characteristics of MCMs suspensions and ascertain whether they sediment slower than carbonyl Fe. For completeness carbonyl Fe suspensions were also measured at the same particle concentration (0.72 g L^{-1}). A sedimentation process was clearly observed in both systems, demonstrated by an increase in the transmission profiles (clarification) at the top of the sample with a concomitant decrease of transmission at the bottom of the sample (Fig. 7a and 7b). Clarification in the whole height of the tube also evidenced aggregation of the dispersed particles in both suspensions, although it was more intense for carbonyl Fe microparticles. Fig. 7c represents the time evolution of the sedimentation front (H/H_0) for the two suspensions at pH = 7. In the case of carbonyl Fe particles, the sedimentation front experienced a marked decrease over time reaching a constant value of 20 % after 5 min whereas MCMs showed a much lower sedimentation over time reaching the constant value of 20 % after 30 min.

a)



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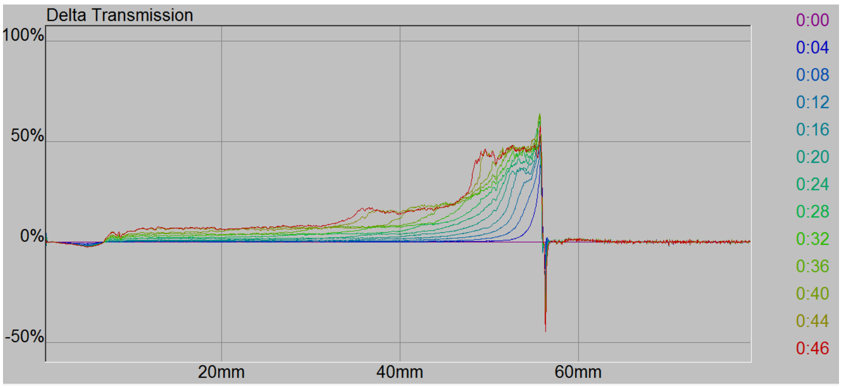

c)

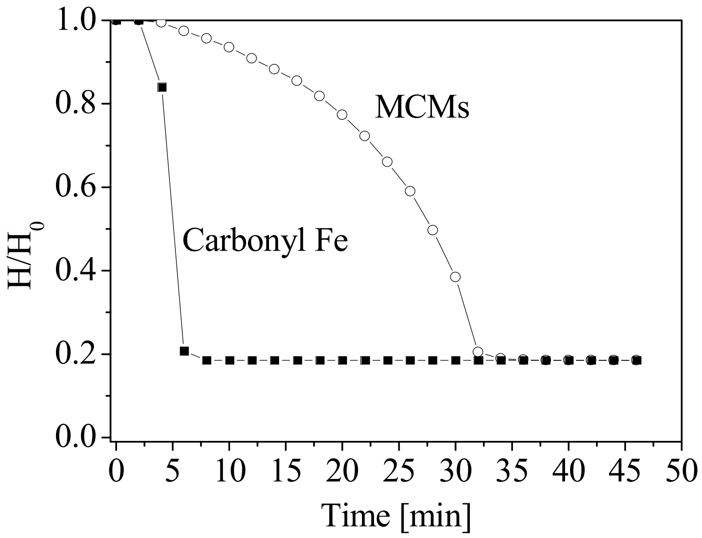

Fig. 7. Transmission measurements (in %) as a function of sample height (mm) in reference mode (by subtracting curve at time zero) for: (a) carbonyl Fe particles, and (b)

MCMs. (c) Sedimentation ratio of carbonyl Fe microparticles (closed squares), and MCMs (open circles). H = sediment front determined at a constant value of transmission (5 %). H_0 = initial sample height (at time zero). Concentration: 0.72 g L^{-1} of carbonyl Fe particles/MCMs.

These results demonstrate that the sedimentation rate of MCMs is slower than carbonyl Fe microparticles despite of their higher particle diameter (average diameter: carbonyl Fe microparticles = 800 nm; MCMs = 4800 nm). This is explained by the lower density of MCMs compared to carbonyl Fe microparticles.

3.3 P removal efficiency by MCMs: effect of pH

In general, pH has an important influence on the nature of the adsorbent and adsorbate [13]. Therefore, the P removal efficiency by MCMs is expected to be influenced by the pH. Results obtained for MCMs are summarized in Fig. 8. This figure shows that the highest P removal efficiency (75 % P removal) corresponds to pH = 7. A significant reduction in P uptake (ANOVA; $F_{2,6} = 441.2$, p value < 0.001) was found with increasing pH value to pH = 8 (58 % P removal) and pH = 9 (24 % P removal). Different P adsorption efficiencies are reported in the literature for neutral pH conditions. A lower P reduction (23 %), but the same decreasing tendency with pH, was found by Yao et al., [56] when using ammonium-functionalized magnetic chitosan. Filipkowska et al., [57] found a 40 % of P reduction when using glutaraldehyde cross-linked chitosan. Dixon [58] reported a high P removal (85 %) by Fe_3O_4 that decreased with raising pH. Contrary to the results for MCMs, P adsorption efficiency by bare carbonyl Fe microparticles (HQ grade from BASF SE) is not pH dependent in the pH range (6-10) leading to a high P reduction (80 % P removal) whatever the pH [14]. These results were explained because of specific adsorption onto the particles. It is worthy to highlight that initial P concentrations used by some of the mentioned authors were much higher than those used in this work.

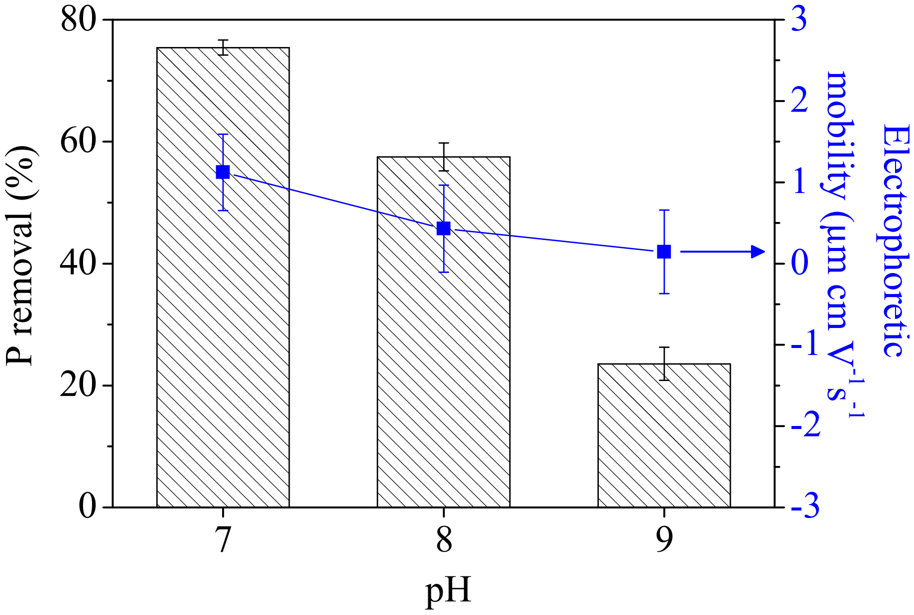

Fig. 8. Effect of pH on P removal efficiency (bars) and on electrophoretic mobility (square symbols) of MCMs. Initial concentrations: 0.72 g MCMs L^{-1} and 0.01 mM P. Standard deviation is represented by vertical error bars.

Our results pointed out that pH dependency of the P adsorption onto MCMs is due to the surface charge of the adsorbent and speciation of the adsorbate. MCMs are positively charged in the pH range of 7-9 but the magnitude of charge decreases with pH (see electrophoretic mobility data in Fig. 8). Electrophoretic mobility measurements seem to be related to the P removal efficiency of the particles. Huang et al., [59] also reported positively charged magnetic chitosan nanopaticles in pure water. At lower pH, amino groups of chitosan are more easily protonated than at higher pH values [60]. At low pHs, P exist as both anions, dyhydrogen phosphate ($H_2PO_4^-$) and hydrogen phosphate (HPO_4^{2-}) [61] thus leading to the conclusion that P adsorption onto MCMs is dominated by electrostatic interaction.

3.4 P maximum adsorption capacity

Adsorption isotherm models describe how adsorbate interact with the adsorbent materials, and thus are critical for optimization of the adsorption mechanism pathways and effective design of the adsorption systems [62]. The adsorption data of the different adsorbents used in this study were fitted to Langmuir isotherm:

$$q = \frac{K_L M_L C_e}{1+K_L C_e} \qquad [3]$$

where K_L is an adsorption constant related to energy of adsorption (L g^{-1}) and M_L is an empirical saturation constant that represents the maximum adsorption capacity (mg g^{-1}). Langmuir isotherm is frequently used when adsorbate uptake occurs by monolayer adsorption assuming no further adsorption when all sites are occupied, thus reaching a saturation value [62]. Moreover, this model considers that active sites are energetically equivalent and homogeneously distributed within the adsorbent [63].

In the three cases, the Langmuir model showed a satisfactory fit to the experimental data. In the case of MCMs and CMs (Fig. 9), at low phosphate concentrations, the adsorption increased when increasing concentration but tended to reach a constant value at higher phosphate concentrations in accordance to Langmuir adsorption model [64]. Although the same adsorption mechanism could be applied to Fe_3O_4, the saturation value was reached very quickly compared to MCMs and CMs (Fig. 9). Table 2 shows the best fitting parameters to Langmuir equation for Fe_3O_4, MCMs and CMs, and the corresponding correlation coefficients. Note that for comparative purposes, in these experiments, the magnetite concentration was the same (constant) in Fe_3O_4 and MCMs suspensions. Also, the chitosan concentration in CMs and MCMs suspensions was kept the same. The highest maximum P adsorption capacity (M_L) was reported by MCMs (4.84 mg g^{-1}) followed by CMs (2.41 mg g^{-1}) and Fe_3O_4 (2.27 mg g^{-1}). Table 2 also summarizes maximum P adsorption capacities of different adsorbents of interest found in literature. P adsorption capacity values of Phoslock® [65] and [66], Al [67] or some clays such as Zenit/Fe [65] and clinoptilolite [68] are closer to values obtained for MCMs. On the contrary, synthetic zeolite HUD [69] and CFH-12® [70] present much higher P sorption capacities compared to MCMs. Isotherm data (data not shown) pointed out a P removal efficiency by MCMs higher than 45 % for P concentrations ranging from 10 to 160 μmol P L^{-1} when using a concentration of 0.72 g MCMs L^{-1}. Then, it can be stated that MCMs would be potentially able to remove 60 % of P from water column of hypertrophic water bodies whose average water column TP have been determined to be higher than 100 μg L^{-1} (3.23 μmol P L^{-1}) [71].

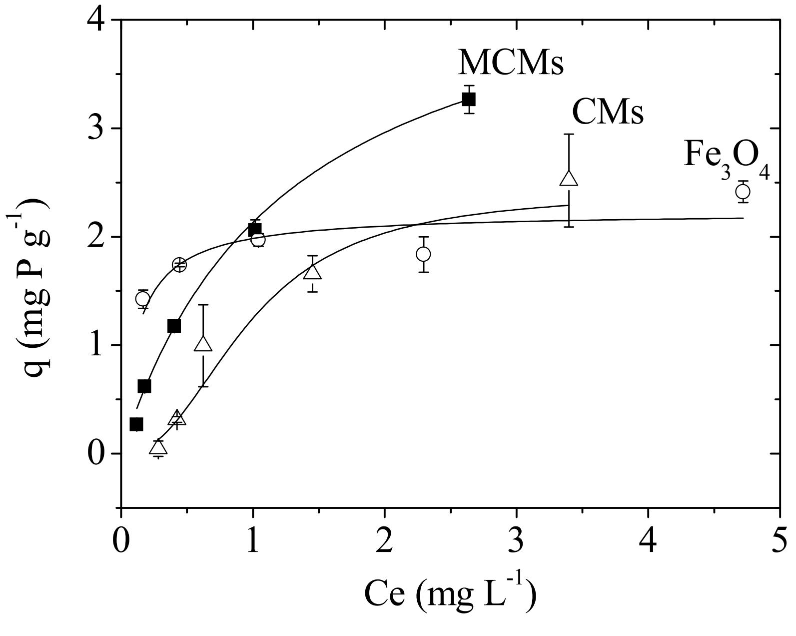

Fig. 9. Adsorption isotherm for Fe_3O_4 (open circles), MCMs (closed squares) and CMs (open triangles) at pH 7. Initial concentrations: 0.1 g Fe_3O_4 L^{-1}, 0.72 g MCMs L^{-1}, 0.62 g CMs L^{-1} and 0.01-0.16 mM P. Standard deviation is represented by vertical error bars.

Differences in P adsorption capacity of Fe_3O_4, MCMs and CMs can be explained by the different surface characteristics of the systems. The low P adsorption capacity found for Fe_3O_4 is in the range of that found by Daou et al., [18] (although at a different pH value; pH = 3) who reported a maximum P adsorption capacity of 5.2 mg P g^{-1} (Table 2) when using a positively charged Fe_3O_4 with higher surface area (40 nm average particle size). However, a much higher P adsorption capacity is also reported in the literature for the same pH conditions to our study. de Vicente et al., [14] found that negatively charged Fe_3O_4 (90 nm) was able to adsorb a maximum of 27.15 mg P g^{-1} (Table 2). At pH = 7, our Fe_3O_4 exhibits a negative surface charge (electrophoretic mobility -2.7 ± 0.45 µm cm V^{-1} s^{-1}) which suggest that P adsorption mechanism is driven by specific interactions (and not by electrostatic interactions). The comparatively low P adsorption capacity found in our Fe_3O_4 can be explained by the organic layer (supported by TGA and FT-IR analysis) covering Fe_3O_4 surface that may difficult P adsorption onto OH^- functional groups present on its surface [72] and [73].

Table 2. Langmuir model parameters, M_L, K_L and R^2 for P adsorption onto different adsorbents.

Sorbent	**Langmuir parameters**			**Ref.**
	M_L(mg g^{-1})	K_L	R^2	
MCMs	4.84	0.79	0.99	this study
CMs	2.41	1.07	0.95	this study
Fe_3O_4	2.27	7.44	0.83	this study
Carbonyl Fe	18.83	-	-	[14]
Fe_3O_4	27.15	-	-	[14]
Fe_3O_4	5.20	-	-	[18]
CFH-12®	27.59	-	-	[70]
Phoslock®	11.60	0.80	1.00	[65]
Phoslock®	9.34	134.00	1.00	[66]
Zenit/Fe	11.15	0.60	0.99	[65]
Clinoptilolite (zeolite)	2.15	44.20	0.97	[68]
Synthetic zeolite HUD	79.40	0.03	0.90	[69]
$AlCl_3$ $6H_2O$	15.00	-	-	[67]
Protonated cross-linked chitosan	58.50	0.01	1,00	[74]
Cross-linked chitosan	104.20	0.01	0.99	[57]

CMs adsorption capacity reported in this work is also low compared to other studies employing cross-linked chitosan composites with the same pH conditions as the cases of Sowmya and Meenakshi [74] and Filipkowska et al., [57] who reported a P maximum adsorption capacity of 58.5 mg g^{-1} and 108.2 mg g^{-1}, respectively (Table 2). Synthesized CMs showed a very slight negative surface charge as indicated by electrophoretic mobility (-0.84 ± 0.65 µm cm V^{-1} s^{-1}) similarly to other cross-linked chitosan composites [56]. Indeed the point of zero charge (PZC) for most chitosan particles has been previously reported to range between 6.2 and 6.7 [75] [76]. P adsorption onto CMs is thought to be due to physical adsorption or intraparticle diffusion through the chains network of the particle [60].

It is noteworthy that MCMs are positively charged despite of the fact that Fe_3O_4 and CMs are negatively charged at pH = 7. Although the interaction mechanism between the cross-linker, chitosan and magnetite is not fully understood, a possible explanation for the different surface charges in CMs and MCMs could be the following: surface charge of chitosan is determined by protonation/deprotonation of its surface functional groups (amino and hydroxyl groups) depending on pH [30], [77]. During the synthesis process in acidic media, aldehyde groups of glutaraldehyde mainly reacts with protonated amino groups, but it can also form covalent bonds with protonated hydroxyl groups [60]. The positive charge of MCMs comes from the interference of magnetite in the crosslinking process and could be explained by a higher presence of unreacted (free) protonated amino and hydroxyl groups on the MCMs particles if compared to CMs. This is demonstrated by an increase in the intensity of the broad peak at 3437 cm^{-1} (c.f. Fig. 4) [45]. On the contrary, the negligible negative surface charge observed in CMs comes from the absence of free functional groups as a result of a complete reaction of amino groups with the crosslinker. This explanation seems to be in agreement with the appearance of a peak at 1720 cm^{-1} in Fig. 4 presumably from free aldehydic groups of glutaraldehyde [Monier, 2012].

Finally, the possibility of reusing MCMs was also investigated. For this aim, a conventional washing protocol was applied that consisted in washing twice with 1M NaOH and 3 times with 3 mM $NaHCO_3$ using magnetic separation for 5 min to separate supernatant from the pellet. This particular protocol was very successful in the case of magnetite and carbonyl Fe particles [14]. However, this particular process failed in the case of hybrid magnetic chitosan particles. Further research is needed since no protocol has been set-up yet to regenerate the adsorbent.

4. Conclusions

We have synthesized hybrid magnetic particles by a reverse-phase suspension cross-linking technique. We demonstrate that these particles are constituted by well dispersed magnetite multicores within an spherical chitosan microparticle (approx. 10 % w/w) that is biocompatible and non toxic. These particles do exhibit a sufficient P adsorption capacity (M_L = 4.84 mg g^{-1}) to be used in eutrophic systems at conventional pH levels. The mechanism behind the adsorption seems to be dual: electrostatic for the case of chitosan matrix and surface specific for the case of magnetite grains. Because of their large size and magnetite loading, these hybrid microparticles can be easily separated using magnetophoresis.

Undoubtedly, the major advantage of these particles is that, once settled from the water column, they have the ability to remain in the upper layers of the sediment where the target sedimentary P pool in eutrophic lakes (P mobile) concentrates. This is due to their lower particle density compared to other adsorbents previously proposed for lake restoration (e. g. carbonyl Fe particles). Further investigation about MCMs reutilization is still needed in order to make these particles cost-effective as P adsorbents.

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Chapter V: Evaluation of dried amorphous CFH-12® and freshly formed $Fe(OH)_3$ as agents for binding bioavailable phosphorus in lake sediments

Fuchs, E., **Funes**, A., Saar, K., Reitzel, K., Jensen, H.S. Evaluation of dried amorphous CFH-12® and freshly formed $Fe(OH)_3$ as agents for binding bioavailable phosphorus in lake sediments. Environ. Sci. Tech. (in preparation to be submitted).

Abstract

Previous studies showed that as well Aluminum (Al) as also Iron (Fe) hydroxides can act as Phosphorus (P) adsorbents for lake restoration. Despite its higher affinity towards P, the high redox sensitivity of Fe limited its application so far and the main focus was set on the usage of Al hydroxides, not least due to its insensitivity towards redox potential changes. In this study CFH-12®, a dried, amorphous Fe-oxide (Kemira), is presented as a promising agent for lake restoration, which does not change the pH of the water during addition and shows with a Fe:P binding molar ratio of 7.6-8:1 a significant reduction of DIP efflux from three different lake sediments (43 % Lake Sønderby, 70 % Lake Hampen, 60 % Lake Hostrup). Furthermore, a significantly lower Fe^{2+} efflux from sediment cores treated with CFH-12® during anoxic periods compared to cores treated with freshly formed $Fe(OH)_3$ was observed. Seemingly, CFH-12® possesses only little redox sensitivity and should be therefore hardly subject to reduction and microbial respiration in lake sediments. Moreover, ageing studies on CFH-12® showed, in contrast to freshly formed $Fe(OH)_3$, no change of its P binding capability.

1. Introduction

The trophic state of a lake is an important indicator for the quality and diversity of the aquatic environment. Although eutrophication of aquatic systems occurs naturally, high additional nutrient input by human activities such as fertilizer run-off, insufficient waste water treatment etc. can lead to hypertrophy of the lake, resulting in biodiversity loss, toxic cyanobacteria-blooms and severe changes within the function of the ecosystem (Dobson and Frid, 2009; Jeppesen et al., 2009; Jeppesen et al., 2012). Phosphorus (P) represents the major limiting nutrient in freshwaters and thus, its reduction constitutes the main target in lake restoration (Hupfer and Hilt, 2008; Dobson and Frid, 2009; de Vicente et al., 2010). In many places measures have been taken to reduce the external P load, however, sediment-released P (internal P load) can be high enough to delay the improvement of the trophic state of the lake after reducing external P inputs (e.g. Søndergaard et al., 2003). With the establishment of the WFD (European Water Framework Directive) in 2000, the need for further research in the field of lake restoration increased, as 36 % of the European lakes can still not fulfill the directive (Spears et al., 2013).

The potential of iron (Fe) to adsorb P has been already shown in several studies (e.g. Gunnars et al., 2002; Kleeberg et al., 2013; Rothe et al., 2014). As for $Al(OH)_3$, Fe hydroxides possess a high affinity for P (e.g. Jeppesen et al., 2009) and most of the P efflux from lake sediment is controlled by Fe when present in a high enough Fe:P ratio (Jensen et al., 1992). The addition of Fe also leads to the formation of a barrier on top of the lake sediment, which prevents further internal loading (Immers et al., 2013).

Several previous studies showed a reduction in the total P concentrations after the addition of Fe (e.g. Kleeberg et al., 2013; Kowalczewska-Madura et al., 2015) although so far all documented examples of Fe addition showed only time-limited effects, however, Kleeberg et al. (2013) were able to provide some evidence, that the addition of Fe in high enough dosages can have long-term effects on the burial of P. The use of Fe for the permanent fixation of P requires in most cases continuous complete mixing or aeration (Jaeger, 1994; Jeppesen et al., 2009) due to the fact that both, the Fe in the sediment and the added Fe by $FeCl_3$ or $FeSO_4$, is highly sensitive to redox changes in the sediment (Jeppesen et al., 2009). An important factor affecting the Fe cycle is the amount of fresh organic matter in the sediment, whose aerobic mineralization can lead to the depletion of oxygen at the sediment surface (Kowalczewska-Madura et al., 2015). While $FeOOH\text{-}PO_4$ precipitates when oxygen is present, Fe becomes reduced to Fe^{2+} and releases P into the overlying water under anaerobic conditions (Gunnars et al., 2002; Hupfer and Hilt, 2008). It has been reported that benthic bacteria can also release ortho-P as a result of their metabolism at low redox potential (Gätcher and Meyer, 1993).

Another concern with treatments using Fe-salts is a drop in lake water pH during the formation of $Fe(OH)_3$ floc similar to the addition of alum (Al)-salts, where the adverse effects of the pH drop (e.g. toxicity of dissolved Fe^{2+} or Fe^{3+}) are not well understood (Jeppesen et al., 2009; Immers et al., 2013; Kowalczewska-Madura et al., 2015). Especially in lakes with low-alkaline water the pH effect limits the use of Fe salts and Al salts and in such lakes a solid $Fe(OH)_3$ product with a good P sorbing capability would be beneficial to use for P inactivation in the sediment.

Such a strategy for P removal is for example the recent use of magnetic Fe micro particles as adsorbents which were tested on core samples and removed from the sediment after P adsorption using a magnet, thus leading to a reduction of adverse effects of Fe on the biota, and moreover to the reusability of the magnetic particles (de Vicente et al., 2010; Funes et al., 2016). These authors reported a complete reduction of short-term P efflux under anoxic conditions. However, further research on the adsorption mechanisms involved and the potential chemical interferences on the P adsorption capacity is still needed (de Vicente et al., 2010). Very promising results on another, Fe-based P adsorbent were achieved by Lyngsie et al. (2014a, 2014b), who tested an industrial Fe-oxide based product (CFH-12®, Kemira) for its potential in P adsorption. CFH-12® was shown to be a rather promising P adsorbent, which moreover retains most of P adsorbed, in contrast to all other tested materials. However, those studies were only conducted at aerobic conditions and only for shorter exposure times (up to 2 weeks) for P adsorption. As the high redox sensitivity of Fe has so far been the major drawback for the usage of $Fe(OH)_3$ for long-term P retention in lake sediments this paper evaluates the ability of CFH-12® and $Fe(OH)_3$ in reducing DIP efflux in a 21 weeks core incubation experiment with sediment from three Danish lakes (Lake Sønderby, Lake Hampen and Lake Hostrup), where at the same time also other parameters such as the sediment-water exchange of alternative electron acceptors (NO_3^-, Mn^{2+}, SO_4^{2-}) and the efflux of Fe^{2+} from anoxic sediment surfaces were monitored. The experiment was divided into an oxic and anoxic period in order to see effects of microbial respiration and reducing conditions on the performance of the two compounds. Besides the core incubation experiment also an ageing study on pure phases of CFH-12® and $Fe(OH)_3$ was conducted, which provides more information on eventual ageing effects on the P binding capability of the two compounds.

2. Experimental (Material and Methods)

2.1 Study site

Sediment and water from three shallow Danish lakes were used in the experiments. Lake Sønderby is a small (8 ha; 2.1 m mean depth; 5.7 m max. depth), hardwater (3 meq L^{-1}) and hypertrophic lake (average summer Secchi depth 0.7 m and chlorophyll *a* (Chl *a*) 60 µg L^{-1}, average winter total P (TP) 0.22 mg P L^{-1}). Lake Hampen is a medium (76 ha; 4 m mean depth; 13.2 m max. depth), softwater (0.36 meq L^{-1}) and oligotrophic lake (average summer Secchi depth 4.0 m and Chl *a* 7.5 µg L^{-1}, and average winter TP 0.015 mg P L^{-1}), while Lake Hostrup is a big (210 ha; 2.1m mean depth; 7 m max. depth), and eutrophic lake (average summer Secchi depth 1.1 m and Chl *a* 35 µg L^{-1}, and winter average TP 0.035 mg P L^{-1}). All three lakes experience temporary (days) stratification and increased TP concentrations during summer due to internal P-loading.

2.2 Iron products

CFH-12® is a granulate of dried amorphous ferric oxy-hydroxide manufactured primarily for adsorption of arsenate and P in ground water used for drinking water purposes. CFH-12® has a Fe content of ~ 44 % with 93 % of the weight in a grain size of 0.85 - 2 mm and 6 % smaller than 0.85 mm. Freshly formed $Fe(OH)_3$ was prepared by adding $FeCl_3$ to distilled water without P and neutralizing the H^+ formed by hydrolysis with NaOH. After 2 h of horizontal agitation, the precipitate was recovered by centrifugation and washed 3 times with distilled water before use.

2.3 CFH-12® distribution/extraction

As a pre-experiment to the sediment core experiment described below, in June 2014, 2 small cores (5.2 cm inner diameter, h = 40 cm) and one large core (7.2 inner diameter; h = 40 cm) were collected from Lake Hampen in order to investigate the vertical distribution of CFH-12® within the sediment. The 2 small cores remained untreated (control group) while the large core was treated with 45 g Fe m^{-2}. After three months of settling, the top ten centimeters of all cores were sliced in 1 cm intervals and analyzed for total Fe concentration. In a post-experiment, the efficiency of Fe and P extraction in certain steps of sediment fractionation was tested on CFH-12®, previously saturated with P.

2.4 Ageing effect of $Fe(OH)_3$ and CFH-12® on P adsorption capacity

Four different adsorption experiments in batch mode were run in parallel with 3 replicates per compound and treatment. (1) Suspensions of freshly prepared $Fe(OH)_3$ (P:Fe molar ratio = 0.22, 2.3 mmol Fe) and CFH-12® (P:Fe molar ratio = 0.26, 1.7 mmol Fe) were aged separately in artificial lake water (final volume 500 mL; composition see supplementary information) with (1) 0.96 mM P (high P concentration) or (2) with 0.05 mM P (low P concentration, P:Fe molar ratio = 0.01) in PC bottles. For treatments (1) and (2) dissolved Inorganic P (DIP) was measured regularly for 86 days. (3) The same amount of freshly prepared $Fe(OH)_3$ and CFH-12® as mentioned before was stored with 30 mL of artificial lake water in centrifuge tubes for 21 h, 1, 7, 10, 14, 56, and 113 days without any P present. Suspensions were transferred to PC bottles and exposed to high P concentration for 24 h, after which DIP was measured. (4) $Fe(OH)_3$ and CFH-12® suspensions were stored as in experiment (3) for 3 months and then treated according to the procedure (1) for high P exposure. pH was in all cases adjusted between 6.5 and 7.5 and a natural movement of the particles was ensured using a table shaker (45 rpm, RT). The bottle walls were washed with distilled water and hydrochloric acid and analyzed for DIP to account for possible adsorption, but as the concentrations did not exceed 1% of the total P uptake, the measured values were not corrected.

2.5 Core incubation experiment

In July 2014, 2 large and 12 short cores were sampled in each lake at the deepest location. Bottom and surface lake water was collected from each lake at the same site. At the beginning of the experiment 2 large cores were sectioned in 1 cm intervals from 0-3cm, 2 cm from 3-7 cm and 3 cm from 7-10 cm (initial fractionation). A sequential extraction procedure according to Paludan and Jensen (1995) using several extractants (deionized water, bicarbonate-dithionite (BD), 0.1N NaOH and 0.5 N HCl) defined seven sedimentary P-pools: pore water iP and loosely adsorbed iP ($P_{\rightarrow Water}$), iP bound to Fe ($P_{\rightarrow BD}$), iP bound to clays and Al oxides ($P_{\rightarrow NaOH}$), P bound to humic acids ($P_{\rightarrow NaOH, Humic}$), calcium-bound IP ($P_{\rightarrow HCl}$) and refractory organic P ($P_{\rightarrow HCl, Res}$). Easily-degradable P (Org-P_{Labile}) was calculated as the sum of the organic P fraction in the three first extractions. P_{Mobile} was calculated as the sum of $P_{\rightarrow Water}$, $P_{\rightarrow BD}$, and Org-P_{Labile} within the first 10 cm (Jensen et al., 2015; Reitzel et al., 2005). In the same extractions Fe fractions were measured. Total sediment P (Tot-P_{sed}) and total sediment Fe (Tot-Fe_{sed}) were determined on parallel samples by combusting the sediment at 520 ºC for 5 h prior to an acid hot digestion with 1M HCl. These measurements were conducted in order to calculate the right amount of iron to be added to each sediment targeting a molar ratio between Fe and P_{Mobile} of 7.6 -10.

The other 12 small cores of each lake were divided into 3 different groups of 4 replicates: one group was treated with freshly formed $Fe(OH)_3$ floc ($Fe(OH)_3$ treatment), another one was charged with CFH-12® (CFH-12® treatment) and the third group served as control (control group). Cores were incubated in incubation tanks at 14 ºC in two periods: an oxic period for 78 days followed by an anoxic period for 70 days. During the anoxic period cores were bubbled with N_2 and covered with a lid to prevent oxygenation. By Fe addition to the cores the overlying water was removed prior to mixing the top layer (0-3 cm) of the sediment with the $Fe(OH)_3$ floc or CFH-12® compound, obtaining a 7.6-8:1 Fe:P_{Mobile} molar ratio for CFH-12® group and 10:1 for $Fe(OH)_3$ group as the minimum P trapping ratio suggested by Jensen et al. (1992). After 24 h of sediment settling, the cores were filled up with bottom lake water, equipped with magnetic stirring bars ensuring complete mixture of the water column, and placed in the incubator to start the experiment. Control cores were treated similarly to cores with Fe addition. Fluxes were calculated by analyzing water samples for DIP, TP, Fe^{2+} and total dissolved Fe (TDFe) on days 1, 9, 22, 36, 50, 63, 78, 85, 99, 113, 134 and 148, while SO_4^{2-}, NO_3^-, NH_4^+ and Mn^{2+} were measured on days 50, 63, 78, 85, 99, 113, 134 and 148. After each sampling the water column was removed and replaced with bottom water until 3rd sampling, after which surface lake water was used because of the relatively high DIP concentrations in the bottom water. Lake Sønderby present high DIP concentrations (8-10 µM) compared to low values in the other two lakes (0.06-0.2 µM). NO_3^- concentration is high (7-40 µM) in both Lake Sønderby and Hostrup whereas it is negligible for Lake Hampen, being SO_4^{2-} concentration similar in all three lakes (0.03-0.08 µM). The O_2 consumption in the cores was calculated by the difference between the initial (previously saturated with O_2) and final O_2 concentration (mg L^{-1}) in the overlying water after 3 h incubation at 14 ºC with a lid. In addition, the redox-profile of the cores was measured in the overlying water, the sediment-water-interface and down to 50 mm into the sediment in 5 mm intervals (Unisense PH/mV meter, Unisense 5731). Incubations were all conducted in the dark.

At the end of the incubation period Tot-P_{sed} and Tot-Fe_{sed} were analyzed in sediment cores. Sediment data of final fractionation were corrected using a calculated correction factor to account for the settling or loosening of the sediment during the incubation period. P and Fe were analyzed spectrophotometrically by molybdenum blue colorimetry (Murphy and Riley, 1962) and the ferrozine method (Gibbs, 1979), respectively. SO_4^{2-} was measured using Ion Chromatography with a conductivity cell detector (Dionex ICS 2000), NO_3^- and NH_4^+ with a Flow injection analyzer (Lachat Quick Chem R 8500), while Al^{3+} and Mn^{2+} were measured on an ICP-AES (Perkin Elmer Optima 2100 DV).

Statistical analyses were performed with IBM SPSS Statistics 20 software. Statistically significant differences between the different treatments in the water and sediment samples were assumed when $p < 0.05$ by using One-way ANOVA with Tukey HSD post hoc test.

3. Results

3.1 CFH-12® distribution/extraction

The pre-experiment on the distribution of CFH-12® within the Lake Hampen sediment two month after addition to the sediment surface resulted in the main recovery of CFH-12® in the upper 5 cm of sediment (almost 100 % Fe from CFH-12®), while the highest Fe concentration was found between 2-3 cm (58 % Fe from CFH-12®). The post-experiment on the solubility of CFH-12® within the fractionation procedure revealed, that only 14 % of P and Fe are extracted during the BD extraction, whereas 52 % of P and 32% of Fe were dissolved within the HCl extraction step, giving a total P extraction after both steps of 67% (data not shown).

3.2 Ageing studies

The first condition tested (1) was the ageing effect under exposure to a high P concentration, indicating the maximum adsorption capacity of the two investigated compounds (Fig. 1a). In comparison to CFH-12® (maximum sorption capacity after 28 days: 119.9 µmol P mmol^{-1} Fe) $Fe(OH)_3$ possesses a higher binding capability (maximum sorption capacity after 24 days: 180.0 µmol P mmol^{-1} Fe). However, due to a continuous slight increase in adsorption until the end of the experiment, the maximal adsorption capacity of CFH-12® might be slightly underestimated. The final Fe:P molar ratio after 86 days resulted in 6:1 for $Fe(OH)_3$ and 13:1 for CFH-12®.

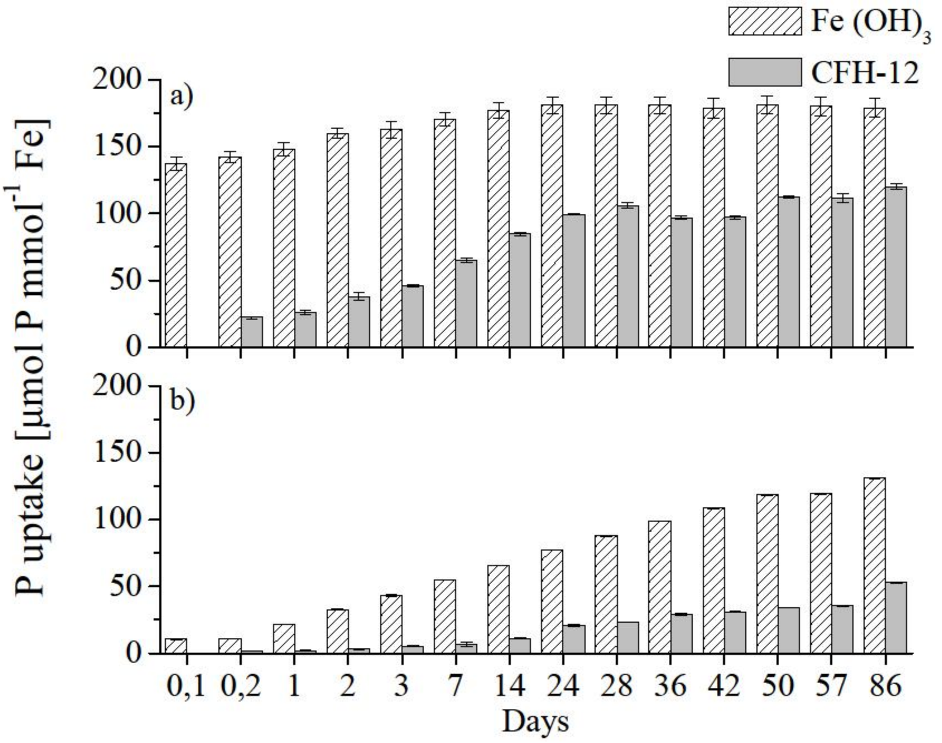


Fig. 1. Ageing effect on adsorption of P to pure phases ($Fe(OH)_3$ and CFH-12®) over time; a) exposure to high P concentration and b) exposure to low P concentration with continuous sampling.

As shown in Fig. 1b the maximum binding capability during the exposure to a low and more natural P concentration (2) was not reached after three months and a steady increase could be observed until the end of the experiment (final adsorption $Fe(OH)_3$: 130.8 µmol P mmol^{-1} Fe; CFH-12®: 53.0 µmol P mmol^{-1} Fe). The final Fe:P molar ratio for the low exposure resulted in 10:1 for $Fe(OH)_3$ and 25:1 for CFH-12®.

When ageing in artificial lake water without any P present (3), $Fe(OH)_3$ showed a clear reduction of its short term (24 h) P binding capability by approx. 30%. When exposing the two compounds after three months of ageing in artificial lake water to a high P concentration with continuous sampling for 79 days (4), a reduction in the maximum adsorption capacity was still observed for $Fe(OH)_3$ (final binding capability: 147.9 µmol P mmol^{-1} Fe), while for CFH-12® a steady uptake of P and no ageing effect was detected (final binding capability: 124.0 µmol P mmol^{-1} Fe).

3.3 Core incubation experiment

3.3.1 DIP effluxes

During the oxic incubation period slight differences in DIP release rates between the three treatments could be observed for all three lakes (Fig. 2). In Lake Hampen -a continuous DIP uptake in all treatments- and in Hostrup -negligible DIP release in all treatments- was observed (Fig. 2b and 2c, respectively). Only in Lake Sønderby a significant difference between the DIP measurements of the control group (8.15 mmol P m^{-2}) and the $Fe(OH)_3$ treatment (-5.52 mmol P m^{-2}) was observed (Fig. 2a; $p < 0.02$).

However, the differences between the treatments become more apparent during the anoxic incubation period, where Lake Sønderby shows a significantly lower DIP release for the $Fe(OH)_3$ treatment (final accumulated DIP release: -12.27 mmol P m^{-2}; $p < 0.05$) and CFH-12® treatment (final accumulated DIP release: 30.87 mmol P m^{-2}; $p < 0.05$) compared to the control group (final accumulated DIP release: 54.46 mmol P m^{-2}). In Lake Hampen a significant decrease in DIP release was observed for the $Fe(OH)_3$ treatment during the anoxic period, (final accumulated DIP release: -1.62 mmol P m^{-2}; $p < 0.05$). Also CFH-12® showed a clear tendency towards lower DIP release (final accumulated DIP release: 2.72 mmol P m^{-2}), although due to the high standard deviation of the control group (final accumulated DIP release: 9.19 mmol P m^{-2}) no significant difference between the two groups could be observed. The same patterns were also observed for Lake Hostrup, where the control group released significantly more DIP into the overlying water in anoxic conditions (final accumulated DIP release: 2.44 mmol P m^{-2}) compared to the $Fe(OH)_3$ treatment (final accumulated DIP release: 0.19 mmol P m^{-2}; $p \leq 0.001$) and the CFH-12® treatment (final accumulated DIP release: 0.99 mmol P m^{-2}; $p \leq 0.001$). In contrast to DIP exchange between sediment and water, the accumulated measurements for DOP showed no significant differences between the treatments in any of the three lakes over the entire core incubation time.

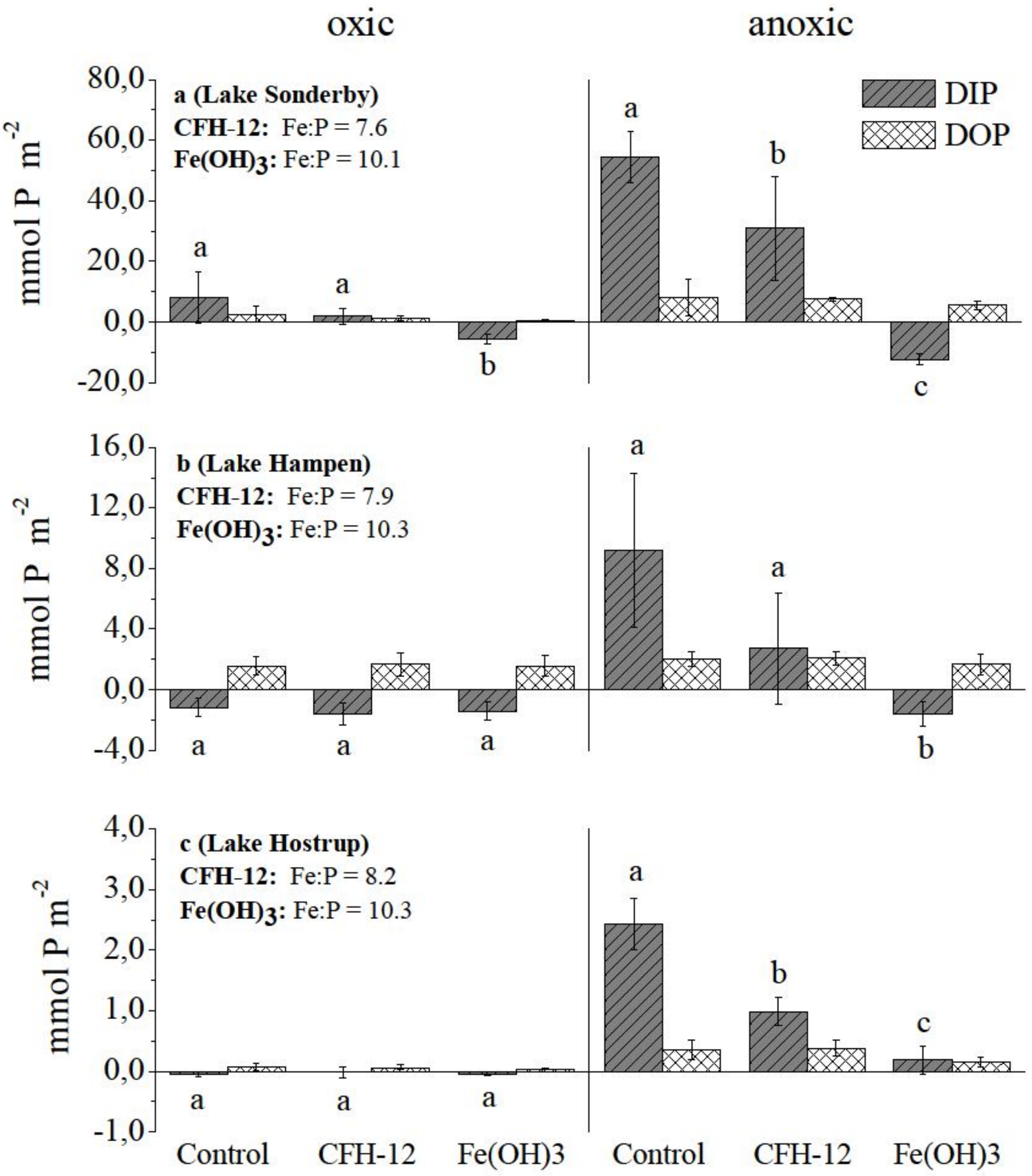
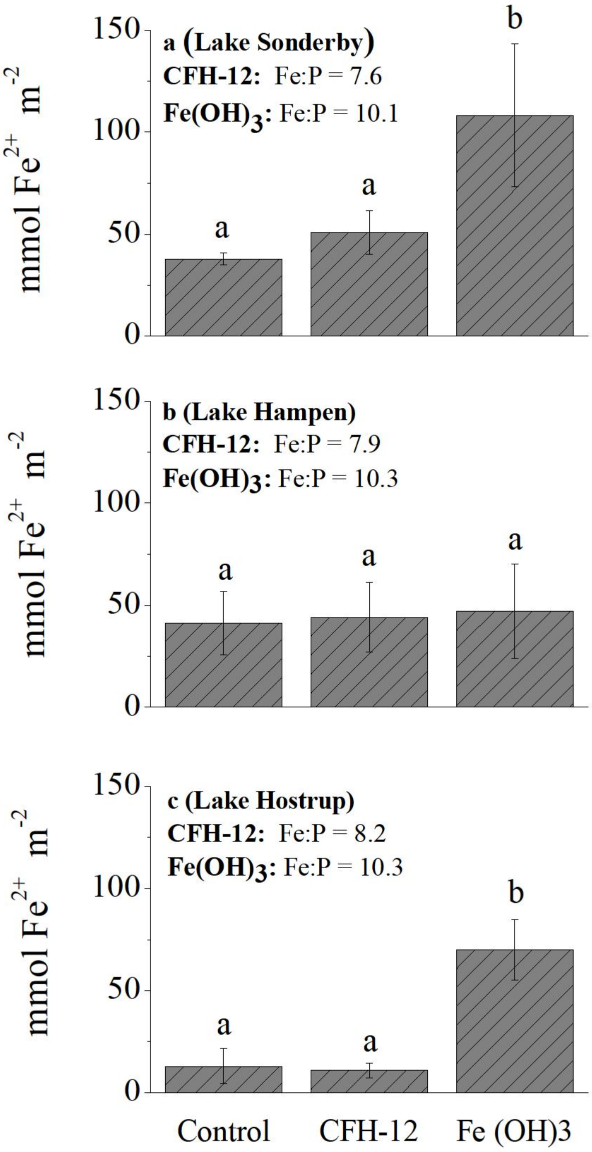


Fig. 2. Accumulated P release (DIP and DOP) from sediment cores during oxic (left) and anoxic (right) period of the core incubation; DIP and DOP fluxes for Lake Sønderby (a), Lake Hampen (b) and lake Hostrup (c).

3.3.2 Fe^{2+} effluxes

Release of reduced Fe was only observed for the anoxic period and all lakes have in common that the highest Fe^{2+} release was observed for the $Fe(OH)_3$ treatment group (Fig. 3). The highest release was measured for Lake Sønderby (Fig. 3a), where the Fe^{2+} release of the $Fe(OH)_3$ treatment (final accumulated Fe^{2+} release: 108.17 mmol Fe m^{-2}) is significantly higher compared to the CFH-12® treatment (final accumulated Fe^{2+} release: 50.81 mmol Fe m^{-2}; $p \leq 0.001$) and the control group (final accumulated Fe^{2+} release: 37.85 mmol Fe m^{-2}; $p \leq 0.001$). In contrast to Lake Sønderby, the measurements for Lake Hampen (Fig. 3b) did not show any significant differences between the three treatments during the anoxic period, although also here the $Fe(OH)_3$ treatment had the highest Fe^{2+} release (46.91 mmol Fe m^{-2}), followed by the CFH-12® treatment (44.21 mmol Fe m^{-2}) and the control group (41.08 mmol Fe m^{-2}). In Lake Hostrup (Fig. 3c) the highest Fe^{2+} release was measured for the $Fe(OH)_3$ treatment group (69.93 mmol Fe m^{-2}) compared to the control group (12.91 mmol Fe m^{-2}; $p \leq 0.001$) and the CFH-12® treatment (10.68 mmol Fe m^{-2}; $p \leq 0.001$). For all lakes and treatments weak Fe^{3+} efflux



was determined, but no significant differences between the different treatments could be obtained (data not shown).

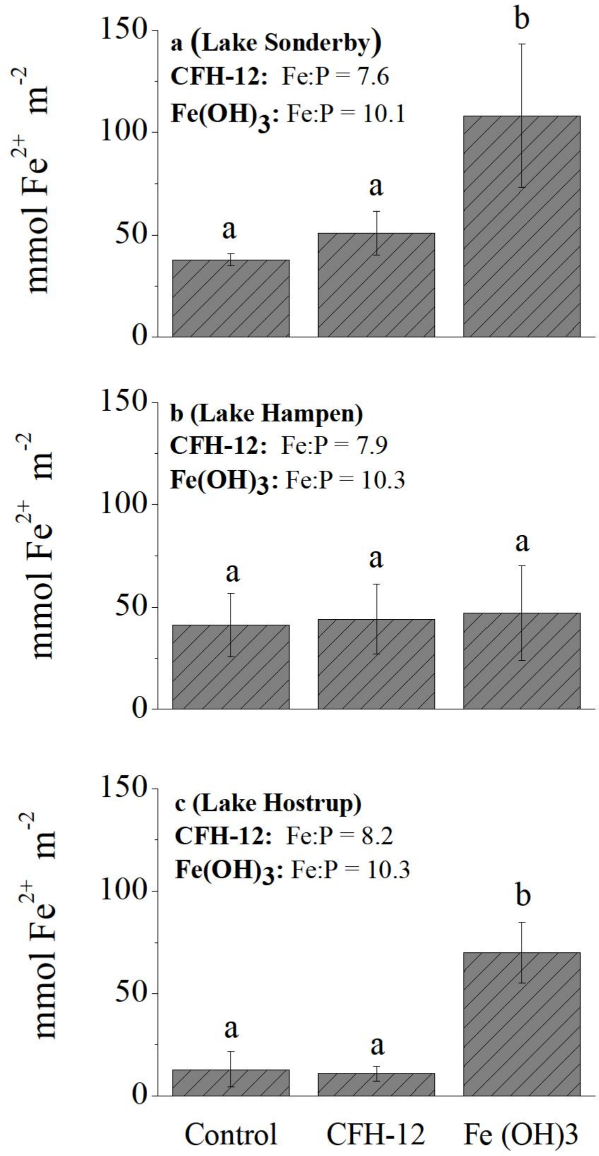


Fig. 3. Accumulated Fe^{2+} release from sediment cores during oxic and anoxic period of the core incubation; Fe^{2+} fluxes for Lake Sønderby (a), Lake Hampen (b) and lake Hostrup (c).

3.3.3 Other parameters monitored during the core incubation

When comparing the release and uptake of NO_3^-, all lakes showed a similar release during the oxic incubation period (10.16 to 23.69 mmol NO_3^- m^{-2}). However, Lake Sonderby and Lake Hostrup showed an increased NO_3^- uptake during the anoxic incubation period (22.27 to 30.24 mmol NO_3^- m^{-2}), while the measurements for Lake Hampen were considerably lower (2.64 to 2.91 mmol NO_3^- m^{-2}). The ammonium release differed considerably between the lakes, while no real differences between the treatments were observed. Lake Hampen had the lowest average NH_4^+ release (65.19 mmol NH_4^+ m^{-2}), similar to the one of Lake Hostrup (105.81 mmol NH_4^+ m^{-2}), while

Lake Sønderby had a considerably higher NH_4^+ release (304.05 mmol NH_4^+ m^{-2}). A similar trend was also observed for Mn^{2+} release during the anoxic incubation period, where again Lake Sønderby showed a considerably higher Mn^{2+} release (14.98 to 24.68 mmol Mn^{2+} m^{-2}) compared to Lake Hampen and Lake Hostrup (2.96 to 6.27 mmol Mn^{2+} m^{-2}). All treatments in the different lakes showed a similar SO_4^{2-} uptake during the anoxic incubation period (12.76 to 42.21 mol SO_4^{2-} m^{-2}).

The oxygen uptake measurements during the oxic period of the core incubation gave the highest uptake rates for Lake Sønderby (7.23 mol m^{-2}), while the measurements for Lake Hostrup (4.18 mol m^{-2}) and Lake Hampen (3.33 mol m^{-2}) were considerably lower. However, also here no differences between the treatments were observed.

The redox potential profiles of the sediment cores showed for all treatments a similar profile. However, in the oxic period the redox potential at the sediment – water interface differed in the three lakes with rather high values for Lake Hampen and Lake Hostrup (450 - 500 mV) and lower measurements for Lake Sønderby (320 - 420 mV). During the anoxic period all treatments of the three lakes showed a similar depth profile with no steep decline of the redox potential and an average redox potential of 50 - 100 mV – only the $Fe(OH)_3$ treatment in Lake Sønderby shows a considerably lower redox potential (average: – 4 mV).

3.3.4 Sediment

The distribution of seven P fractions in the initial fractionation as an average over the top 10 cm of the sediment is shown in Fig. 4. The average Tot-P_{sed} corresponds to 121, 62 and 50 µmol gDW^{-1} in Lake Sønderby, Lake Hampen, and Lake Hostrup, respectively. Lake Sønderby possessed the highest Tot-P_{sed} content for the upper 10 cm (65.0 µmol cm^{-2}) followed by Hampen (37.5 µmol cm^{-2}) and Hostrup (19.4 µmol cm^{-2}). The P_{Mobile} fraction accounted for 50 % in Lake Sonderby, 41 % in Lake Hampen and 45 % in Lake Hostrup, showing a decreasing share of P_{Mobile} with depth in all three lakes. The P fractions distribution, varied slightly between lakes: All three lakes had in common, that the largest fraction of Tot-P_{sed} was Org-P_{Labile} (26-36% of Tot-P_{sed}), which decreased with depth. In Lake Sønderby the $P_{\rightarrow BD}$ fraction (22% of Tot-P_{sed}) was the second largest fraction in contrast to Lake Hampen and Hostrup with $P_{\rightarrow NaOH,Humic}$ (31-35% of Tot-P_{sed}) as second largest fraction. Lake Sønderby showed the highest Tot-Fe_{sed} concentration over the top 10 cm (118.5 µmol cm^{-2}) compared to Lake Hampen (104.4 µmol cm^{-2}) and Lake Hostrup (54.7 µmol cm^{-2}), while the Tot-Fe_{sed} content was rather stable through the depth profile in all three lakes (Lake Sønderby: 221.1 µmol gDW^{-1}, Lake Hampen: 172.6 µmol gDW^{-1}, Lake Hostrup: 139.6 µmol gDW^{-1}). In all three lakes most of the Fe was found in the $Fe_{\rightarrow HCl,Res}$ fraction (41-44% of Tot-Fe_{sed}) increasing with depth and in the $Fe_{\rightarrow BD}$ fraction (18-27 % of Tot-Fe_{sed}) decreasing with depth. The $Fe_{\rightarrow BD}$:$P_{\rightarrow BD}$ molar ratio was 2:1 for Lake Sonderby, 11.3 for Lake Hampen and 7.6 for Lake Hostrup at the start of the experiment.

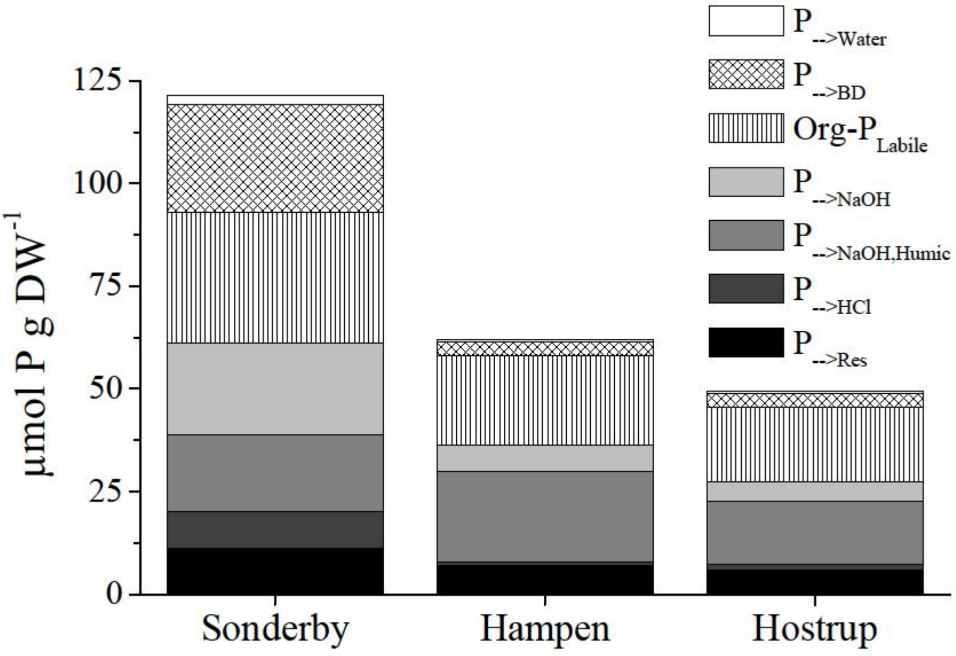


Fig. 4. P pool composition of the three different lake sediments from Lake Sønderby, Lake Hampen and Lake Hostrup calculated as average values for the top 10 cm of sediment (initial fractionation).

The final fractionation of the sediment showed no significant differences in Tot-P_{sed}, between the three treatments in any lake. As in the initial measurement, the highest Tot-P_{sed} was measured in Lake Sønderby (58.3-69.7 µmol cm^{-2}), followed by Lake Hampen (34.5-36.8 µmol cm^{-2}) and Lake Hostrup (17.2-18.8 µmol cm^{-2}).

When comparing the Tot-Fe_{sed} measurements, the $Fe(OH)_3$ treatment in Lake Sønderby showed the highest Tot-Fe_{sed} content (429.3 µmol cm^{-2}) which was 3 times higher than the control group ($p < 0.005$) and 2.5 times higher than the CFH-12® treatment ($p < 0.009$), however, no significant differences between the control group and CFH-12® treatment could be obtained. In Lake Hampen as well the $Fe(OH)_3$ treatment (207.6 µmol cm^{-2}; $p \leq 0.005$) as the CFH-12® treatment (189.6 µmol cm^{-2}; $p < 0.014$) exceeded significantly the Tot-Fe_{sed} content in the control group (114.4 µmol cm^{-2}). For Lake Hostrup no significant differences in Tot-Fe_{sed} between the treatments could be obtained (Tot-Fe_{sed}: 65.9 to 127.2 µmol cm^{-2}).

4. Discussion

4.1 CFH-12® distribution/extraction

The determination of the distribution of CFH-12® throughout the top sediment layer is a key factor to assess the practical use of this compound within lake restoration, due to the fact that the P_{Mobile} pool is mainly situated within the first layers of the sediment and decreases rapidly with depth (Reitzel et al., 2005). It has been shown that most of the Fe in CFH-12® is concentrated within the top 5 cm of the sediment, thus being mostly accessible for P immobilization and increasing the P binding capacity of the sediment (Deppe and Benndorf, 2002; Hupfer and Hilt, 2008). Unlike to what happened to $Fe(OH)_3$ (complete Fe extraction with BD) a low extraction of P and Fe from CFH-12® under either strong reducing or acidic conditions was obtained, which leads to the conclusion that CFH-12® possesses only little redox sensitivity and is therefore also hardly subject to microbial respiration. The low Fe and P dissolution from CFH-12® by BD could be explained by the high content of amorphous iron(hydr)oxides present in CFH-12® which are especially efficient P sorbents (Lyngsie et al., 2014a, Borggaard et al., 2005). In contrast to our results, Lyngsie et al. (2014a) showed a high (almost completely) Fe extraction from CFH-12® when using either an aggressive procedure combining BD with citrate solution at 80 ºC based on Mehra and Jackson (1960) or oxalate reagent, but a low desorption of P from CFH-12® (< 10 %) when applying four extractions with 6 mM KNO_3. At neutral pH (as in this case) an outer sphere complex with subsequent slow migration of P into inner reactive sites is probably the main mechanism explaining the strong bound between P and CFH-12® (Lyngsie et al., 2014b).

4.2 Ageing studies and core incubation experiment

The potential of Fe to adsorb P is already well known (Gunnars et al., 2002; Kleeberg et al., 2013), but so far the P inactivation by Fe has been found to be highly sensitive to redox potential changes in the sediment. In the studies of Lyngsie et al. (2014a, 2014b) it was shown that CFH-12® possesses a good P sorption capability, but no long-term ageing effects or reductive effects on CFH-12® under anoxic conditions were investigated. Due to the fact that these parameters are rather important for the application of CFH-12® as a lake restoration tool, the ageing effects on the adsorption capacities of $Fe(OH)_3$ and CFH-12® were tested under four different ageing conditions in artificial lake water, while the pH was kept between 6.5 and 7.5 due to the pH dependency of P adsorption (de Vicente et al., 2008a). When comparing the maximum adsorption capacity for $Fe(OH)_3$ and the most likely slightly underestimated one for CFH-12®, the one obtained for $Fe(OH)_3$ is approximately one third higher. The Fe:P molar ratio at the end of the high P exposure experiment yielded at approximately 6:1 for $Fe(OH)_3$ and 13:1 for CFH-12®, which is rather similar compared to the Al:P molar ratio of 10:1 measured for $Al(OH)_3$ after six months of ageing or as recovered in lake sediments (de Vicente et al., 2008b). Moreover, a similar response of $Fe(OH)_3$ to ageing in artificial lake water was observed compared to $Al(OH)_3$ - while $Al(OH)_3$ was loosing 70-75 % of its binding capacity within six months (de Vicente et al., 2008b), $Fe(OH)_3$ was loosing about 30 % P binding capacity due to crystallization of $Fe(OH)_3$ and the associated loss of adsorbing surface within 3 months. CFH-12® on the other hand showed no noticeable ageing effect and after three months ageing in artificial lake water the same adsorption profile as in the high P exposure experiment was obtained.

The reductive effect on the two compounds was investigated by performing a core incubation experiment with intact sediment cores. As the microbial respiration is an important factor influencing the P-release rates from the three lakes (e.g. Jensen and Andersen, 1992), the oxygen uptake and several fluxes (such as SO_4^{2-}, NO_3^-, NH_4^+ and Mn^{2+}) were monitored. The highest accumulated oxygen uptake was measured in Lake Sønderby, followed by Lake Hostrup and lastly Lake Hampen, which supported the prediction by the lakes' different trophic states. As the respiration using oxygen represents the most efficient way to degrade organic matter under aerobic conditions (den Heyer and Kalff, 1998), the obtained measurements strengthen the conclusion, that the shallow and hypereutrophic Lake Sønderby possesses the highest mineralization rate followed by Lake Hostrup, while the deep and oligotrophic Lake Hampen shows a significantly lower rate (den Heyer and Kalff, 1998). This is also supported by the NO_3^-, SO_4^{2-}, NH_4^+, Mn^{2+} and Fe^{2+} fluxes during the anoxic incubation period, which shows the highest corresponding uptake or release in Lake Sønderby and the lowest in Lake Hampen. With respect to the magnitude of anoxic P release in control treatments the lakes rank similarly to their pool-sizes of P_{Mobile} and not in the same order as their trophic state. The most interesting result within the flux measurements is, however, the decrease of P release in the cores treated with Fe, together with the efflux of Fe^{2+} during the anoxic period. When looking at the fluxes for DIP it is shown that both, $Fe(OH)_3$ as well as CFH-12®, show a clear reduction of released DIP at the end of the core incubation compared to the control group. While $Fe(OH)_3$ almost completely reduces the release of DIP and TP until the end of the core incubation time (except for Lake Hostrup, where the reduction was 92 % and 90 %, respectively), the DIP/TP reduction rate of CFH-12® was not as high. Nevertheless, CFH-12® reduced the DIP release by 43 % for Lake Sønderby, 59 % for Lake Hostrup and 70 % for Lake Hampen, which corresponds to a TP reduction of 39 %, 55 % and 66 %, respectively. When comparing the results of the accumulated Fe^{2+} release during the anoxic period, the advantages of the usage of CFH-12® becomes even more visible: while the Fe^{2+} release in the cores treated with $Fe(OH)_3$ in the eutrophic Lakes Sønderby and Hostrup is significantly higher compared to the control and CFH-12® treatment group, the CFH-12® treatments show nearly no increase in Fe^{2+} efflux compared to the control groups. The increased Fe^{2+} release in the $Fe(OH)_3$ treatment group of Lake Hostrup is with 442 % considerably higher than in Lake Sønderby with 186 %. This can be partially explained by the higher presence of other electron acceptors (such as NO_3^- and Mn_2^+) in Lake Sønderby, which will normally be used prior to Fe^{3+} for microbial respiration (Boström et al., 1982). In Lake Hostrup the concentration of the mentioned alternative electron acceptors is considerably lower, which leads in return to a higher usage of Fe for microbial respiration. Moreover, our measurements of the redox potential during the anoxic period assured, that all cores have a low enough redox potential to allow for Fe^{3+} reduction throughout the top layer of the sediment during the anoxic period (Boström et al., 1982). The fact, that the cores treated with CFH-12® show no significantly higher Fe^{2+} efflux than the control group strengthens the assumption that CFH-12® is little subject to microbial respiration and chemical reduction. An alternative explanation could be that Fe^{2+} obtained from CFH-12® reduction forms precipitates as e.g. vivianite (Rothe et al., 2014) within the sediment to a larger degree than Fe^{2+} obtained from reduction of the more dispersed $Fe(OH)_3$. The maintenance of pH after CFH-12® addition to water (in contrast to the pH drop reported for the addition of $FeCl_3$ and Al sulfate or poly-Al chloride; Jeppesen et al., 2009; Immers et al., 2013), is also beneficial for the ecotoxicological assessment of the compound due to the known toxicity of Fe^{2+} towards aquatic organisms (Vuori 1995; Kinsman-Costello et al. 2015).

The effect of adding $Fe(OH)_3$ and CFH-12® to the cores in a 10:1 and 7.6-8:1 $Fe:P_{Mobile}$ molar ratio, respectively, have not resulted in significant changes in the sedimentary P pools between the treatments within the final fractionation. A higher concentration of P in the $P_{\rightarrow BD}$ fraction would have been expected for the Fe treatments compared to the control group since these treatments have shown a complete or partially P efflux reduction, in accordance to what found Hansen et al. (2003) when adding $FeCl_3$ to sediment cores to reduce the P efflux. One explanation for not observing this could be a too short incubation period to see changes in sedimentary P pools. Within the initial fractionation, only the $Fe_{\rightarrow BD}:P_{\rightarrow BD}$ molar ratio in Lake Hampen was well above the

8:1 molar ratio proposed by Jensen et al. (1992) to assure P retention in the sediment under oxic conditions. This is also in agreement with the observed P scavenging within the control group of Lake Hampen under oxic conditions, while P release was observed for Lake Sønderby and Hostrup.

As a result of this extensive work, we can state that in lakes with a $Fe_{\rightarrow BD}$:$P_{\rightarrow BD}$ molar ratio less than 8 (as in Lake Sønderby) the addition of $Fe(OH)_3$ and CFH-12® resulted in the suppression/reduction of DIP release from oxic sediment surfaces as a consequence of the higher P binding capacity of the sediment compared to the control group (Jensen et al., 1992, Hansen et al., 2003). Lakes in which $Fe_{\rightarrow BD}$:$P_{\rightarrow BD}$ molar ratio are close or well above 8, as occurred in both Lake Hampen and Hostrup, P binding capacity of sediment under oxic conditions is enough to trap P. Under anoxic conditions, Fe reduction takes place as shown by the redox profile measurements, releasing previously Fe-bound P into the overlying water (Einsele 1936; Mortimer 1941; Gomez et al., 1999).

In conclusion, we have tested the granulate of dried amorphous ferric oxy-hydroxide (CFH-12®) as a P binding agent in three lake sediments and as a pure phase alongside the freshly formed and aged $Fe(OH)_3$, which would be the product of e.g. $FeCl_3$ addition to a lake. Clearly the freshly formed $Fe(OH)_3$ had a higher affinity for DIP and a higher binding capacity for P but with ageing and long-time exposure to P the products appeared more similar. Added to lake sediment CFH-12® sinks ~ 2 cm into the very organic Lake Hampen sediment surface which was mimicked by mixing both CFH-12® and $Fe(OH)_3$ into the top 3 cm of the three types of sediment. In two of the lakes CFH-12® lowered DIP release significantly but in all three lakes $Fe(OH)_3$ was still more efficient, also more than could be expected from the slightly higher dose molar ratio ($Fe:P_{Mobile}$) of $Fe(OH)_3$ than of CFH-12®. Meanwhile, CFH-12® treatments did not release significantly more Fe^{2+} than control sediments during the anoxic period in any of the lakes while $Fe(OH)_3$ treatments did release significantly more Fe^{2+} in two of the lakes. Along with the very modest extraction of Fe from CFH-12® in the reducing BD solution these results suggest that the oxic Fe in CFH-12® is much more resistant to chemical and microbial reduction than freshly formed $Fe(OH)_3$ and that it therefore is able to maintain a P-binding capacity in anoxic freshwater sediments. Since CFH-12® can be added to a lake without affecting lake water pH or alkalinity and since it is only 30% more expensive than solutions of $FeCl_3$ it could be a relevant product to immobilize P in the sediment of eutrophied softwater lakes. Although the difference in Fe:P molar ratio is not ideal for the comparison of the two treatments due to the fact that the performance of CFH-12® will be underestimated, we could state that, in the long run CFH-12® should show considerably beneficial effects compared to $Fe(OH)_3$ but clearly, full scale studies are needed before it can be finally recommended.

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General conclusions

1. For pH values higher than 9, both in the presence and in the absence of bare carbonyl Fe particles, Mn was removed from aqueous solution by an oxidation and subsequent precipitation process with an efficiency of 98%. When magnetic particles were present, most of the MnOx(s) were precipitated on the magnetic particles surface being easily removed from solution by magnetic field gradients. The use of MnOx(s)-coated magnetic particles resulted in the best alternative to improve Mn removal efficiency at intermediate pH values (7-9) which was always higher than 80 %. Adsorption isotherm of MnOx(s)-coated magnetic particles did not reach saturation due to a multilayer adsorption mechanism in which adsorption capacity was continuously regenerated. Desorption studies suggested that MnOx(s) precipitated on magnetic particles were stable compounds over a wide range of pH (6-9) and reutilization studies pointed out the feasibility of reusing magnetic particles without losing adsorption capacity compared to the new ones.

2. In oxic conditions when sedimentary Fe:P molar ratio is well above 8:1, the addition of carbonyl Fe particles in the cores had negligible effect on P efflux. However, in anoxic conditions when sediment looses adsorption capacity due to the solubilisation of natural Fe, the addition on magnetic particles resulted in partial/complete reduction of P efflux indicating that magnetic particles were effective in one of the most adverse conditions (anoxia). Magnetic particles also caused a reduction in sedimentary P_{Mobile} of 22-25 % in anoxic and of 12-16 % in oxic conditions within the first 4 cm of sediment. The suppression of P efflux and the reduction of sedimentary P_{Mobile} meant that the addition of magnetic particles increased P export from the system which was not available anymore to be release to the water column under changing physicochemical conditions.

3. The addition of carbonyl Fe particles in a Fe:P_{Mobile} molar ratio of 85:1 in the enclosures caused a significant reduction of DIP for the oxic (68 % average reduction) and for the anoxic period (80% average reduction). N cycle was not affected by magnetic particles amendment, however, Si was reduced by half and DOC was decreased 15 %, indicating that both Si and DOC interfered in P adsorption by magnetic particles. Although DIP was significantly reduced, Chl *a* did not decrease because TP was still high (> 200 µg L^{-1}) after magnetic particles treatments. Despite the fact that lake water used in the enclosures was brackish, no interferences of major cations and anions were observed in P adsorption by magnetic particles. From the point of view of magnetic particles recovery efficiency, we recommend the addition of magnetic particles over the surface water (90 % of recovery) in contrast to the addition of magnetic particles over the sediment surface since lower efficiency was obtained (30 %).

4. It has been carried out, in laboratory conditions, the successful synthesis of hybrid magnetic particles which consisted of magnetic multicores homogeneously distributed within a spherical chitosan matrix. These particles are easily separated from the media by applying a magnetic separation gradient and are non toxic, but the most outstanding advantage is that they present the capability of remaining in the upper layers of sediment due to a lower density compared with carbonyl Fe particles. This fact favours the availability of the new chitosan-modified magnetic particles for P adsorption (which mostly concentrates in the top sediment), reduces resuspension during their removal with a magnet and makes the magnetic particles recovery more efficient. Although lower than carbonyl Fe particles (18.83 mg g^{-1}), P adsorption capacity of chitosan-modified magnetic particles (4.84 mg g^{-1}) is by far high enough to be used as P sorbents in hypertrophic lakes where TP is higher than 100 µg L^{-1}.

5. From the comparison of $Fe(OH)_3$ floc and CFH-12®, we concluded that $Fe(OH)_3$ floc presented more P adsorption capacity than CFH-12®. However, with ageing and long time exposure to P, the performance of both products was alike. Contrarily to what occurred to $Fe(OH)_3$ floc, CFH-12® neither lost P adsorption capacity as a consequence of ageing nor caused a pH drop in lake water after its addition. Even more, although both compounds caused a significant reduction of DIP fluxes in oxic and also anoxic conditions with a $Fe:P_{Mobile}$ molar ratio of 7.6-10:1, CFH-12® showed little Fe^{2+} release to water column in anoxic conditions and thus little redox sensitivity that was also supported by a modest extraction of the Fe presents in its composition under strong reducing conditions (BD reagent) compared to $Fe(OH)_3$ floc.

6. The three adsorbents proposed in this PhD dissertation have been determined to be promising lake restoration tools since they overcome the most important limitations of traditional adsorbents. Briefly, they were capable of reducing/suppressing internal P load in the short and in the long-term and, specifically magnetic particles, were also able to increase P export from the system. Likewise, all of them were efficient P adsorbents when working in the most undesirable conditions such as anoxic conditions and did not cause a pH drop in lake water when adding to the lake. Additionally, the possibility to recover the adsorbent (i.e. magnetic particles) through magnetic separation makes this technique more profitable in the long-term and makes it also possible to recover P as well. This P could be further used as a fertilizer.

Conclusiones generales

1. Para pH mayores a 9, tanto en ausencia como en presencia de partículas de Fe carbonilo sin recubrir, el Mn fue eliminado de la solución acuosa mediante un proceso de oxidación y precipitación con una eficiencia del 98%. Cuando las partículas magnéticas estaban presentes, la mayoría de MnOx(s) precipitaron sobre su superficie siendo fácilmente retiradas de la solución mediante gradientes de campo magnético. El uso de partículas magnéticas recubiertas de MnOx(s) resultó ser la mejor alternativa para mejorar la eficiencia de retirada de Mn a valores de pH intermedios (7-9) la cual fue mayor al 80%. La isoterma de adsorción de las partículas magnéticas recubiertas de MnOx(s) no alcanzó la saturación debido a un mecanismo de adsorción en multicapa en el cual la capacidad de adsorción era continuamente regenerada. Los estudios de desorción sugirieron que los MnOx(s) precipitados sobre las partículas magnéticas eran compuestos estables en un amplio rango de pH (6-9) mientras que los estudios de reutilización señalaron la factibilidad de reutilizar las partículas magnéticas sin perder capacidad de adsorción comparadas con las nuevas.

2. En condiciones óxicas cuando el ratio molar Fe:P está por encima de 8:1, la adición de partículas de Fe carbonilo a los cores de sedimento tuvo un efecto despreciable sobre el flujo de P. Sin embargo, en condiciones anóxicas cuando el sedimento pierde capacidad de adsorción debido a la solubilización del Fe presente de forma natural en el sedimento, la adición de partículas magnéticas provocó la reducción parcial/completa del flujo de P desde el sedimento al agua indicando que las partículas magnéticas eran efectivas en las condiciones más adversas (anoxia). Las partículas magnéticas también provocaron la reducción de P móvil del sedimento en un 22-25% en condiciones anóxicas y en un 12-16% en condiciones óxicas en los primeros 4 cm del sedimento. La supresión del flujo de P y la reducción de P móvil del sedimento significó que la adición de partículas magnéticas produjo una eliminación P del sistema el cual ya no estaría disponible nunca más para ser liberado a la columna de agua cuando cambiasen las condiciones físico-químicas.

3. La adición de partículas de Fe carbonilo en un ratio molar Fe:P móvil 85:1 en los cerramientos causó una reducción significativa del P inorgánico soluble tanto en el período óxico (68% de reducción promedio) como anóxico (80% de reducción promedio). El ciclo del N no fue afectado por la adición de partículas magnéticas, sin embargo, el Si fue reducido a la mitad y el DOC fue reducido un 15%, indicando que tanto el Si como el DOC interfirieron en la adsorción de P por las partículas magnéticas. Aunque el P inorgánico disuelto fue significativamente reducido, la Chl *a* no disminuyó debido a que el TP aún era elevado (>200 µg L^{-1}) después de la aplicación de los tratamientos con partículas. A pesar del hecho que el agua de la laguna utilizada en los cerramientos era salobre, no se observaron interferencias de los cationes y aniones mayoritarios en la adsorción de P por las partículas magnéticas.

4. Se ha llevado a cabo, en condiciones de laboratorio, la síntesis exitosa de partículas magnéticas híbridas las cuales consistían en varios núcleos magnéticos homogéneamente distribuidos en una matriz esférica de quitosano. Las partículas resultantes son fácilmente separadas del medio mediante la aplicación de un gradiente de separación magnética y no presentan toxicidad, aunque la ventaja más destacable es que presentan la capacidad de permanecer en las capas más superficiales del sedimento debido a que poseen una menor densidad comparadas con las partículas de Fe carbonilo. Este hecho favorece la disponibilidad de las nuevas partículas modificadas con quitosano para la adsorción de P (el cual se concentra en su mayor parte en la superficie del sedimento), así como reduce la resuspensión durante la retirada de las mismas con un imán y hace el proceso de recuperación más eficiente. Aunque la capacidad de adsorción de P por parte de las partículas magnéticas modificadas con quitosano (4.84 mg g^{-1}) es menor a la de las partículas de Fe carbonilo (18.83 mg g^{-1}), es suficientemente alta como para puedan ser utilizadas como adsorbentes de P en lagos hipertróficos donde la concentración de TP es superior a 100 µg L^{-}

5. De la comparación del fóculo $Fe(OH)_3$ y CFH-12®, se concluyó que el flóculo de $Fe(OH)_3$ presenta mayor capacidad de adsorción de P que el CFH-12®. Sin embargo, si consideramos el envejecimiento de ambos compuestos y la exposición de los mismos al P a largo plazo, la eficiencia de ambos parece similar. Contrariamente a lo que le ocurrió al flóculo de $Fe(OH)_3$, el CFH-12® ni mostró pérdida de la capacidad de adsorción como consecuencia de su envejecimiento con el tiempo ni causó una caída del pH del agua del lago después de su adicción. Lo que es más, aunque ambos compuestos causaron una reducción significativa de los flujos de P inorgánico disuelto tanto en condicione óxicas como anóxicas con un ratio molar Fe:P móvil de 7.6-10:1, el CFH-12® mostró una escasa liberación de Fe^{2+} a la columna de agua en condiciones anóxicas y por tanto una escasa sensibilidad redox, hipótesis que fue también apoyada por una modesta extracción del Fe presente su composición en condiciones fuertemente reductoras (reactivo BD) comparado con el flóculo de $Fe(OH)_3$.

6. Los tres adsorbentes propuestos en esta tesis doctoral han demostrado ser unas herramientas prometedoras para la restauración de lagos ya que no presentan las limitaciones que poseen los adsorbentes tradicionales. Brevemente, los tres adsorbentes son capaces de reducir/suprimir la carga interna de P tanto a corto como a largo plazo, y específicamente las partículas magnéticas, también son capaces de eliminar P del sistema. Asimismo, todos ellos son adsorbentes eficientes de P incluso trabajando en las condiciones más indeseables como son las condiciones anóxicas y no causan reducción del pH del agua cuando son añadidos al lago. Además, la posibilidad de recuperar el adsorbente (como en el caso de las partículas magnéticas) a través de técnicas de separación magnética hace esta técnica más rentable a largo plazo así como también hace posible la recuperación de P para ser utilizado posteriormente como fertilizante.

Scientific production and activity

- **PUBLICATIONS**

Funes, A., de Vicente, J., Cruz-Pizarro, L., de Vicente, I., 2014. The influence of pH on manganese removal by magnetic microparticles in solution. Water Res. 53, 110–122. doi:10.1016/j.watres.2014.01.029

Funes, A., de Vicente, J., Cruz-Pizarro, L., Álvarez-Manzaneda, I., de Vicente, I., 2016. Magnetic microparticles as a new tool for lake restoration: A microcosm experiment for evaluating the impact on phosphorus fluxes and sedimentary phosphorus pools. Water Res. 89, 366–374. doi:10.1016/j.watres.2015.11.067

Funes, A., del Arco, A., Álvarez-Manzaneda Salcedo, I., de Vicente, J., de Vicente, I., 2016. An enclosure experiment for testing consequences of using magnetic microparticles on water quality and sediment phosphorus pools. Water Res. (in preparation to be submitted).

Funes, A., de Vicente, J., de Vicente, I., 2016. Hybrid magnetic chitosan microspheres: synthesis, characterization and applications in lake restoration. J Hazard Mater. (submitted for publication).

- **ASSISTANCE TO NATIONAL AND INTERNATIONAL CONGRESS**

Poster presentation: Improving water quality by magnetic microparticles: the case of manganese removal
Authors: **A. Funes**, J. de Vicente, and I. de Vicente
Name of the congress: 3th Workshop on Advanced in Colloidal Materials
Location: Granada, Spain
Date: October 25th, 2013

Oral Presentation: Coating magnetic particles with MnOx(s) enhances manganese removal efficiency. Implications for water quality
Authors: **A. Funes**, J. de Vicente, and I. de Vicente
Name of the congress: II Reunión de Jóvenes Investigadores en Coloides e Interfases (JICI II)
Location: Granada, Spain
Date: April 27-30th, 2014

Poster Presentation: Using magnetic particles for improving water quality and lake restoration
Authors: **A. Funes**, J. de Vicente, A. Merino-Martos, L. Cruz-Pizarro, and I. de Vicente
Name of the congress: XVII Congress of the Iberian Association of Limnology (AIL 2014)
Location: Santander, Spain
Date: July 6-11th, 2014

Poster Presentation: Iron magnetic particles as a new tool for lake restoration. A microcosm experiment as a previous step for future in situ application
Authors: **A. Funes**, J. de Vicente, I. Álvarez-Manzaneda, L. Cruz-Pizarro, and I. de Vicente
Name of the congress: Association for the Sciences of Limnology and Oceanography (ASLO 2015). Aquatic Sciences Meeting
Location: Granada, Spain
Date: February 22-27th, 2015

Oral Presentation: Evaluating phosphorus removal efficiency by iron magnetic microparticles with special consideration of chemical interferences in a context of lake restoration
Authors: A. Funes, I. Álvarez-Manzaneda, A. del Arco, J. de Vicente, and I. de Vicente
Name of the congress: XXXIII Congress of the International Society of Limnology (SIL 2016)
Location: Turin, Italy
Date: July 31th- August 5th, 2016

- **RESEARCH VISITS**

Host University: University of Southern Denmark
Tutor: Henning S. Jensen
Department: Biology
Location: Odense, Denmark
Dates: July 30th-October 31th, 2014

Host University: University of Southern Denmark
Tutor: Henning S. Jensen
Department: Biology
Location: Odense, Denmark
Dates: January 18th-February 16th, 2015

- **GRANTS**

Name: Beca F.P.D.I. Proyectos de Excelencia. Personal investigador en formación correspondiente a los proyectos de investigación de excelencia. Proyecto de Investigación de excelencia P10-RNM-6630.
Objective: Predoctoral
Organization: Consejería de economía, innovación y ciencia, Junta de Andalucía.
Dates: February 1st, 2012-January 31th, 2016

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