Universidade de Aveiro
2020

Ana Rita Correia Fernandes

Remediation of P-rich eutrophic water using anionic nanoclays

Remediação de água eutrófica rica em P utilizando nanoargilas aniónicas

Universidade de Aveiro
2020

Ana Rita Correia Fernandes

Remediation of P-rich eutrophic water using anionic nanoclays

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Eco-Toxicologia e Análise de Risco, realizada sob a orientação científica do Doutor Roberto Carlos Domingues Martins, Investigador Auxiliar do Departamento de Biologia da Universidade de Aveiro, e coorientação do Doutor João Tedim, Professor Auxiliar do Departamento de Engenharia Materiais e Cerâmica da Universidade de Aveiro.

Para a minha irmã,
Na esperança que tenha sempre mais sucesso que eu.

"Ostra feliz não faz pérolas"

o júri

Presidente	Prof. Doutor Carlos Miguel Miguez Barroso Professor Auxiliar, Centro de Estudos do Ambiente e do Mar (CESAM) e Departamento de Biologia, da Universidade de Aveiro, Portugal
Arguente	Doutor Frederico Calheiros Maia CEO e Diretor de I&D, Smallmatek - Small Materials and Technologies, Lda., Portugal
Orientador	Doutor Roberto Carlos Domingues Martins Investigador Auxiliar, Centro de Estudos do Ambiente e do Mar (CESAM) e Departamento de Biologia, Universidade de Aveiro, Portugal

agradecimentos

Começo por agradecer aos meus orientadores, Roberto Martins e João Tedim, por me darem a oportunidade incrível de trabalhar com algo que eu realmente gosto muito e por sempre se mostrarem disponíveis para me auxiliar quando precisei. Um especial agradecimento ao Roberto, que sempre foi muito compreensível, encorajador e nunca desistiu de mim, mesmo que a minha tese tenha sido uma dor de cabeça enorme.

Aos meus pais, por todos os sacrifícios que fizeram ao longo destes anos para que eu tivesse a oportunidade de estudar e tentar alcançar algo que para eles nunca foi opção. À minha irmã, por ser o melhor presente que eu alguma vez recebi e por me impulsionar a ser um exemplo e a minha melhor versão de mim mesma. À minha restante família, por terem percorrido este percurso ao meu lado, levantando-me quando caí e festejando comigo todas as vitórias. Nunca serei capaz de vos agradecer devidamente, mas ainda assim eu tento: Obrigada!

À Diana Carneiro, por tanto… Por tudo! Por me acompanhar em todos os momentos e todas as etapas ao longo de seis incríveis e esgotantes anos. Por nunca me deixar fraquejar. Por me fazer regressar à Terra e continuar. Por tantas vezes que, literalmente, me salvou a vida e ajudou em tudo o que precisei. Por todas as ideias que eu não tive. Por querer sempre o meu melhor e por me ensinar tanto. Obrigada, mana!

À Patricia Louro e à Inês Fonseca, por me apoiarem, ouvirem as minhas reclamações, terem sempre uma palavra de encorajamento e por fazerem o esforço de me ajudar a solucionar os problemas que me foram surgindo pelo caminho. Obrigada por cuidarem de mim, meninas!

À Andreia Silva, Eva Lopes, Sara Marques e Teresa Garrido quero agradecer, principalmente, por se interessarem pelo meu trabalho. Ainda que muitas das vezes eu não fizesse sentido, nunca me deixaram entrar em pânico sozinha e sempre tiveram palavras de encorajamento para me oferecer. Mil obrigadas por serem apoios incansáveis!

Ao Telmo Correia que esteve comigo durante o tempo pós-desconfinamento. A tua companhia foi imprescindível nas horas a fio que passei no laboratório.

À Sara Silva, que foi uma ajuda indispensável na luta contra o HACH. Obrigada por teres estado do outro lado e me ajudares sempre que precisei.

Por fim, mas não menos importante, a todos os colegas de laboratório, em especial à Catarina Malheiro, Marija Prodana, Fábio Campos, Paula Silva, Rita Silva e Sandra Gonçalves, que me ajudaram a solucionar todos os dramas que vivi no maravilhoso Edifício 37.

palavras-chave

Remediação, hidróxidos duplos lamelares, fosfato, nitrato, ensaios ecotoxicológicos, *Raphidocelis subcapitata*, eutrofização

resumo

A eutrofização de corpos de água é um fenómeno comum resultante da disponibilidade de nutrientes inorgânicos, normalmente relacionada com atividades antropogénicas, tais como agricultura, descargas de esgoto, mineração, indústria, entre outros. Os recentes esforços para controlar e remediar o processo de eutrofização incluem a utilização da nanotecnologia. Os hidróxidos duplos lamelares (LDH) de Zn-Al são nanoargilas aniónicas não tóxicas que conseguem trocar o anião estabilizador por outros que podem estar presentes na água, como é o caso do fosfato. Deste modo, o presente estudo teve como objetivo avaliar a eficácia do Zn-Al LDH-NO_3 como um adsorvente de baixa toxicidade para a remediação de massas de água ricas em P, comparativamente a um material de referência: LDH calcinado. Para tal, três concentrações de LDH (5, 50 e 500 mg/L) foram adicionados a soluções que mimetizaram a concentração de fosfato máxima recomendada em água potável (0.4 mg P/L) e água de ETAR (10 mg P/L), de acordo com a legislação Portuguesa (DL 236/98), e ainda a água recolhida de um lago artificial eutrofizado (Aveiro, Portugal), num cenário real. Os nitratos e fosfatos foram medidos periodicamente de forma a avaliar a capacidade do nanomaterial remover o conteúdo de fosfato durante um período de 7 dias. A eficácia da remediação aos 7 dias foi igualmente avaliada em termos da toxicidade da água remediada através de testes de inibição de crescimento da microalga de água doce *Raphidocelis subcapitata.*

Os materiais testados demonstraram uma grande eficiência na remoção de fosfatos, em todos os meios testados. No geral, quanto maior a concentração de LDH, maior a velocidade de remoção de P, maior a quantidade de nitratos libertados (inferior ao limite para água potável: 25 mg NO_3/L) e maior são os efeitos de inibição de crescimento na microalga *R. subcapitata.* A intercalação de fosfatos foi confirmada através da difração de raio-X (DRX). Em conclusão, os resultados sugerem que Zn-Al LDH-NO_3 é uma solução nanotecnológica ambientalmente promissora para a remediação de P, preferencialmente se utilizada durante curtos períodos de tempo (p.e. a adição de 500 mg LDH/L na solução de 10 mg P/L removeu 99.9% de P em apenas 5 minutos, libertando apenas 6 mg NO_3/L).

keywords

Remediation, layered double hydroxides, phosphate, nitrate, ecotoxicological assays, *Raphidocelis subcapitata*, eutrophication

abstract

Eutrophication of water bodies is a common phenomenon resulting from the surplus of inorganic nutrients, usually related to anthropogenic activities, such as, agriculture, sewage discharges, mining, overconsumption, among others. Recent efforts to control and remediate eutrophication processes include the use of green nanotechnology. Zn-Al layered double hydroxides (LDH) are non-toxic hydrotalcite-like anionic nanoclays that can exchange the stabilizer anion by others that can be present in the water, such as phosphates. Therefore, the present study aimed to assess the efficacy of Zn-Al LDH-NO_3 as a low toxic adsorbent for the remediation of P-rich water bodies (benchmarked with calcined LDH). For this purpose, three concentrations of LDHs (5, 50 and 500 mg/L) were added to P-rich solutions mimicking the maximum recommended concentration of phosphorus on drinking water (0.4 mg P/L) and wastewater (10 mg P/L), according to the Portuguese legislation (DL 236/98) and to water collected from an eutrophic artificial lake (Aveiro, Portugal), simulating a real scenario. Nitrates and phosphates were periodically measured to evaluate the nanomaterial capacity to remove phosphates' content for a period of 7 days. The remediation efficacy was also assessed in terms of the toxicity of remediated water at the end of the remediation period through growth inhibition tests using the freshwater microalgae *Raphidocelis subcapitata*.

Tested materials showed a great efficacy on the removal of phosphates, in all tested media. Overall, the higher the LDH tested concentration, the higher the speed on P removal, the higher the nitrates released (below the recommended threshold for drinking water, 25 mg/L) and the higher the growth inhibition effects on the green microalgae *R. subcapitata*. The intercalation of phosphates was confirmed through X-ray diffraction (XRD). In conclusion, the results suggest that Zn-Al LDH-NO_3 is a technological and environmentally friendly promising solution for phosphates remediation, preferably if used for very short-periods (e.g. the addition of 500 mg LDH/L into the solution of 10 mg P/L removes 99.9% of the P in just 5 minutes, releasing only 6 mg NO_3/L).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA: One-way analysis of variance

ELV: Emission limit value

K_2HPO_4: Dipotassium hydrogen phosphate

LDH: Layered double hydroxides

MAC: Maximum allowable concentration

NPs: Nanoparticles

XRD: X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1. State of the art

The rapid worldwide population growth in the last centuries has caused severe and undeniable changes in both terrestrial and aquatic ecosystems. Also, the biogeochemical cycles of nitrogen, carbon, and phosphorus, nutrients present in aquatic systems, have been demonstrating profound changes (Schlesinger and Bernhardt, 1991; Vitousek *et al*., 1997a; Vitousek *et al*., 1997b).

Humans are responsible for the introduction of massive quantities of nitrogen into the environment coming from various sources, such as deficiently controlled agriculture practices (Carpenter *et al*., 1998). In this particular case, nitrogen may accumulate in the soil, enter the atmosphere through the production of nitrous oxide and/or ammonia volatilization or in the surface water or groundwater (Nolan *et al*., 1997; Vitousek *et al*., 1997a; Carpenter *et al*., 1998). Also, the combustion of fossil fuels is an activity that affects the nitrogen cycle as this nutrient return to the aquatic and terrestrial surface by dry and wet deposition (Vitousek *et al*., 1997b). However, it is in phosphorus that great concerns arise because of its excessive bioavailability in water bodies. Besides that, phosphorus is considered a raw material for the EU manufacturing industry, so it is urgent to ensure its sustainable and secure supply, and it is therefore important to develop ways to certify a permanently effective solution for its removal from water bodies (National Research Council, 2000; Comissão Europeia, 2017). When phosphorus enters into the water bodies two situations can happen: phosphorus sinks or returns to the column of water. Thus, the internal charge can be maintained in the system or promoted even when the external phosphorus charge is reduced which continues to stimulate the eutrophication process for decades (Wang *et al*., 2009).

The Portuguese Decree-Law No. 236/98 of August 1st and Council Directive 98/83/EC of November 3rd establishes quality criteria, standards and objectives intending to improve water quality according to its uses, and also protecting the aquatic environment. That said, the maximum allowable concentration (MAC) of phosphates in surface waters for human consumption (class A1, that is, with physical treatment and disinfection intended to produce drinkable water) is 0.4 mg/L. In wastewater, the emission limit value (ELV) of phosphorus increases considerably, being, therefore, 10 mg/L.

Eutrophication is a phenomenon caused by the proliferation of primary producers as a result from the availability of inorganic nutrients (Thoman and Mueller, 1987; de Varennes, 2003). The eutrophication process is very slow and can take thousands of years to occur, allowing the plant and animal communities to develop in a diversified and balanced manner (Thoman and Mueller, 1987). However, aquatic plants (such as cyanobacteria, phytoplankton, and algae) can develop over several years if optimal nutrient availability, light and temperature conditions are met, which can irreversibly unbalance the ecosystem (Cardoso *et al.*, 2001; Santos *et al*., 2004).

In fact, it has been found that human activity is often responsible for increasing the availability of nutrients in aquatic systems, which may come from point or non-point sources, leading to eutrophication (Tchobanoglow and Burton, 1991; Valsami-Jones, 2004). Some examples of human activities responsible for eutrophication are soil erosion, agriculture (excessive and improper use of fertilizers and manure), sewage discharges (industrial or municipal), mining, overconsumption, among others (Figure 1) (Howarth *et al.*, 2000; de Varennes, 2003).

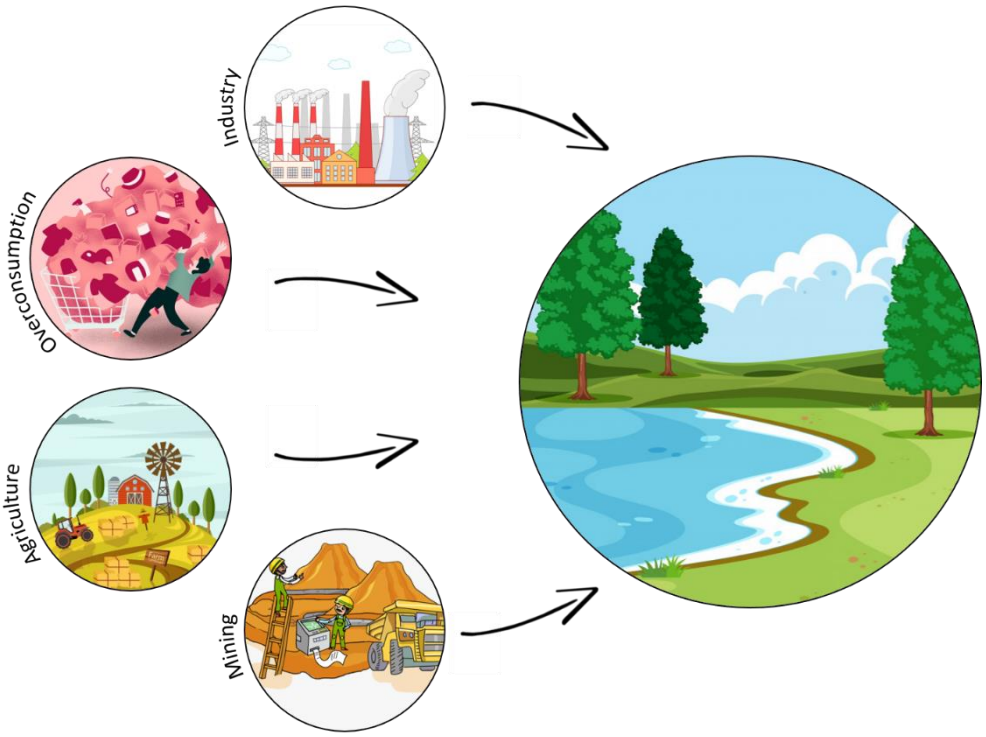

Figure 1 | Schematic representation of some examples of human activities responsible for P supply, such as industry, overconsumption, agriculture and mining (Adapted from Othman *et al*., 2018).

The eutrophication process is also followed by deterioration of water quality as organic matter decomposes, leading to a decrease in dissolved oxygen concentration and light in the water, which may lead to the formation of "hypoxic zones" harming aquatic organisms including lethality on fish (Anderson *et al*., 2008; Diaz and Rosenberg, 2008; Heisler *et al*., 2008; Hautier, Niklaus and Hector, 2009; Li *et al*., 2014). Besides, a long-term decrease in biodiversity may also occur if the aquatic system is dominated by cyanobacteria as they may release toxic compounds that negatively affect aquatic biota (Skulberg, Codd and Carmichael, 1984; Carmichael, 1991; Vasconcelos *et al*., 1996; Glasgow and Burkholder, 2000; de Figueiredo *et al*., 2006; Dai *et al*., 2008). Activities such as swimming, fishing and boating in eutrophic waters can also pose a risk to human health (Dodds *et al*., 2009). In fact, ingestion or exposure to eutrophic water can cause, in humans, tumor lysis syndrome, rhabdomyolysis, impaired renal function or, in the worst-case scenario, death (Razzaque, 2011). There are also economic consequences, as the values of eutrophic waterside properties may decrease due to unpleasant odors or decreased clarity (Dodds *et al*., 2009).

There are three types of remediation methods usually used to remediate eutrophic water bodies, being them broadly categorized in physical, chemical and biological methods (Table 1).

Due to the large variety of existing pollutants, different types of efficient techniques have been developed for the remediation of eutrophic water bodies, such as chemical precipitation, crystallization, biological processes (using photosynthetic organisms, like planktonic bacteria, algae or plants), absorption, solvent extraction, coagulation, flocculation, filtration and ion exchange (Table 2) (Carillo and Griego, 2012; Othman e*t al.*, 2018). The use of sorbents, such as clays, zeolites, and activated alumina, is an alternative that, in addition to its simple design, is also very effective, making it among the most used, even though its operational cost is considerably high (Kasprzyk-Hordern, 2004; Cornejo *et al.*, 2008; Misaelides, 2011; Carillo and Griego, 2012).

Some technologies are already in the market for the remediation of eutrophic water bodies. As an example, when placed in water, Phoslock^{TM} (a modified clay product that comprise of 95% of bentonite and 5% of lanthanum) forms a compound with low solubility thus functioning as a phosphate reservoir because lanthanum (held in the bentonite structure) is able to bind phosphate (Yuan and Wu, 2007). However, this

situation is not a fully effective solution since phosphate can be released back into the system (Yuan and Wu, 2007). Alum (aluminium sulfate), one of the most used compounds in the treatment and remediation of nutrient-rich waters, can form, on the surface layer of sediments, a P-adsorbing floc cap or can alternatively mix within the sediments (Cooke *et al.*, 1993; Pilgrim, Huser and Brezonik, 2007) (Table 3). Despite being very effective in the remediation of eutrophic water bodies, negative effects have been observed in fish, macroinvertebrates and other organisms (Smeltzer, 1990; Smeltzer, Kirn and Fiske, 1999). Beyond that, this compound can lead to situations of aluminium toxicity and secondary physiological effects since can reduce the pH of water bodies (Morris *et al*., 1989; Gensemer and Playle, 1999; Klapper, 2003). Z2G1 is a modified zeolite product produced by amending natural zeolite with an aluminosilicate polymer that provides an improved P-binding (Hickey and Gibbs, 2009). Z2G1 has been demonstrated (under laboratory conditions) to be a very effective sediment capping agent in blocking the release of P from sediments (under anoxic conditions) (Gibbs and Özkundakci, 2011). In addition, Z2G1 is, as far as we are concerned, the only one sediment capping agent that inactivates both N and P (Gibbs and Özkundakci, 2011).

Table 1 | Description, advantages and disadvantages of the major types of remediation methods.

Remediation methods	Description	Advantages	Disadvantages	Reference
Biological	• Key method for restoration of natural cycles. • Used to adjust the stability of the water body. • Microorganisms, aquatic plants and animals are used to transform, degrade and absorb the water body nutrients.	• Sustainable. • Cost-effective. • Can achieve complete remediation. • Less residues and secondary pollutants.	• Needs a long performance period.	Schindler *et al*., 2008 Li *et al*., 2010 Zhang *et al.*, 2020
Chemical	• Usually used in emergency situations. • Suitable for water bodies with a serious imbalance of nutrients (which results in uncontrolled blue-green algae). • First attempts to remedy eutrophic water bodies mainly involve herbicides, algicide and copper sulfate ($CuSO_4$).	• Quick and obvious effects on temporary solutions. • Can result when conventional treatments fail to sufficiently reduce nutrient concentrations in the water body. • Used in emergency situations. • Simple.	• Expensive. • Incomplete. • Can produce residues and secondary pollutants very easily. • May cause, to many non-target aquatic organisms, cumulative and/or immediate harmful side-effects.	Carmichael and Li, 2006 Schindler, 2006 Hanson and Stefan, 2010
Physical	• Called engineering methods. • Dilution and flushing, sediment dredging and deep aeration are some examples of techniques that are used.	• Quick and obvious effects on temporary solutions. • Simple.	• Expensive. • Incomplete. • Can't solve the cause of the problem. • Very dependent on machinery and manpower.	Foster, 2010 Estrada *et al*., 2011 Kuha *et al.*, 2016 Nygrén *et al.*, 2017

Table 2 | Description, advantages and disadvantages of the different types of chemical methods.

Method	Description	Advantages	Disadvantages	References
Absorption	• Reactive components (e.g. iron) presents in the solution cause the inorganic P to move and accumulate in their body or surface.	• Absorptive media can be manufactured from man-made, natural or industrial waste products.	• Long-term performance needs more investigation.	Quan *et al.*, 2016 Bunce *et al.*, 2018
Coagulation	• Metal salts are added, as coagulant, in the water and dissolved inorganic phosphate is transformed into their particulate form that is, later, separated by gravity.	• Remarkable removal of P by Al coagulation.	• Necessity to investigate the coupled removal of phosphorus.	Morse *et al.*, 1998 Aguillar *et al.*, 2002 Mortula *et al.*, 2011
Crystallization	• Addition of seeding crystals and consequent removal of phosphorus from the waste water.	• Already applied in full scale in Netherlands. • Allows phosphorus recycling.	• Only applied in alkaline environment. • The dissolved CO_2 in the water has to be, previously, removed.	Donnert *et al.*, 2002
Ion Exchange	• Process that exchange ions held in insoluble materials with other ions presents in solutions.	• Highly selective nature. • Can be effective in removal and recovery of P-rich water bodies. • Effective for selection of P anions over others ions.	• Expensive. • Not suitable for long-term scenarios.	Zarrabi *et al.*, 2014
Precipitation	• P in wastewater is precipitated when metal salt is added and from that results solids residuals that are removed by filtration or settling.	• Cost-benefit to the amount of salt and the method of results solids that are used. • Reliable.	• Requires high doses of chemicals. • Can produce residues. • Phosphorus recyclability us variable.	Jenkins, Fergurson and Menar, 1971 Morse *et al.*, 1998

Table 3 | Nanotechnology-based methods used for eutrophic water remediation. NPs - nanoparticles.

Method	Description/Advantages	Efficiency	Ecotoxicity	References
Fe NPs	• Green synthetized using extracts of natural products. • Environmentally friendly. • Cost effective.	• Good removal longevity and capability. • Antioxidants or polyphenols protect the particles from aggregation and oxidation. • Great potential for in situ remediation of wastewater.	• Low toxicity.	Soliemanzadeh *et al.*, 2015
SiO_2 NPs	• Silica-based material. • Pores with sizes between 2 and 50 nm. • Structural stability. • Chemical resistance. • Good selectivity when exposed to competitive anions.	• High and rapid adsorption capacity.	• Low toxicity.	Lai *et al.*, 2016
TiO_2 NPs	• Small particles size. • High adsorption capacity. • High specific surface area. • Strong electrostatic attraction at the surface area.	• Can be effective in reducing and/or controlling algae growth.	• Deleterious effects on non-target species (e.g. pelagic organisms).	Da Silva *et al.*, 2016 Cekli *et al.*, 2015 Luo *et al.*, 2010
Allophane (nano)	• High specific surface area. • Strongly react with metal cations, dissolved anions, water, organic molecules and soil minerals. • Natural, inexpensive and can be recovered from wastewater. • Environmentally friendly.	• Ineffective in P adsorption at high pH.	• Data not found	Yuan and Wu, 2007
Alum (nano)	• Most common lake water treatment. • Removes impurities by forming a capping layered over the sediment.	• Works effectively, except in alkaline water.	• Data not found	Pilgrim, Huser and Brezonik, 2007

Nonetheless, researchers continue to work on new strategies to tackle excess of P in water bodies. Some interesting alternatives based on nanotechnology emerged as possible innovative ways to remediate eutrophic waters. Nanotechnology is the science, engineering and technology that can work on an atomic and molecular level (on a scale from 1 to 100 nm) to understand, produce and use nanomaterials with unique functions and properties, thus proving to be an area of great interest, as it operates in the industry, medicine, human-care and environmental protection standing out in the remediation process (Roco, 2003). Nanomaterials are very versatile and can be manipulated in terms of size, porosity, morphology and chemical composition, among other properties (Guerra *et al*., 2018). Some nanomaterials have additional advantages, such as storing and controlled release capacity of active compounds immobilized within the nanostructure (Zheludkevich, Tedim and Ferreira, 2012). Not surprisingly, some classes of engineered nanomaterials have been proposed as interesting and suitable candidates for water remediation processes, such as TiO_2, Si O_2 or Fe nanoparticles, allophane and carbon-based materials (such as graphene, carbon nanotubes and activated carbon), LDHs, among others (Yuan and Wu, 2007; Vallet-Regí, 2014; Wang *et al.*, 2014; da Silva *et al.*, 2016; Whitley, 2017; Bunce *et al*., 2018). TiO_2 nanoparticles, despite showing effective results in the control of algal blooms, can impact the biota of the aquatic system, particularly in non-target species (da Silva *et al.*, 2016). Green Fe nanoparticles synthesized using extracts of natural products are simple, environmentally-friendly and cost-effective, and also have great potential for remediation of wastewater (Smuleac *et al*., 2011; Kumar *et al*., 2013; Machado *et al*., 2013; Wang *et al.,* 2014). SiO_2 nanomaterials are silica-based materials that show chemical resistance, low toxicity, structural stability and porosity particularly advantageous to be used as a porous sorbent (Vallet-Regí, 2014). Allophane is a natural, environmentally-friendly and inexpensive nanoclay that can be regarded as effective in the remediation of P-enriched eutrophic water bodies, since they have a strong-propensity for absorbing phosphate (Yuan and Wu, 2007). Carbon-based materials are very promising in wastewater treatment. Graphene and carbon nanotubes have many useful features, such as high specific surface area, high thermal and chemical stability, oxygen functionalities and abundant binding sites (Bradder *et al.*, 2011; Wang *et al.*, 2013; Mubarak *et al.*, 2014; Namvari and Namazi, 2014). Activated carbon is an adsorbent used very often, produced

by physical processes or activation of carbon-containing materials, that have a highly porous adsorptive structure, fast adsorption kinetics and high specific surface area (Dias *et al.*, 2007). Biomass-derived sorbents are normally synthesized from renewable and available raw material (like eggshell waste, refined aspen wood fiber, *Staphylococcus xylosus* biomass, among others) and have advantageous features, such as, large surface area, high porosity and good capacity to remove phosphorus from water in an environmentally-friendly way (Eberhardt and Min, 2008; Mezenner and Bensmaili, 2009; Aryal and Liakopoulou-Kyriakides, 2011; Bunce *et al.*, 2018).

In this research work plan, Zn-Al Layered Double Hydroxides (LDH) (Figure 2) stabilized with nitrates (in the anionic form) are firstly proposed as a versatile nanomaterial for the remediation of P-rich water bodies. Zn-Al LDHs-chlorides/carbonates have been already proposed as efficient solutions to remove phosphates or sulphates from water bodies (Iftekhar et al., 2018). However, the low (eco-)toxicity of Zn-Al LDHs-nitrates supported the decision to use them as an innovative and truly low toxic remediation nano-based technology. LDHs are a family of nanomaterials whose physical and chemical properties are similar to naturally occurring mineral clays, presenting a two-dimensional structure that derives from brucite or hydrotalcite (Vaccari, 1999; Carillo and Griego, 2012). LDHs have some impressive features, such as layered structure, microscopic controllability of the layers chemical composition (i.e. structural divalent and trivalent metals in the positively-charged layer), a reactive interlayer gallery with water molecules and stabilizing anions (e.g. carbonates that are not readily exchangeable, or nitrates and chlorides that can exchange with anions present in the surrounding media, such as phosphates), catalytic activity, rheological and colloidal properties, variable layer charge density and the ability to swell in water (Newman and Jones, 1998; Vaccari, 1999; Khan and O'Hare, 2002; Goh, Lim and Dong, 2008; Bergaya and Lagaly, 2013). LDHs can be represented by the following general formula:

$$[M^{II}_{1-x}\, M^{III}_{x}\, (OH)_2](X^{q-}_{x/q} \bullet n\, H_2O)$$

where M^{II} represents any divalent metal cation (such as Zn^{2+}, Mg^{2+}, Cu^{2+}, Cd^{2+}, Ca^{2+}, …), M^{III} any trivalent metal cation (for example Al^{3+}, Cr^{3+}, Fe^{3+}, …), both of them occupying octahedral positions, and X^{q-} represents an inorganic (e.g. carbonate or nitrate) or

organic (e.g. biocides or pharmaceutical drugs) anion (Carillo and Griego, 2012; Bergaya and Lagaly, 2013; Avelelas et al., 2017; Martins et al., 2017; Gutner-Hoch et al., 2018).

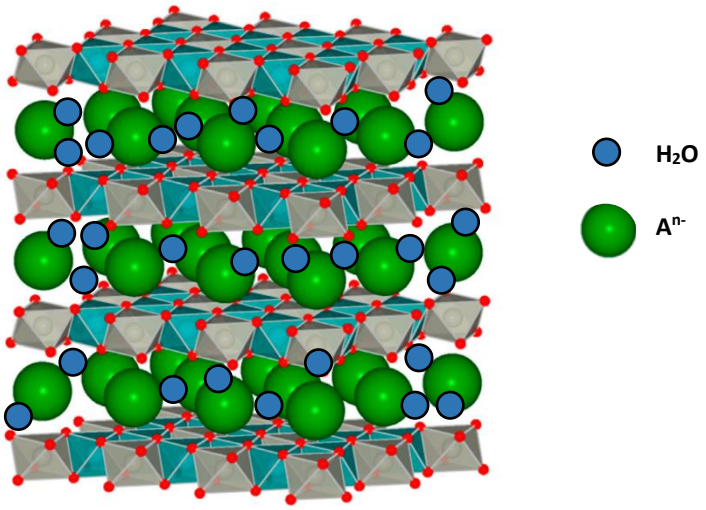

Figure 2 | Schematic representation of "layered double hydroxides" (LDHs) (Adapted from Bergaya and Lagaly, 2013).

LDHs can be of natural origin (e.g. hydrotalcite and manassite), where usually carbonate is the intermediate anion, or of synthetic origin (Carillo and Griego, 2012), as proposed in the present work plan having nitrates as an intermediate anion. These engineered nanoclays have a hexagonal shape, lateral size of 20 to 40 nm and width with hundreds of nm or few µm (Newman and Jones, 1998). LDHs have been regarded as one of the most technologically promising inorganic host-guest materials (Duan et al., 2017) attracting high interest in multiple areas, such as industry, corrosion science, optics and medical science, pharmaceutical and separation technology (Nalawade *et al.*, 2009; Tedim e*t al.*, 2010; Zheludkevich, Tedim and Ferreira, 2012). The use of these materials as drug delivery systems for humans is based in their low toxicity and reactivity in humans and mammals, both *in vivo* and *in vitro*. Recent studies highlighted that Zn-Al LDH-nitrates are low toxic for marine species, namely microalgae, bivalves, bryozoans, crustaceans and echinoderms (Avelelas *et al*., 2017; Martins *et al*., 2017; Gutner-Hoch *et al.*, 2018; Gutner-Hoch *et al.*, 2019). There is no published information in terms of their effects on freshwater organisms, however preliminary studies by our R&D group confirmed the low toxicity of Zn-Al LDH-NO_3 on microalgae, crustaceans and fish, supporting our research plan.

1.2. Objectives

The main objectives of this thesis are:

i) Evaluate the effectiveness of LDHs on the remediation of phosphates-rich waters, namely the "smart" anionic nanoclay Zn-Al LDH-NO_3 (non-calcined) by doing a chemical assessment of remediated water (phosphates uptake studies, phosphates removal efficacy, nitrates release studies and x-ray diffraction) and ecotoxicological assessment of remediated water using the freshwater green microalgae *R. subcapitata*.

ii) Compare the effectiveness of the Zn-Al LDH-NO_3 with a reference nanomaterial (calcined Zn-Al LDH) on the remediation of phosphates-rich waters by doing a chemical assessment of remediated water (phosphates uptake studies, phosphates removal efficacy, nitrates release studies and x-ray diffraction) and ecotoxicological assessment of remediated water using the freshwater green microalgae *R. subcapitata*.

CHAPTER 2

MATERIALS AND METHODS

2.1. Remediation effectiveness assays

2.1.1. Sample preparation and collection

In this study, P-rich solutions prepared in the lab were tested intended to mimic the maximum recommended concentration of phosphorus on drinking water (0.4 mg P/L) and wastewater (10 mg P/L), according to the Portuguese legislation (DL 236/98). Besides that, to simulate a real scenario, sample water from a eutrophic artificial lake (40° 38' 07.0" N; 8° 39' 31.6" W - Aveiro, Portugal) was also collected (August 22nd 2020) (Figure 3) and tested.

Figure 3 | Water sampling site (University of Aveiro lake, Aveiro, Portugal).

Phosphate solutions were prepared in the laboratory using dipotassium hydrogen phosphate (K_2HPO_4) and milli-Q water. The 10 mg P/L solution was prepared by adding 29 mg of K_2PO_4 to 1 L of ultra-pure water in a volumetric flask. The solution was shaken until the compound K_2HPO_4 was completely dissolved. The 0.4 mg P/L solution was prepared by dilution (from the 10 mg P/L). All solutions were prepared in triplicate (Figure 4). Physic-chemical parameters were measured immediately after solutions preparation, namely pH, nitrates and phosphates (pls. cf. details in the next sub-section; Figure 5). The pH was measured with a pH meter Mettler Toledo mod. Five Easy. The concentration of phosphates and nitrates were measured with a spectrophotometer HACH DR/2000.

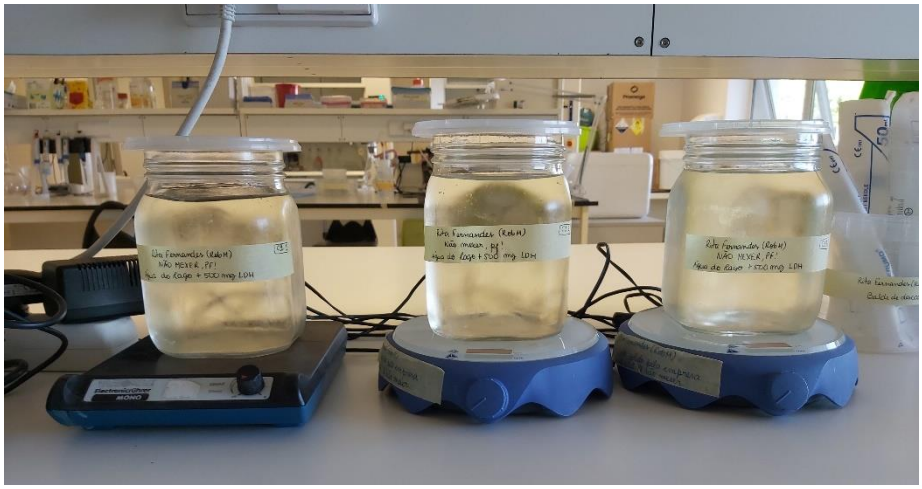

Figure 4 | Set preparation for remediation effectiveness assays.

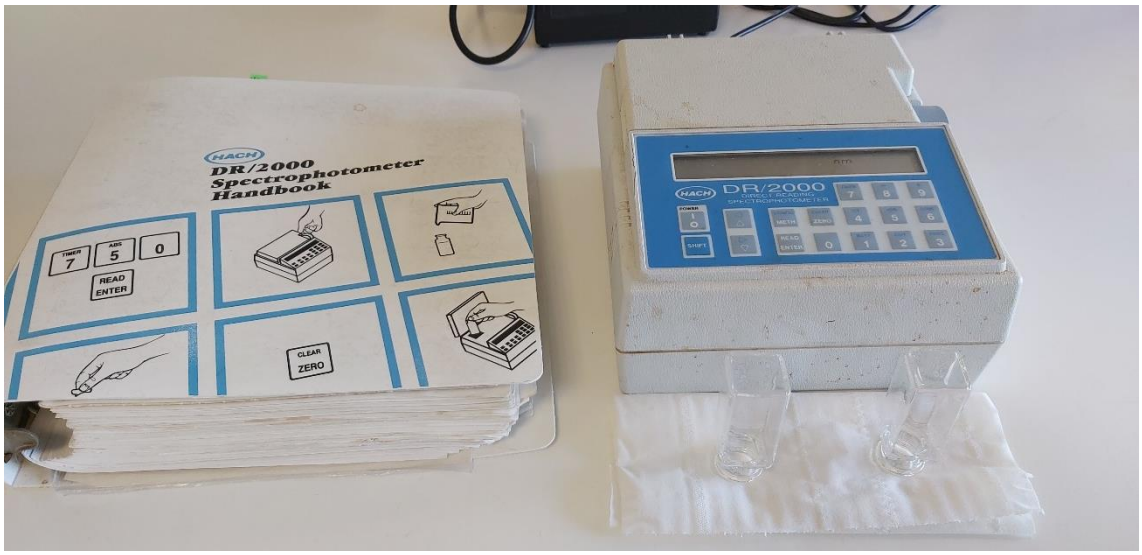

Figure 5 | HACH DR/2000.

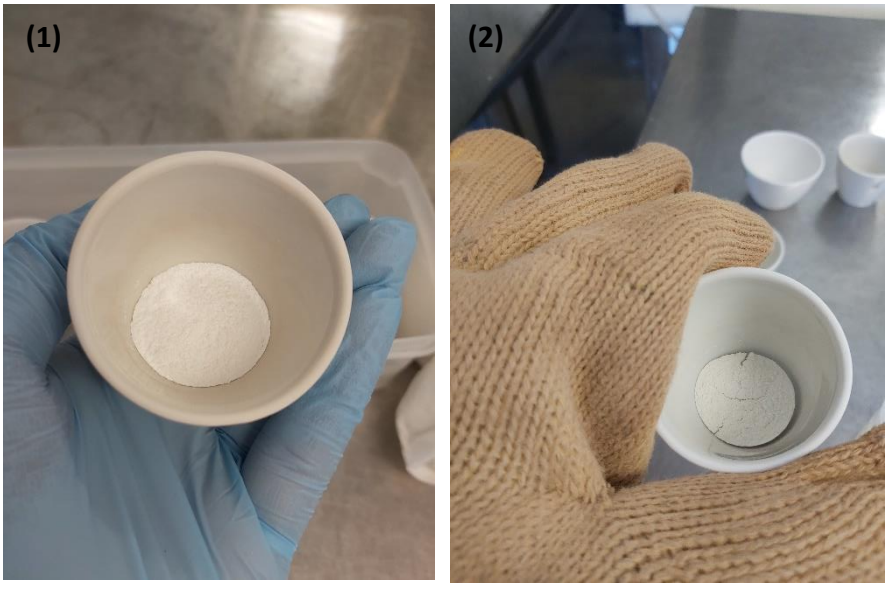

Figure 6 | Non-calcined (1) and calcined (2) Zn-Al LDH-NO_3.

Tested nanomaterials Zn-Al LDH-NO_3, prepared through a co-precipitation method, were kindly provided by the company Smallmatek, Small Materials and Technologies, Lda., headquartered in Aveiro, Portugal.

Zn-Al LDH-NO_3 was calcined at the laboratory using a furnace (Ceramifor mod. MEC-18 1200 °C) at 450 °C for approximately one hour and twenty minutes. Calcined LDHs lose organic content and water molecules and have been already purposed as efficient adsorbents. Thus, calcined Zn-Al LDH was used as reference material for benchmarking purposes.

2.1.2 Remediation effectiveness: chemical assessment

Different amounts of Zn-Al LDH were used to assess the ability to remove phosphates from the aqueous environment: 5, 50 and 500 mg Zn-Al LDH-NO_3 /L and 250 and 2500 mg of calcined Zn-Al LDH.

Powder materials were weighted and added to the glass vessels containing the P-rich solutions (cf. Figure 4) which were kept under continuous magnetic agitation at 350 rpm (VARIOMAG Electronicrührer MONO magnetic stir). In order to assess the removal efficiency of phosphates and the increase of nitrates (nitrates leave the nanostructure exchanging with phosphates that enter and replace the “space” left by the stabilizing anion), several measurements were made for a period of time of one week. Thus, a sample of 50 mL of water for phosphates and nitrates measurements were collected from the glass vessels in the following timepoints: in the initial solution of P free of LDHs (i.e. before adding LDH), in the moment “0” (i.e. as soon as LDHs were added), 0.25, 0.5, 1, 3, 24, 48, 72 and 144 hours. All treatments were prepared in triplicate, therefore 3 indepedent samples were collected in each timepoint. In order to “stop” the anion-exchange process, all samples were filtrated immediately after sampling and kept at 4 °C upon nutrients measurements (no more than one week).

2.1.2.1. Quantification of phosphates

Phosphates concentration in water samples was measured with a spectrophotometer HACH DR/2000, based on the protocol “Phosphorus reactive” which

is used for water, wastewater and seawater media (0 to 2.5 mg/L PO_4^{3-}) (HACH DR/2000 Spectrophotometer Handbook, pages 131 and 132).

The program “490” was selected and adjusted to the wavelength to 890 nm.

First, a total of 25 ml of water sample was transferred with a micropipette to the glass measuring cell. The content of one PhosVer® 3 Phosphate Reagent powder pillow was placed in the glass cell, followed by a brief dispersion (15 seconds) in the ultrasonic sonifier (Sonifier Branson S-250A). After that, the button “SHIFT” and “TIMER” were pressed to start a two minutes timer (Figure 7), in which the cells remained stationary. The blank (cell with 25 mL of sample without reagent) was firstly placed in the cell holder, the light shield closed and the button “SHIFT” and “ZERO” pressed. The display showed 0.0 mg/L PO_4^{3-} (and the process repeated if this was not the case). Then, the homogenized sample (with reagent) was “READ” and the display value annotated as the phosphates concentration present (expressed as mg/L PO_4^{3-}).

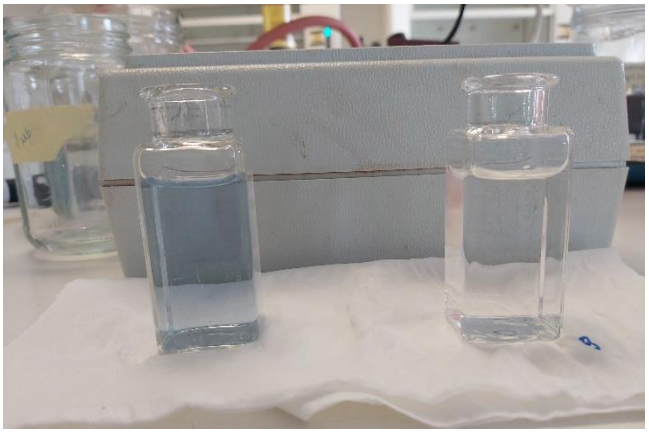

Figure 7 | Cells containing test solution with powder pillow and blank. In the presence of phosphate, PhosVer causes the solution to turn blue. The bluer it is, the more phosphate is present in the solution.

2.1.2.2. Quantification of nitrates

Nitrates concentration in water samples was measured with a spectrophotometer HACH DR/2000, based on the “Nitrate, MR” protocol, which is used for water, wastewater and seawater media (0 to 4.5 mg/L NO_3^- - N) (HACH DR/2000 Spectrophotometer Handbook, pages 297 and 298).

The program “353” was selected and adjusted to the wavelength to 400 nm.

First, a total of 25 ml of water sample was transferred with a micropipette to the glass measuring cell followed by the addition of the content of one NitraVer® 5 Nitrate Reagent powder pillow. After that, the button “SHIFT” and “TIMER” were pressed to

start a one-minute timer (Figure 7) and, in the end, the cells were shake. Then, "SHIFT" and "TIMER" buttons were pressed again to start a five-minute timer, where cells remained stationary (Figure 8). The blank (cell with 25 mL of ultra-pure water and without reagent) was firstly placed in the cell holder, the light shield closed and the button “SHIFT” and “ZERO” pressed. The display showed 0.0 mg/L NO_3^- (and the process was repeated if this was not the case). Then, the sample (with reagent) was “READ” and the display value annotated as the nitrates concentration present (expressed as mg/L NO_3^- - N).

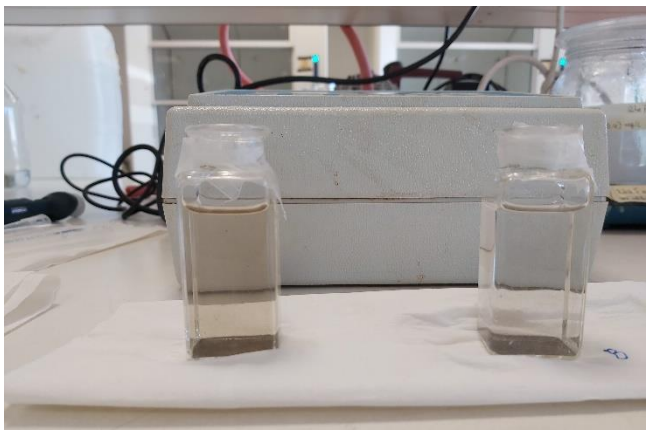

Figure 8 | Cells containing test solution with powder pillow and blank. In the presence of nitrate, NitraVer causes the solution to turn brown. The browner it is, the more nitrate is present in the solution.

2.1.2.3. Adsorption studies

The amount of PO_4^{3-} - P adsorbed was given as following equation:

$$q_e = \frac{(C_0 - C_e).V}{m}$$

where q_e is the equilibrium adsorption capacity (mg/g), C_0 e C_e are the initial and equilibrium concentrations of PO_4^{3-} - P in the solution (mg/L) respectively, V is the volume of the solution (L) and m is the adsorbent dry weight (g).

Based on the previous equation, the adsorption isotherms of Zn-Al LDH were studied at room temperature. To describe the adsorption balance, the experimental Langmuir (1) and Freundlich (2) models were used:

(1) $$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m}$$ (2) $$\log q_e = \log K_f + \frac{1}{\mathrm{n}} \log C_e$$

where C_e is the equilibrium concentration of phosphate in the solution (mg/L), q_e is the amount of phosphate adsorbed at equilibrium (mg/kg), K_L is Freundlich adsorption

equilibrium constant (L/mg), q_m is the theoretical maximum sorption capacity (mg/kg), K_f is Freundlich adsorption equilibrium constant and n is a constant which represent the Freundlich isotherm curvature (Freundlich, 1906; Langmuir, 1918).

2.1.2.4 X-Ray Diffraction (XRD)

X-ray diffraction was used to determine the chemical structure of LDH powders, as-received and after calcination, as well as to investigate the structural changes in LDHs which occurred with the immobilization of phosphates after the remediation process.

2.1.2.4.1 Sample preparation

Samples were prepared from the remaining water dispersions (section 2.1.2), in the end of each test which was decanted, transferred to previously labelled Petri dishes and dried for, at least, 48 hours, in an oven (MMM mod. Venticell 222). Petri dishes remained at room temperature (Figure 9) until analysis.

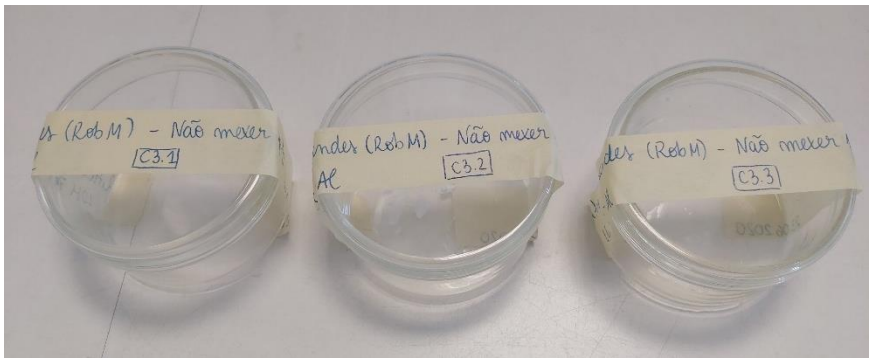

Figure 9 | LDH samples prepared for XRD tests.

2.1.2.4.2. XRD measurements and data analysis

The XRD measurements were performed using a PANalytical X'Pert Powder diffractometer (Ni filtered Cu Kα radiation, a tube power of 45 kV and 40 mA) coupled with a PIXcell1D detector, and an exposition time of 6 s per step of 0.02° over an angular range (2θ) between 4° and 65°.

The obtained XRD patterns qualitatively compared with information available in the literature for Zn-Al LDHs with similar compositions (Tedim *et al.*, 2010; Abderrazek, Najoua and Srasra, 2016). Specifically, the comparison of XRD patterns was restricted to the main peaks identifying LDH phases, namely peaks occurring at low 2θ angles associated with reflection by planes (003), (006) and (009), which are associated with

layer stacking, size and orientation of the anions within the LDH galleries. The LDH gallery height can be calculated by subtracting the thickness of cationic sheets (approximated to the thickness of brucite layer (Newman and Jones, 1998)) to the basal spacing d, the latter being calculated from the position of (003) peak, based on Bragg's law. Additionally, the peak (110) occurring at ≈ 60º is determined by the size and ratio of metal cations in the LDH hydroxide layers and thus allows us to infer whether structural changes may occur in the cationic sheets with anion exchange and/or thermal treatments (Novell-Leruth *et al.*, 2020). Table 4 summarizes literature data regarding the basal spacing for different Zn(2)-Al LDHs (Tedim *et al.*, 2010; Kuznetsova, 2011).

Table 4 | Position of (003) peak and corresponding basal spacing d (Tedim *et al.*, 2010; Kuznetsova, 2020; Novell-Leruth *et al.*, 2020).

Composition	Position of (003)/degrees	d_{003}/nm
Zn(2)-Al-NO_3	9.88	0.89
Zn(2)-Al-Cl	11.26	0.78
Zn(2)-Al-CO_3	11.59	0.76
LDH-OH	11.75	0.75
LDH-HPO_4	7.75	1.14

2.1.3 Remediation effectiveness: ecotoxicological assessment

The ecotoxicity of remediated waters by LDHs (non-calcined and calcined forms) was assessed through microalgae growth inhibition tests using the green microalgae *Raphidocelis subcapitata* (previously named *Pseudokirscheneriella subcapitata*).

2.1.3.1. Test organisms and culture maintenance

The microalgae *R. subcapitata* is a sensitive planktonic and photosynthetic freshwater species, representative of primary producers. Organisms of this species are often used in toxicity tests, since they are easy to obtain, cultivate and maintain. They have a short life cycle and high capacity for photosynthesis and they are very sensitive to a wide range of xenobiotics (Wells, Lee and Blaise, 1998; Sipaúba-Tavares and Rocha, 2003; Machado, Lopes and Soares, 2015). Cultures used in the present study were maintained in the dedicated laboratory of the Centre for Environmental and Marine Studies (CESAM) of the University of Aveiro. All tests were carried out when the microalgae were in exponential growth phase. Thus, the inoculum culture was prepared

at least four days in advance. Microalgae grew in cotton-stoppered Erlenmeyer flasks at 20 °C ± 2 °C, with continuous aeration and light (white fluorescent light) (Figure 10).

Figure 10 | Inocula of green freshwater microalgae *R. subcapitata*.

To prepare tested solutions in these bioassays, 5 µL of the 1 to 13 reagents and 10 µL of reagent nr. 14 of the growth mean (MBL) were added to 3 mL of the solution, previously saved from the chemical tests (Table 5). After all reagents have been placed in the falcon, we make up to 5 mL with the solution (previously saved from the chemical tests). This procedure was performed for all stored solutions. Afterwards, solutions were sterilized in an autoclave for 20 min., at 120 °C. Sterile solutions were left to stand at room temperature for, at least, one day, and then 0.625 µL of vitamin concentrated stock were added to the falcons under aseptic conditions (using flame).

Table 5 | Stock solutions used in MBL medium preparation.

Stock solutions		
1	Calcium chloride dihydrate	$CaCl_2 \bullet 2H_2O$
2	Magnesium sufate heptahydrate	$MgSO_4 \bullet 7H_2O$
3	Sodium hydrogen carbonate	$NaHCO_3$
4	Dipotassium hydrogenphosphate	K_2HPO_4
5	Sodium nitrate	$NaNO_3$
6	Sodium metasilicate nonahydrate	$NaSiO_3 \bullet 9H_2O$
7	Ethylenediaminetetraacetic acid,	$Na_2EDTA \bullet 2H_2O$
8	Iron (III) chloride hexahydrate	$FeCl_3 \bullet 6H_2O$
9	Copper sulfate pentahydrate	$CuSO_4 \bullet 5H_2O$
10	Zinc sulfate heptahydrate	$ZnSO_4 \bullet 7H_2O$
11	Cobaltous dichloride hexahydrate	$CoCl_2 \bullet 6H_2O$
12	Manganesium dichloride dihydrate	$MnCl_2 \bullet 2H_2O$
13	Sodium molybdate dihydrate	$Na_2MoO_4 \bullet 2H_2O$
14	TRIS	

2.1.3.2. Growth inhibition testing

The determination of the effects of the remediated waters on the microalgae growth for 72 h of exposure followed the OECD guideline 201 (OECD, 2011), adapted to 24-well microplates (Geis *et al.*, 2000).

Tests started only when cultures were in the exponential growth phase. MBL medium (stored at 4 °C) was used to dilute the inoculum, so the cell density could be 5 × 10^4 cells/mL at the beginning of every test (Figure 11).

Regarding the experimental design, wells on the edges of the microplate ("blue" wells: 2; Figures 12 and 13) were filled with 1 mL of milli-Q water to minimize the occurrence of evaporation during the test period. The negative control corresponded to 1 mL of MBL and microalgae ("orange" wells: 2; Figures 12 and 13). The experimental treatments ("green" wells: 3; Figures 12 and 13) corresponded to the remediated water in different concentrations of both calcined and non-calcined Zn-Al LDH. The adjusted microalgae cell density in every well correspond to 5 × 10^4 cells/mL.

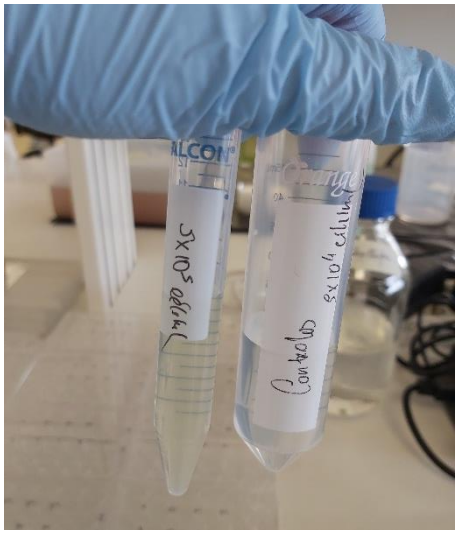

Figure 11 | Control solution for the growth inhibition tests with *R. subcapitata*.

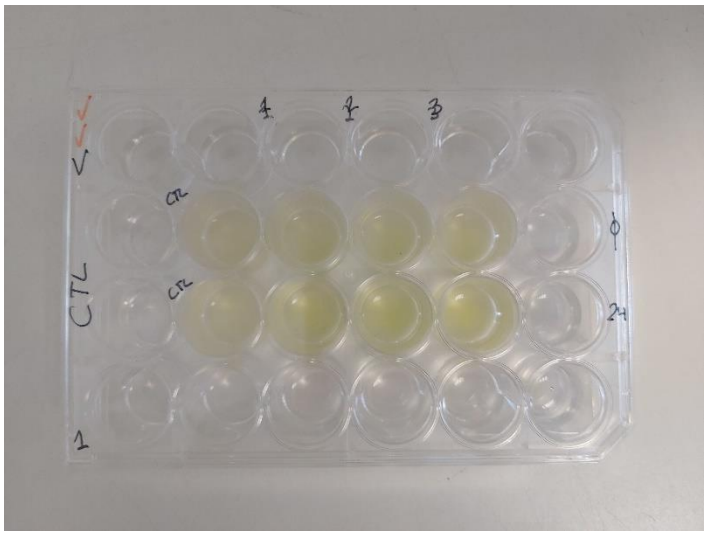

Figure 12 | Growth inhibition test with *R. subcapitata*.

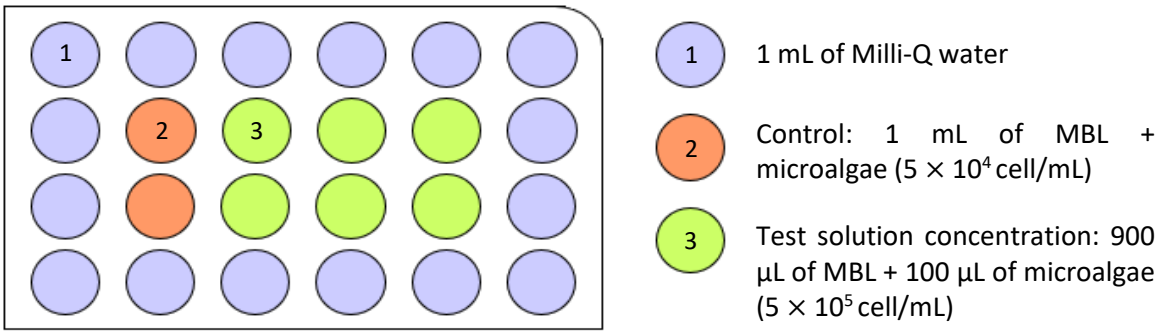

Figure 13 | Schematic representation of a 24-well microplate. Blue wells were filled with mili-Q water to minimize the evaporation rate during the test realization. Orange wells were filled with the control (MBL and microalgae). Green wells were filled with the test solutions.

Growth inhibition tests took place in a chamber (Binder mod. B28), with controlled temperature 23 °C, continuous light (intensity between 4440 and 8880 lux) and constant shaking (350 rpm), in disposable and sterile microplates.

Optical density (440 nm) was measured daily using a microplate reader (Thermo Scientific, Multiskan Spectrum). Cell density was then estimated as follows:

$$Cell/mL = -17107.5 + ABS \times 7925350$$

where ABS corresponds to the optical density measured in each well.

2.1.3.3. Data analysis

Data normality and homoscedasticity were analyzed using the Shapiro-Wilks and Levene tests ($p < 0.05$). Significant differences between treatments and control were analyzed using a one-way analysis of variance (one-way ANOVA). Then, whenever significant differences were observed ($p < 00.5$), a Dunnett's multiple comparison test was carried out.

CHAPTER 3

RESULTS

3.1. Characterization of non-calcined and calcined Zn-Al LDH

3.1.1. XRD

In order to be able to determine if the phosphate was captured by Zn-Al LDH, XRD analyzes were performed for the two types of LDHs used before exposure to phosphate solutions: Zn-Al LDH with nitrates intercalated and calcined LDHs. The corresponding XRD patterns are depicted in Figure 14.

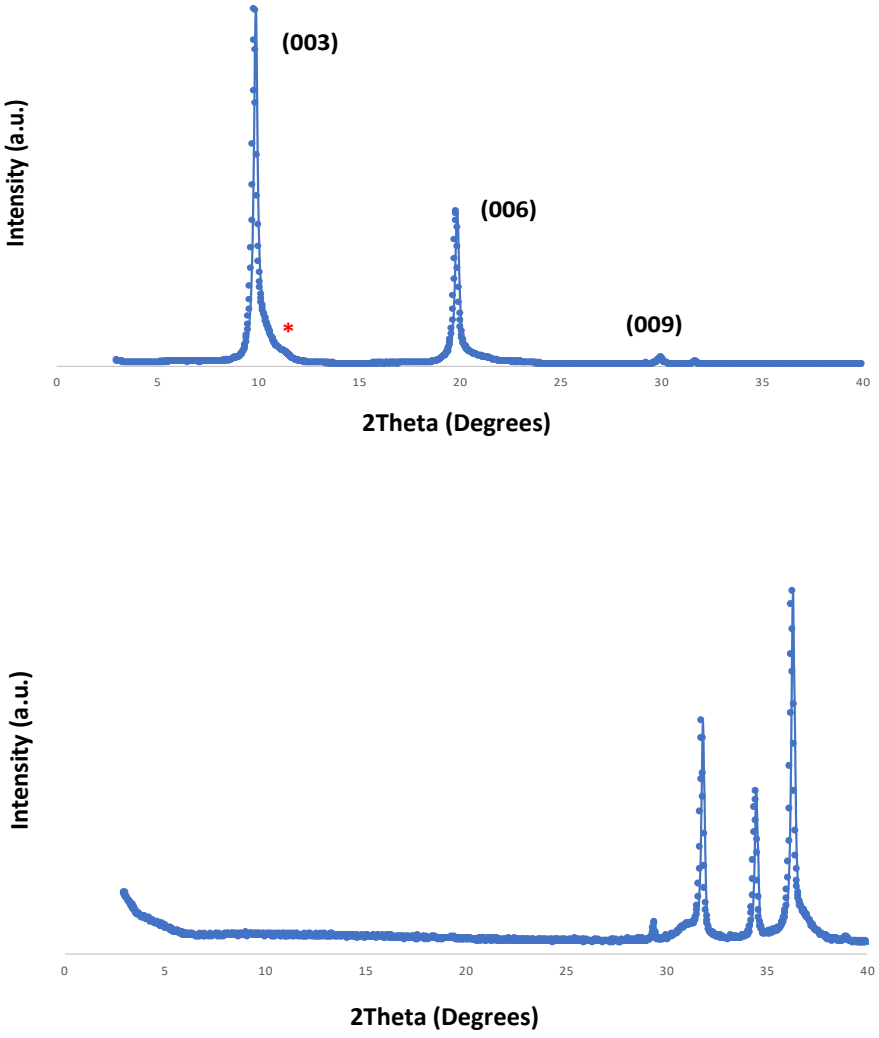

Figure 14 | XRD patterns of Zn-Al LDHs intercalated with nitrate as-received (above) and after calcination (below).

Zn-Al LDHs interlayered with nitrates show peaks at low 2Θ angles at 9.86°, 19.87° and 30.08° that correspond to reflection planes (003), (006) and (009). The position of these peaks is consistent with intercalation of nitrates (recall Table 4 in the

M&M section). In addition, it is possible to observe a secondary LDH phase around 11.65º (marked with an asterisk in Figure 14) possibly due to the presence of carbonates (Tedim *et al*., 2010). Moreover, upon thermal treatment (calcination) the LDH structure is destroyed and XRD peaks associated with LDHs at low 2theta angles is no longer observed. This is expected and consistent with data available in the literature (Abderrazek, Najoua and Srasra, 2016).

3.1.2. Ecotoxicological effects of LDHs on freshwater green microalgae

Ecotoxicological effects of both calcined and non-calcined LDHs in MBL were assessed prior the ecotoxicological evaluation of the treated waters.

The LDH exposure caused no significant effects on the microalgae growth at 5 and 50 mg of non-calcined Zn-AL LDH tests. However, exposure to high concentrations of LDHs, namely 500 mg of non-calcined Zn-AL LDH and both 250 and 2500 mg of calcined Zn-AL LDH caused significant growth inhibition on *R. subcapitata* (Figure 15).

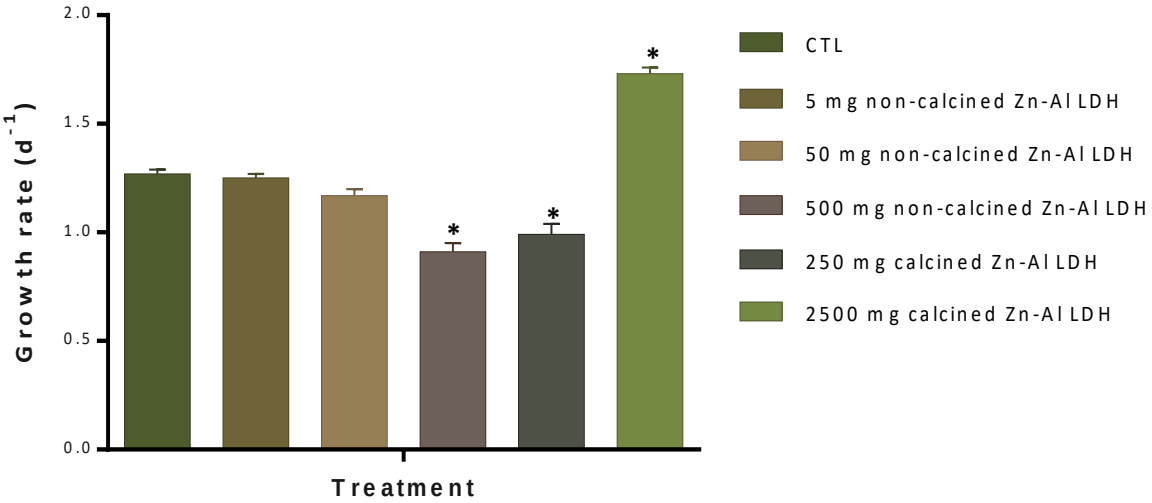

Figure 15 | Growth rate of *R. subcapitata* with different types and concentrations of Zn-Al LDHs tested.

3.2. Evaluation of the effectiveness of LDHs on the remediation of phosphates-rich waters

3.2.1. The “smart” anionic nanoclay Zn-Al LDH-NO_3 (non-calcined)

3.2.1.1. Remediation of a solution of 0.4 mg phosphates/L

3.2.1.1.1. Chemical assessment of remediated water

3.2.1.1.1.1. Phosphates uptake studies

In the control test, phosphates concentration remains, as expected, considerably constant, close to 0.4 mg PO_4^{3-}/L (Figure 16).

When 5 mg of Zn-Al LDH-NO_3 was added, a decrease of 0.09 mg/L in the concentration of phosphates was immediately noticed (up to 3 h). After 144 h, the quantity of phosphates present in the solution was 0.17 mg/L (Figure 17).

By adding 50 and 500 mg of Zn-Al LDH-NO_3, phosphates decreased much faster and in the end of both tests, the concentration was close to 0 mg PO_4^{3-}/L (Figure 18 and 19).

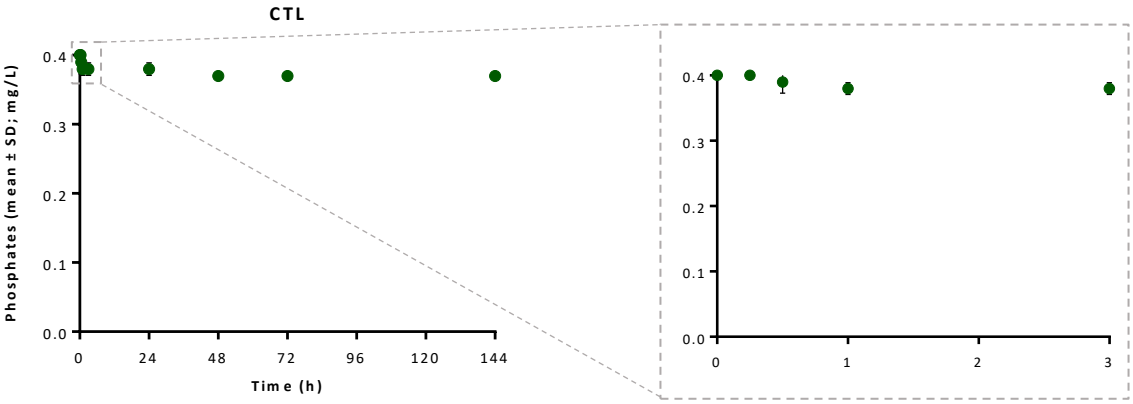

Figure 16 | Phosphates concentration during the control test with 0.4 mg PO_4^{3-}/L. of phosphates.

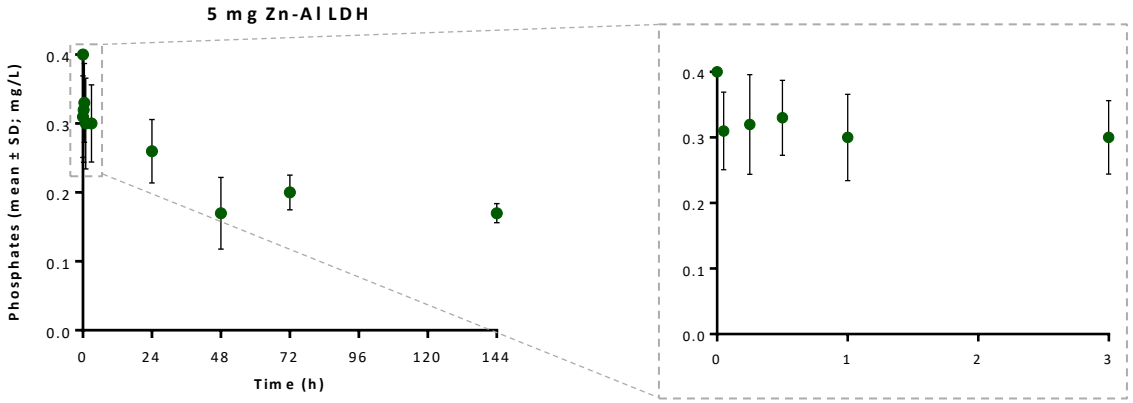

Figure 17 | Phosphates concentration during the test with 0.4 mg PO_4^{3-}/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

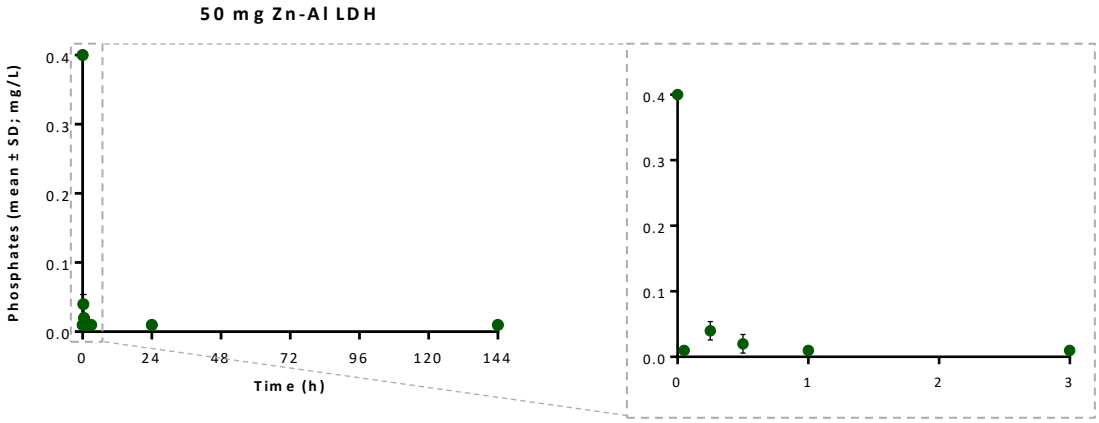

Figure 18 | Phosphates concentration during the test with 0.4 mg PO_4^{3-}/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

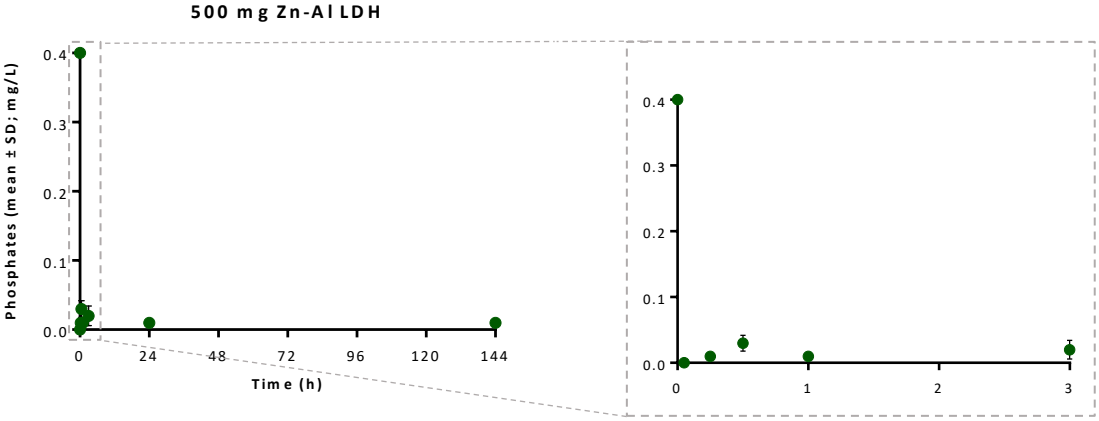

Figure 19 | Phosphates concentration during the test with 0.4 mg PO_4^{3-}/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.1.1.2. Phosphates removal efficacy

In the end of the test with 5 mg Zn-Al LDH-NO_3/L, the phosphate removal rate was approximately 58% (Figure 20). However, when 50 and 500 mg of Zn-Al LDH-NO_3 were added, the removal rate was very high (between 97 and 99%) even in the first minutes, remaining stable until 144h (Figures 21 and 22).

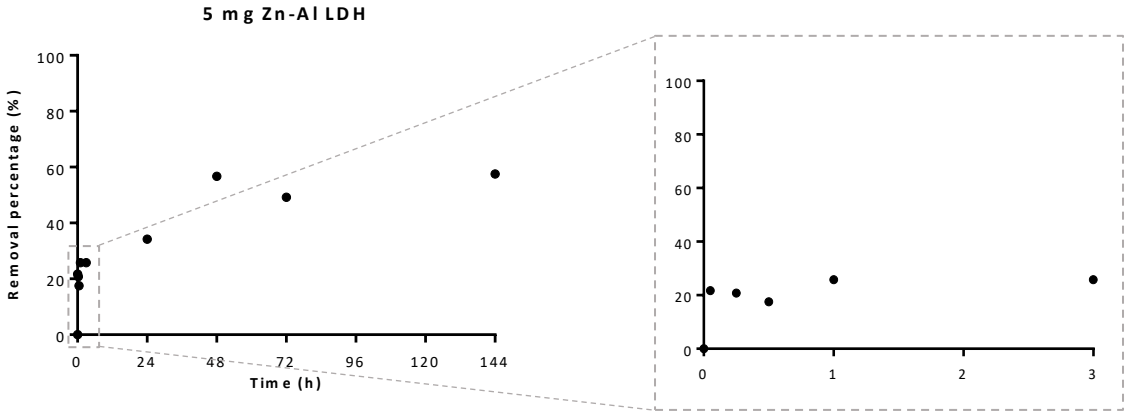

Figure 20 | Percentage of phosphates removed during the test with 0.4 mg PO_4^{3-}/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

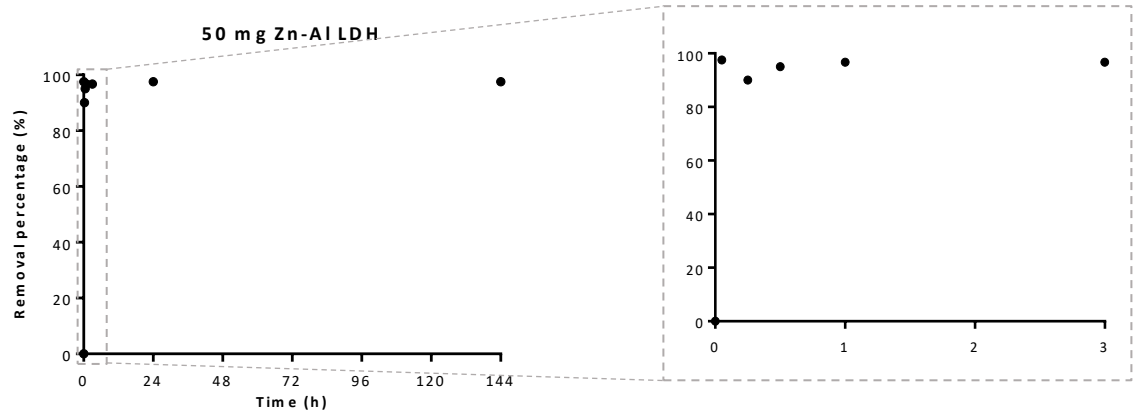

Figure 21 | Percentage of phosphates removed during the test with 0.4 mg PO_4^{3-}/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

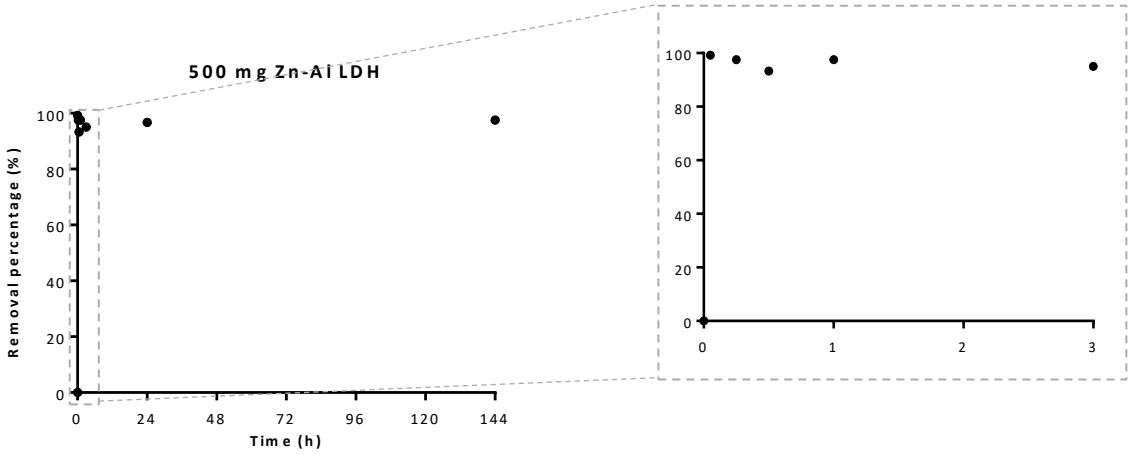

Figure 22 | Percentage of phosphates removed during the test with 0.4 mg PO_4^{3-}/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.1.1.3. Nitrates release studies

In the control test, nitrates concentration remained constant in time (0.0 mg/L; Figure 23).

When 5 mg of Zn-Al LDH-NO_3 was added to the solution, an increase of approximately 0.13 mg/L of nitrates was detected, and kept stable until the end of the test (Figure 24).

The addition of 50 mg/L of Zn-Al LDH-NO_3 caused an increase of 1.00 mg nitrates/L. After 144 hours, the nitrates concentration was 1.60 mg/L (Figure 25).

When 500 mg of Zn-Al LDH-NO_3 was added, an immediate increase of nitrates was detected (3.30 mg/L), increasing up to 14.83 mg/L at 144 hours (Figure 26).

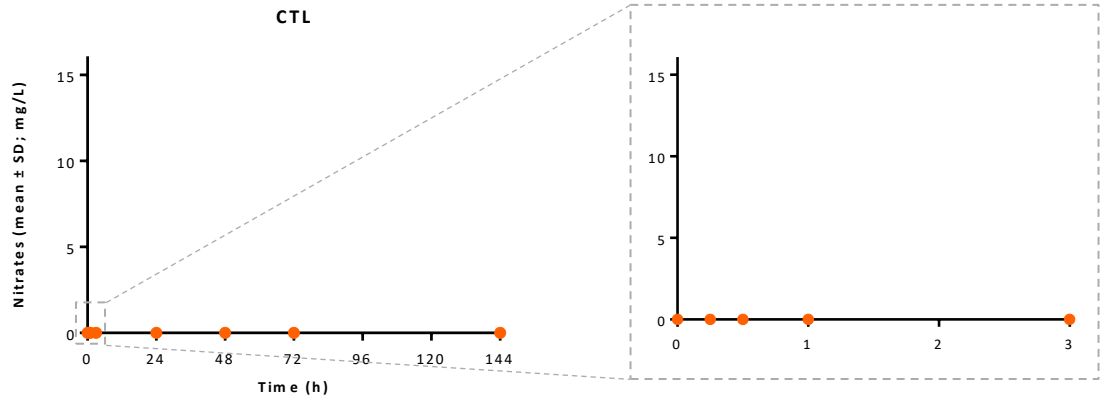

Figure 23 | Nitrates concentration during the control test with 0.4 mg/L of phosphates.

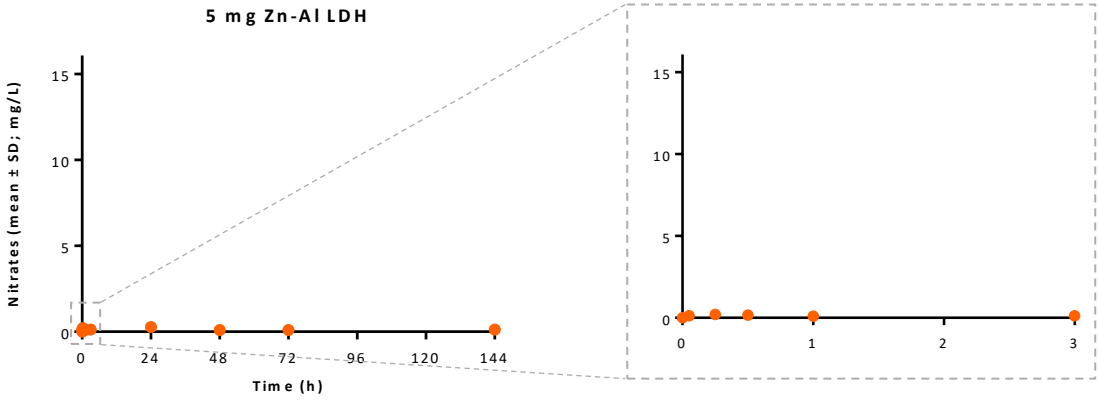

Figure 24 | Nitrates concentration during the test with 0.4 mg/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

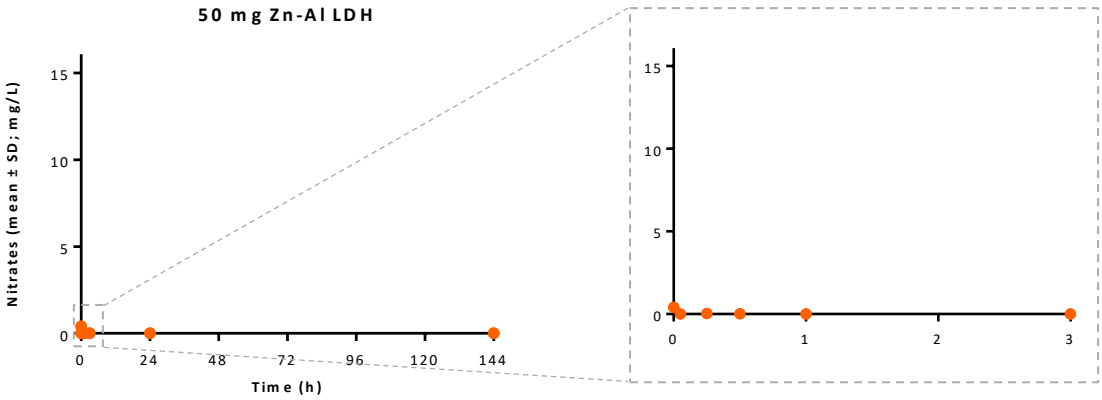

Figure 25 | Nitrates concentration during the test with 0.4 mg/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

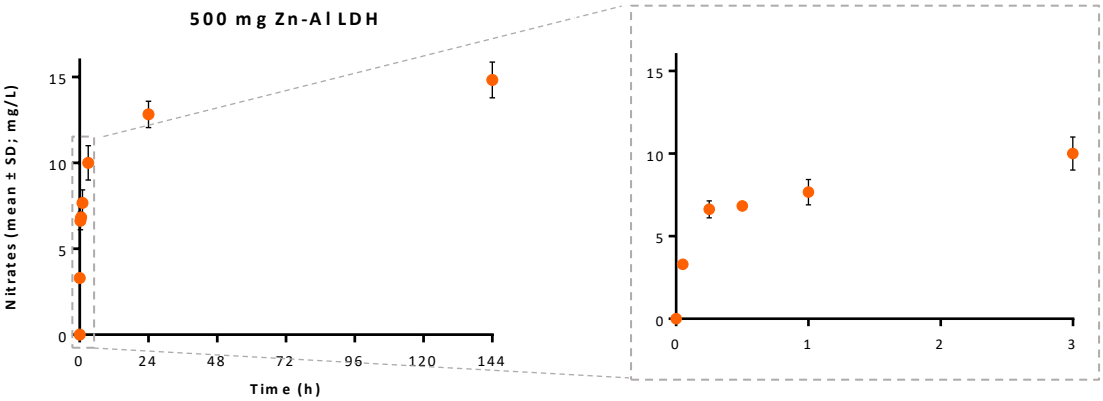

Figure 26 | Nitrates concentration during the test with 0.4 mg/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.1.2. Ecotoxicological assessment of remediated water

Non-treated solution (just 0.4 mg P/L), along the different timepoints, caused no significant effects on the microalgae growth. Remediated water with 5 mg Zn-Al LDH-NO_3 along the different timepoints, caused no significant effects on the microalgae growth. Samples treated with 50 and 500 mg/L of Zn-Al LDH-NO_3 caused significant growth inhibition effects on *R. subcapitata* except the water samples treated with 50 mg/L at time 0 which caused an increase on the microalgae growth (Figure 27).

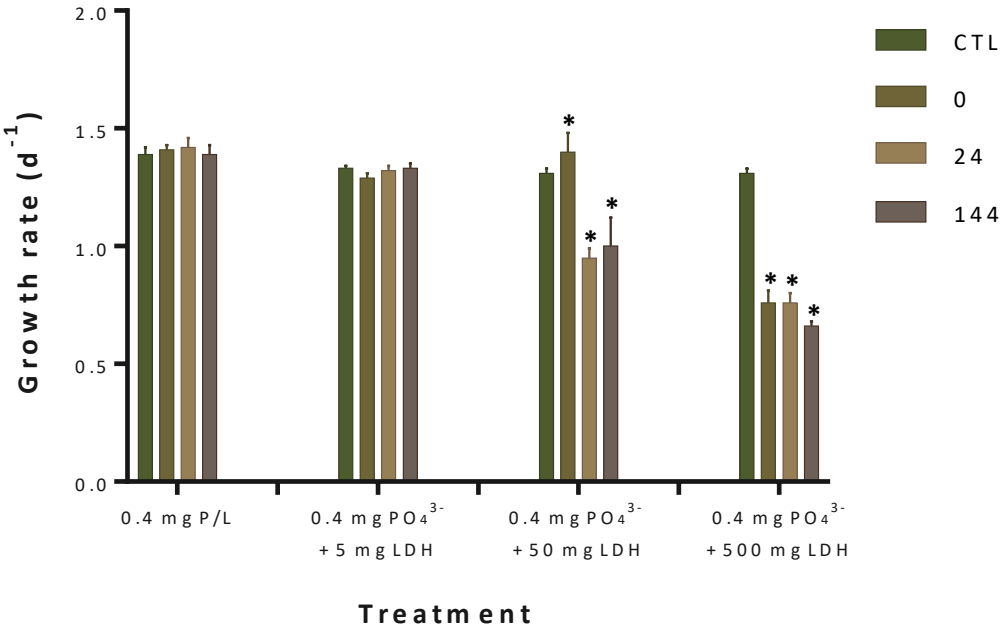

Figure 27 | Effects on the microalgae *Raphidocelis subcapitata* growth rate upon 72 h of exposure to phosphates-rich water (0.4 mg PO_4^{3-}/L) and the same solution remediated with 5, 50 and 500 mg/L of Zn-Al layered double hydroxides (LDH; stabilized with nitrates) for a period of one week. CTL – only culture media; 0, 24 and 144 – sample collected at time 0, 24 or 144 h, respectively, from the P-rich solution ("0.4 mg PO_4^{3-}/L") or from P-rich solution remediated with LDH ("PO_4^{3-}/L + 5, 50 or 500 mg LDH").

3.2.1.2. Remediation of a solution of 10 mg phosphates/L

3.2.1.2.1. Chemical assessment of remediated water

3.2.1.2.1.1. Phosphates uptake studies

In the control test, phosphates concentration remains, as expected, considerably constant, close to 10 mg PO_4^{3-}/L (Figure 28).

When 5 mg of Zn-Al LDH-NO_3 was added, a decrease in the concentration of phosphates was noticed and after 144 h, the quantity of phosphates present in the solution was 8.20 mg PO_4^{3-}/L (Figure 29).

When 50 mg of Zn-Al LDH-NO_3 was added, decrease of 3.63 mg PO_4^{3-}/L in the concentration of phosphates was immediately noticed (up to 3 h). After 144 h, the quantity of phosphates present in the solution was 5.37 mg PO_4^{3-}/L (Figure 30).

By adding 500 mg of Zn-Al LDH-NO_3, phosphates decreased much faster and in the end of the test, the concentration was close to 0 mg PO_4^{3-}/L (Figure 31).

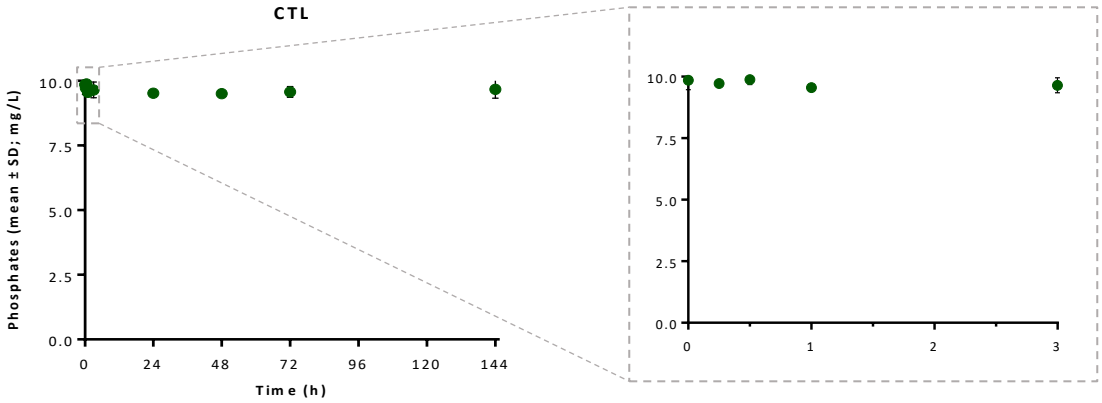

Figure 28 | Phosphates concentration during the control test with 10 mg PO_4^{3-}/L of phosphates.

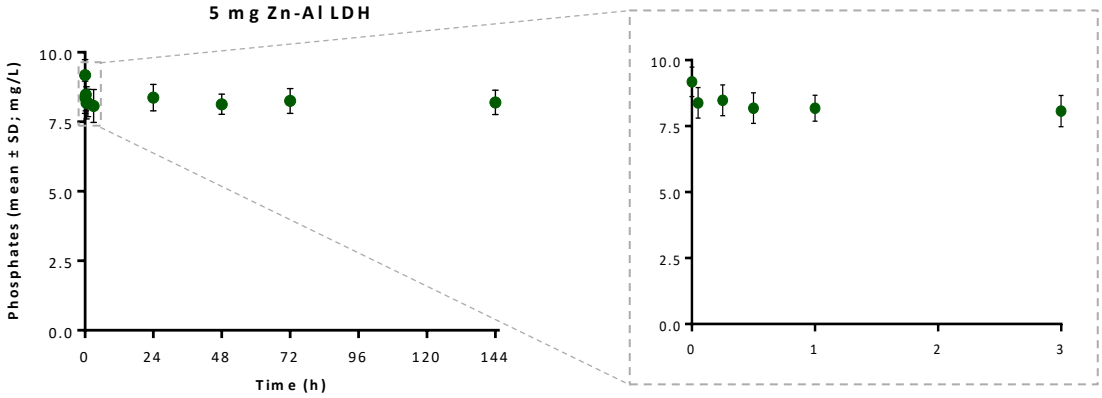

Figure 29 | Phosphates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

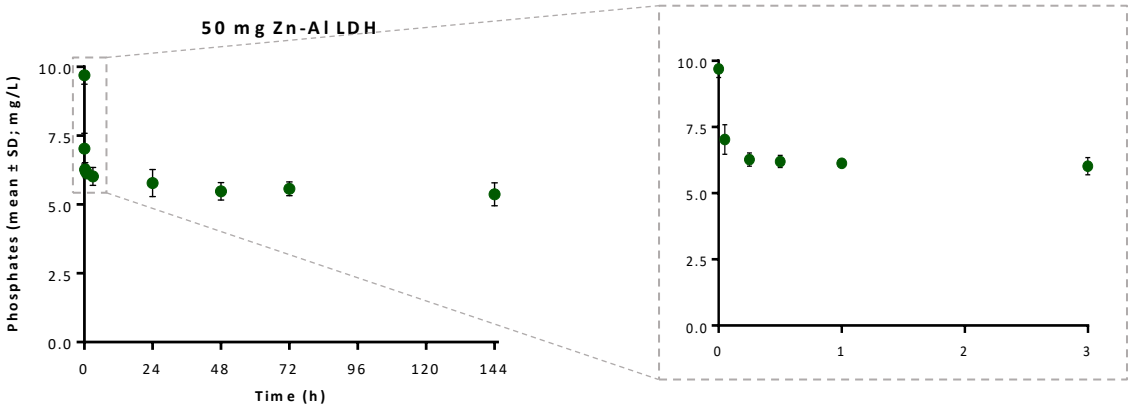

Figure 30 | Phosphates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

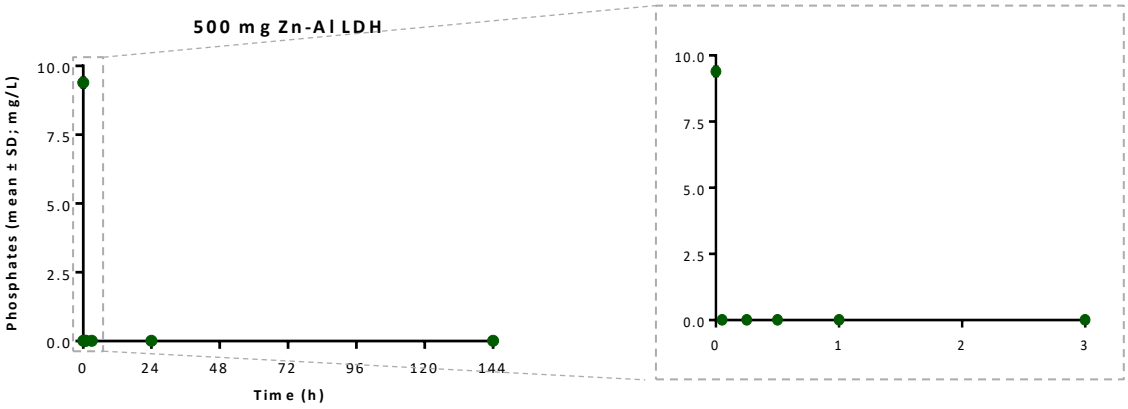

Figure 31 | Phosphates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.2.1.2. Phosphates removal efficacy

In the end of the test with 5 mg Zn-Al LDH-NO_3/L, the phosphate removal rate was approximately 11% (Figure 32). In the end of the test with 50 mg Zn-Al LDH-NO_3/L, the phosphate removal rate was approximately 45% (Figure 33). However, when 500 mg of Zn-Al LDH-NO_3 was added, the removal rate was very high (almost 100%) even in the first minutes, remaining stable until 144h (Figure 34).

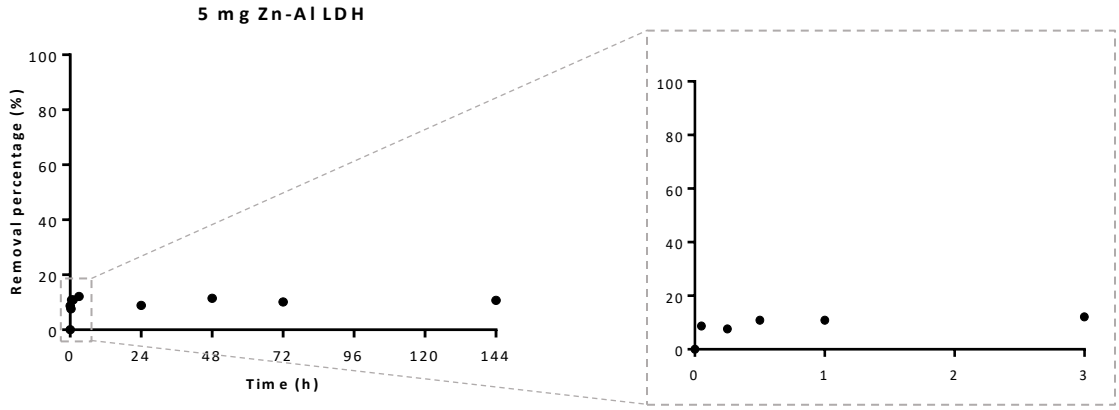

Figure 32 | Percentage of phosphates removed during the test with 10 mg PO_4^{3-}/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

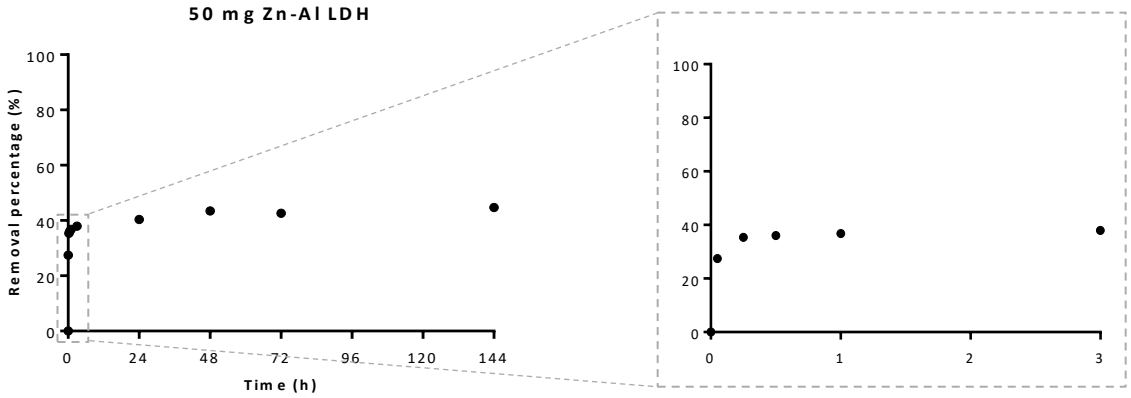

Figure 33 | Percentage of phosphates removed during the test with 10 mg PO_4^{3-}/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

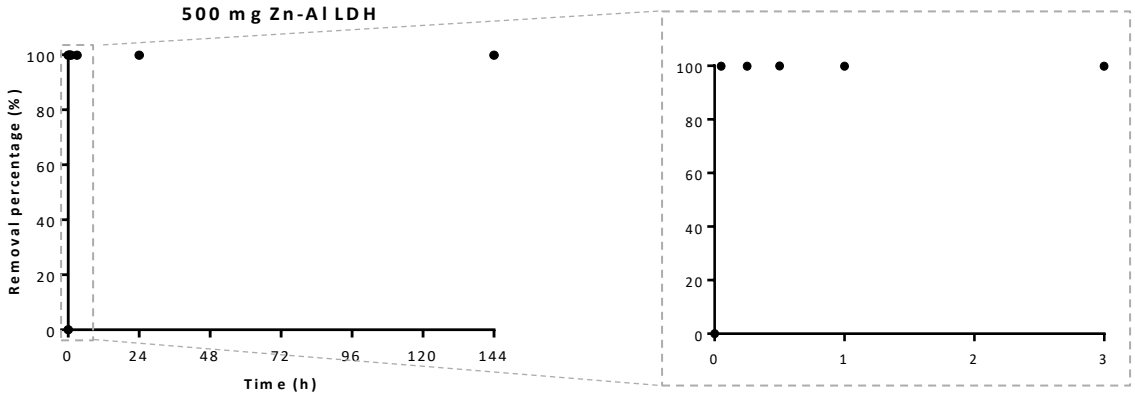

Figure 34 | Percentage of phosphates removed during the test with 10 mg PO_4^{3-}/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.2.1.3. Nitrates release studies

In the control test, nitrates concentration remained constant in time (0.0 mg/L; Figure 35).

When 5 mg of Zn-Al LDH-NO_3 was added to the solution, an increase of approximately 0.1 mg/L of nitrates was detected, and kept stable until the end of the test (Figure 36).

The addition of 50 mg/L of Zn-Al LDH-NO_3 caused an increase of 2.0 mg nitrates/L and kept stable until the end of the test (Figure 37).

When 500 mg of Zn-Al LDH-NO_3 was added, an immediate increase of nitrates was detected (6 mg/L), increasing up to 16.8 mg/L at 144 hours (Figure 38).

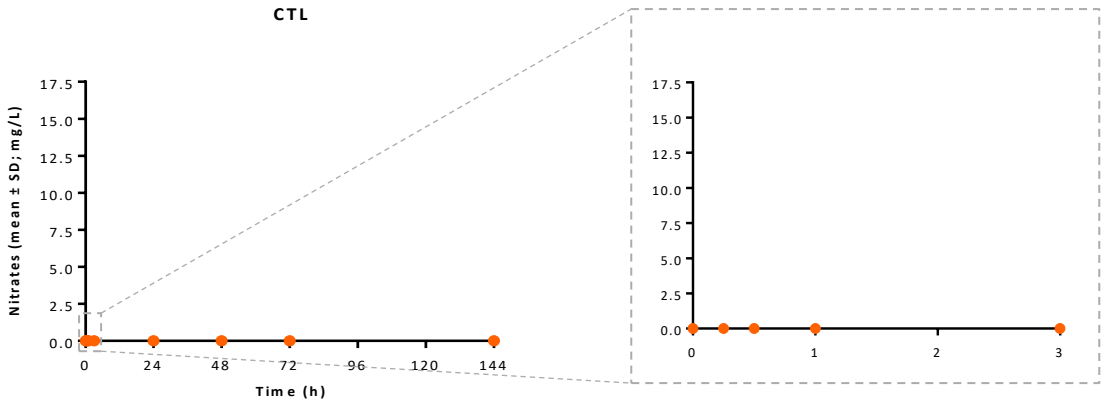

Figure 35 | Nitrates concentration during the control test with 10 mg/L of phosphates.

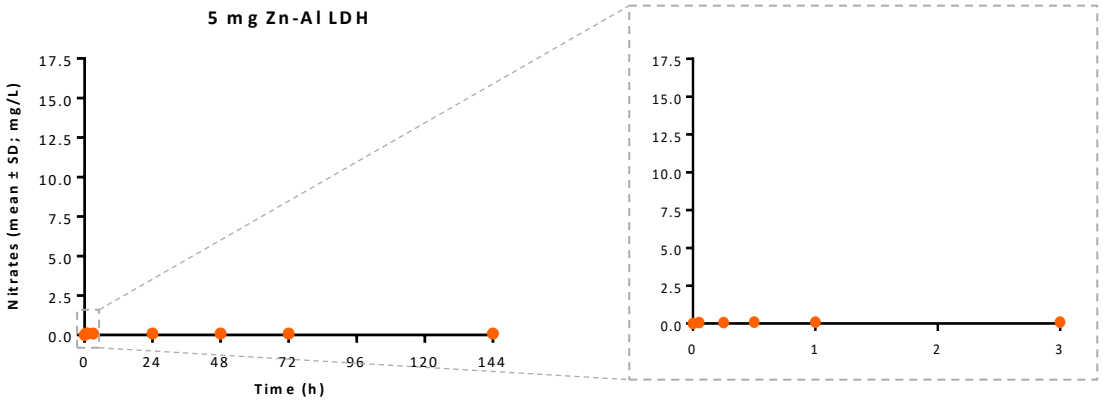

Figure 36 | Nitrates concentration during the test with 10 mg/L of phosphates and 5 mg of non-calcined Zn-Al LDH-NO_3.

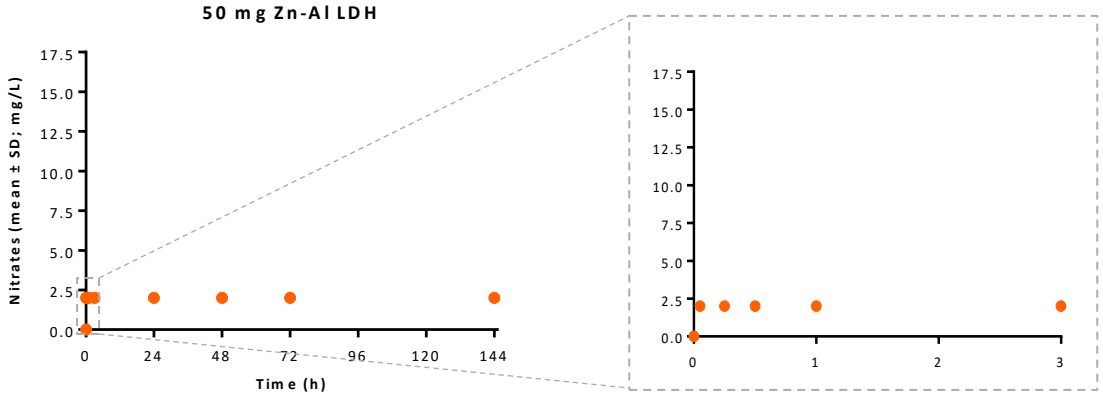

Figure 37 | Nitrates concentration during the test with 10 mg/L of phosphates and 50 mg of non-calcined Zn-Al LDH-NO_3.

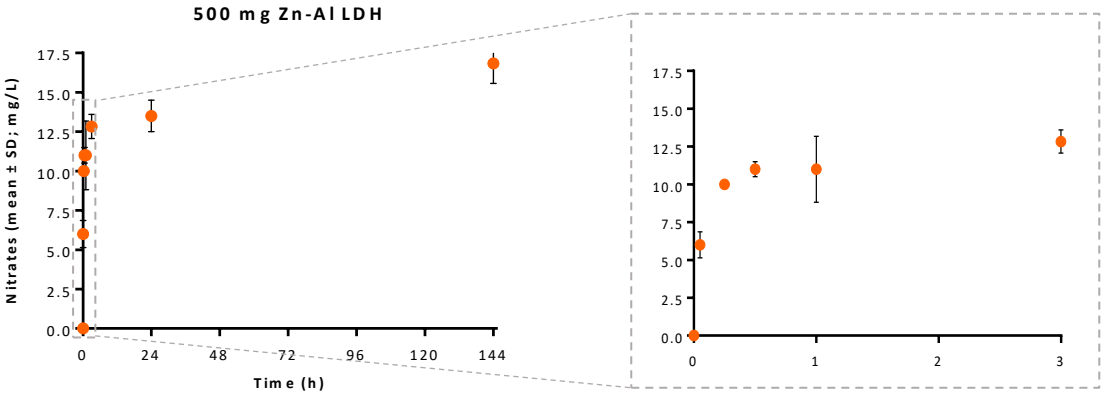

Figure 38 | Nitrates concentration during the test with 10 mg/L of phosphates and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.2.2. <u>Ecotoxicological assessment of remediated water</u>

Non-treated solution (just 10 mg P/L), along the different timepoints, caused no significant effects on the microalgae growth. Remediated water with 5 mg Zn-Al LDH-NO_3 along the different timepoints, caused no significant effects on the microalgae growth. Samples treated with 50 mg/L of Zn-Al LDH-NO_3 along the different timepoints, caused no significant effects on the microalgae growth, except at time 0 which caused an increase on the microalgae growth. Samples treated with 500 mg/L of Zn-Al LDH-NO_3 caused significant growth inhibition effects on *R. subcapitata* (Figure 39).

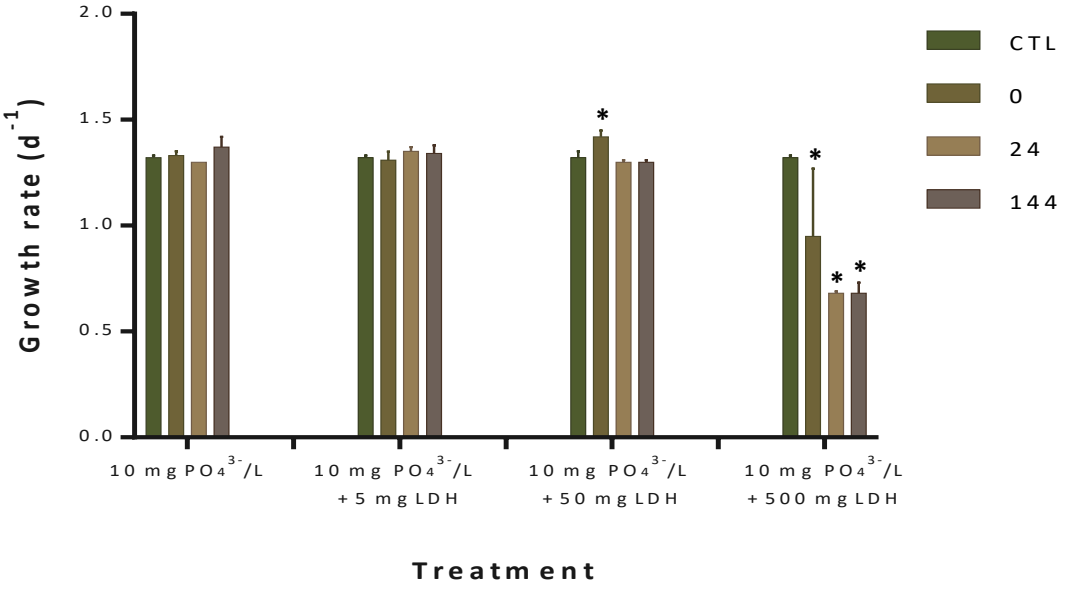

Figure 39 | Effects on the microalgae *Raphidocelis subcapitata* growth rate upon 72 h of exposure to phosphates-rich water (10 mg PO_4^{3-}/L) and the same solution remediated with 5, 50 and 500 mg/L of Zn-Al layered double hydroxides (LDH; stabilized with nitrates) for a period of one week. CTL – only culture media; 0, 24 and 144 – sample collected at time 0, 24 or 144 h, respectively, from the P-rich solution ("10 mg PO_4^{3-}/L") or from P-rich solution remediated with LDH ("10 mg PO_4^{3-} + 5, 50 or 500 mg LDH").

3.2.1.2.3. XRD

For the 10 mg PO_4^{3-}/L of phosphate tests, XRD analysis were performed only with the non-calcined Zn-Al LDH-NO_3 remained from the 500 mg assay (Figure 40). In all the replicates studied the results were similar, so only the XRD corresponding to one of them is presented.

The changes which occurred on the LDHs upon exposure to the phosphate solution are consistent with a displacement of reflections associated with LDHs towards higher 2theta angles. Based on Table 5, the positions of the peaks are consistent with the formation of LDHs intercalated with hydroxides (HO^-) or carbonates (CO_3^{2-}). It is worth to mention that in the first peak there is an overlap with another peak (around 10°). This may be due to incomplete replacement of nitrates with carbonates or hydroxides. In addition, there one additional peak around 18° that has a different shape from the others (hence corresponding to a different phase) but not ascribed to LDH

phases. It may result from some sort of product formation between LDHs and the phosphate salt used.

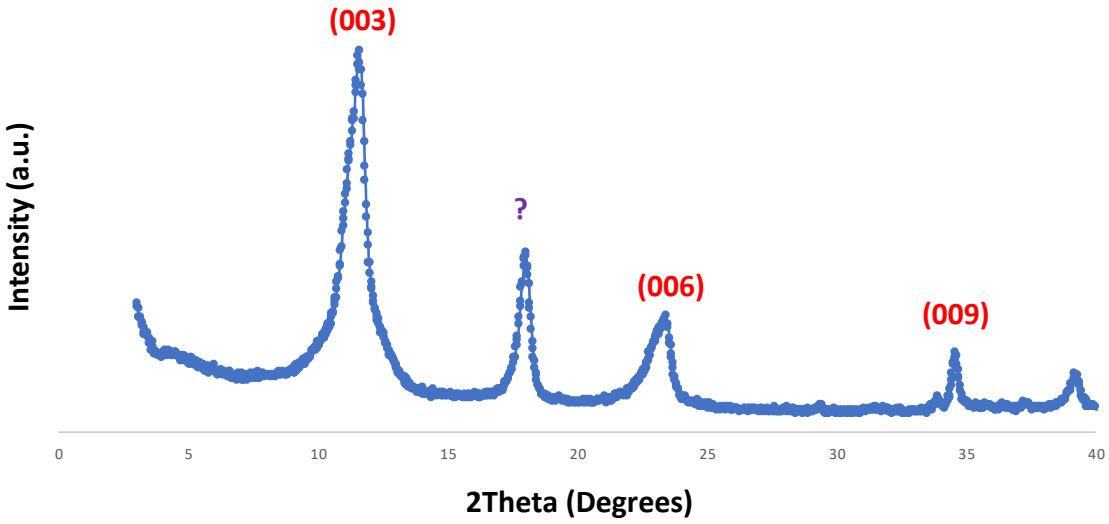

Figure 40 | XRD pattern of Zn-Al LDHs-NO_3 after exposure to 10 mg/L phosphate solution.

3.2.1.3. Remediation of a solution of lake water

3.2.1.3.1. Chemical assessment of remediated water

3.2.1.3.1.1. Phosphates uptake studies

In the control test, phosphates concentration remains, as expected, considerably constant, close to 7.65 mg PO_4^{3-}/L (Figure 41).

When 50 mg of Zn-Al LDH-NO_3 was added, a decrease of 2.00 mg PO_4^{3-}/L in the concentration of phosphates was immediately noticed (up to 3 h). After 144 h, the quantity of phosphates present in the solution was 4.85 mg PO_4^{3-}/L (Figure 42).

By adding 500 mg of Zn-Al LDH-NO_3, phosphates decreased much faster and in the end the test, the concentration was close to 0.50 mg PO_4^{3-}/L (Figure 43).

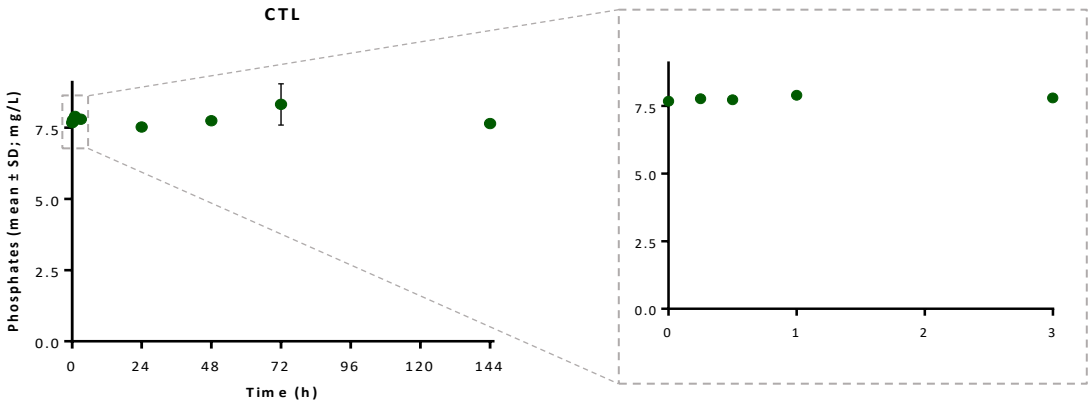

Figure 41 | Phosphates concentration during the control test with lake water.

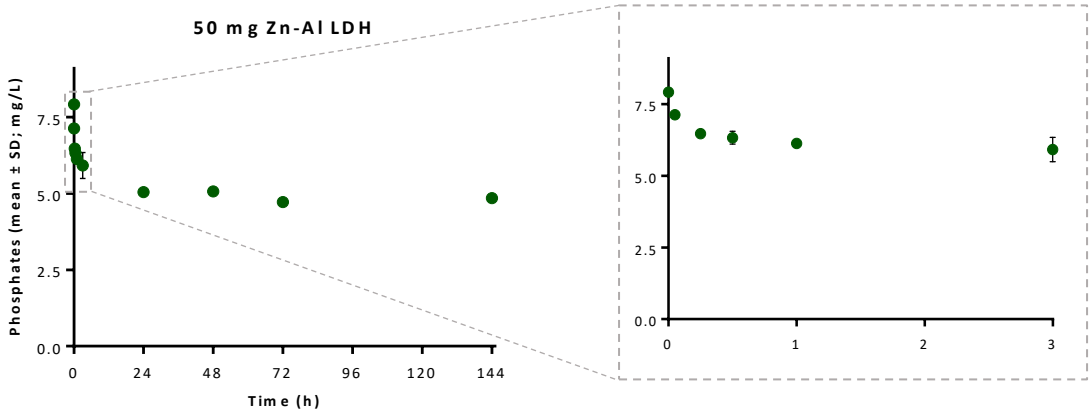

Figure 42 | Phosphates concentration during the test with lake water and 50 mg of non-calcined Zn-Al LDH-NO_3.

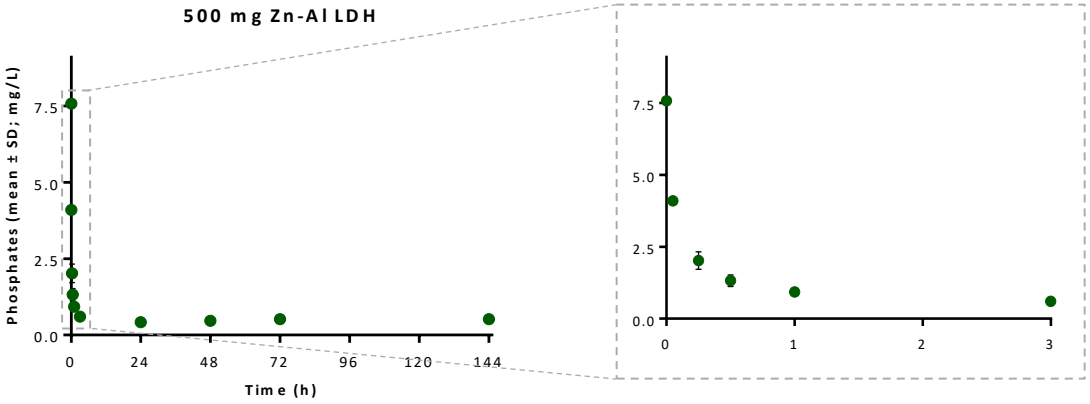

Figure 43 | Phosphates concentration during the test with lake water and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.3.1.2. Phosphates removal efficacy

In the end of the test with 50 mg Zn-Al LDH-NO_3/L, the phosphate removal rate was approximately 39% (Figure 44). However, when 500 mg of Zn-Al LDH-NO_3 was added, the removal rate was very high (almost 93%) (Figure 45).

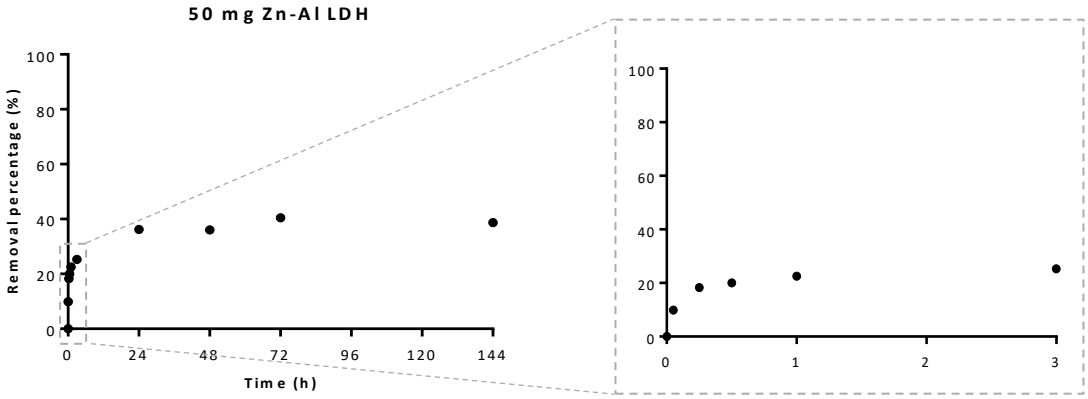

Figure 44 | Percentage of phosphates removed during the test with lake water and 50 mg of non-calcined Zn-Al LDH-NO_3.

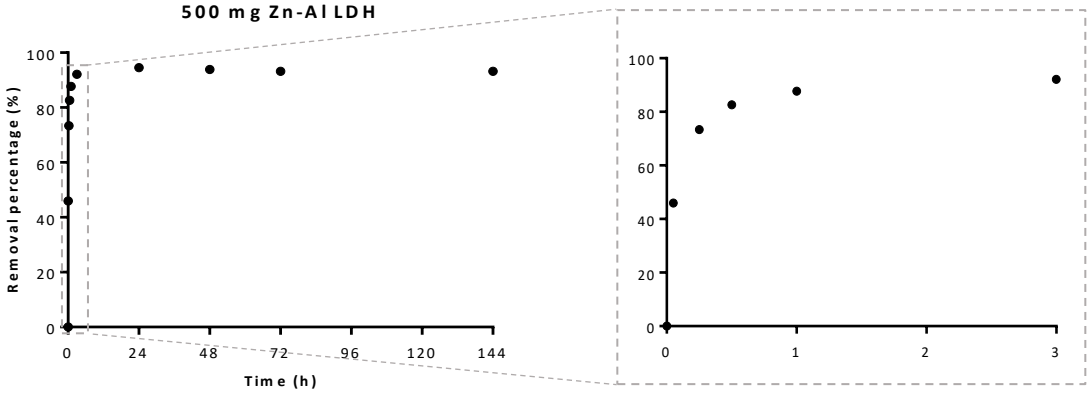

Figure 45 | Percentage of phosphates removed during the test with lake water and 50 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.3.1.3. Nitrates release studies

In the control test, nitrates concentration remained constant in time (0.0 mg/L; Figure 46).

The addition of 50 mg/L of Zn-Al LDH-NO_3 caused an increase of 2.2 mg nitrates/L increasing up to 2.8 mg/L at 144 hours (Figure 47).

When 500 mg of Zn-Al LDH-NO_3 was added, an immediate increase of nitrates was detected (8.5 mg/L), increasing up to 14.3 mg/L at 144 hours (Figure 48).

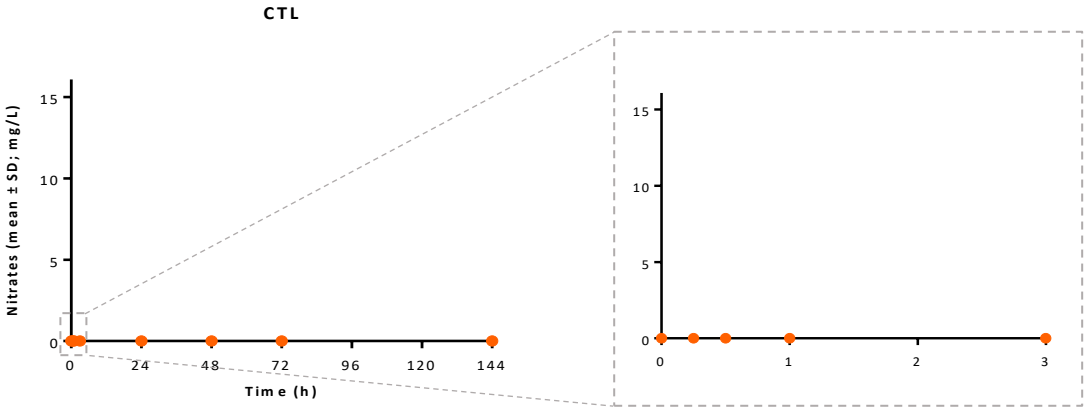

Figure 46 | Nitrates concentration during the control test with lake water.

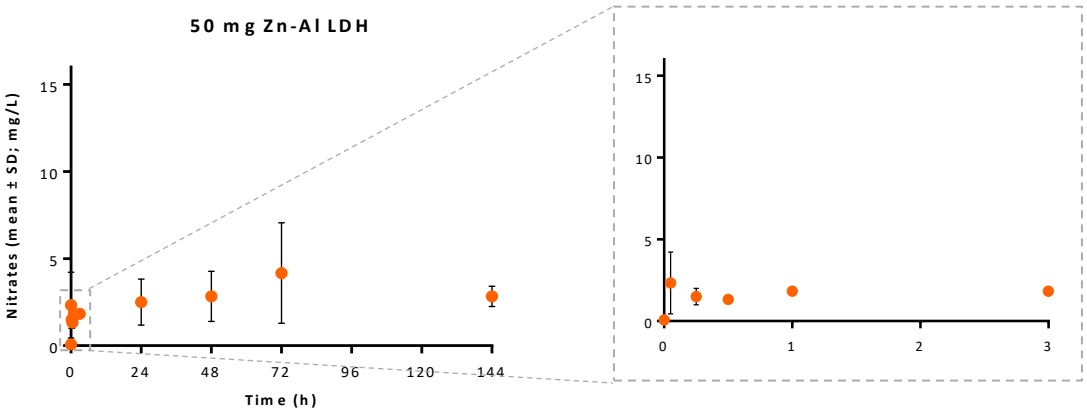

Figure 47 | Nitrates concentration during the test with lake water and 50 mg of non-calcined Zn-Al LDH-NO_3.

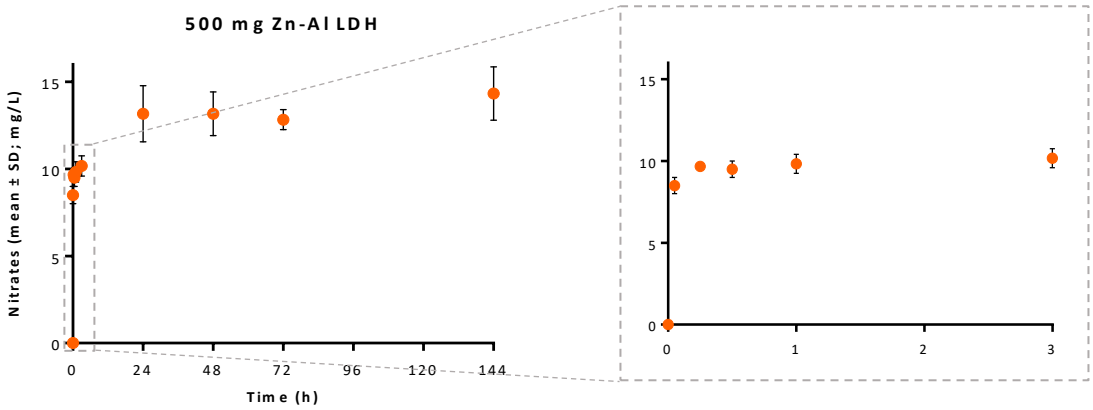

Figure 48 | Nitrates concentration during the test with lake water and 500 mg of non-calcined Zn-Al LDH-NO_3.

3.2.1.3.2. Ecotoxicological assessment of remediated water

Non-treated solution (just lake water), along the different timepoints, caused significant effects on the microalgae growth. Samples treated with 50 and 500 mg/L of Zn-Al LDH-NO_3 caused an increase on the microalgae growth (Figure 49).

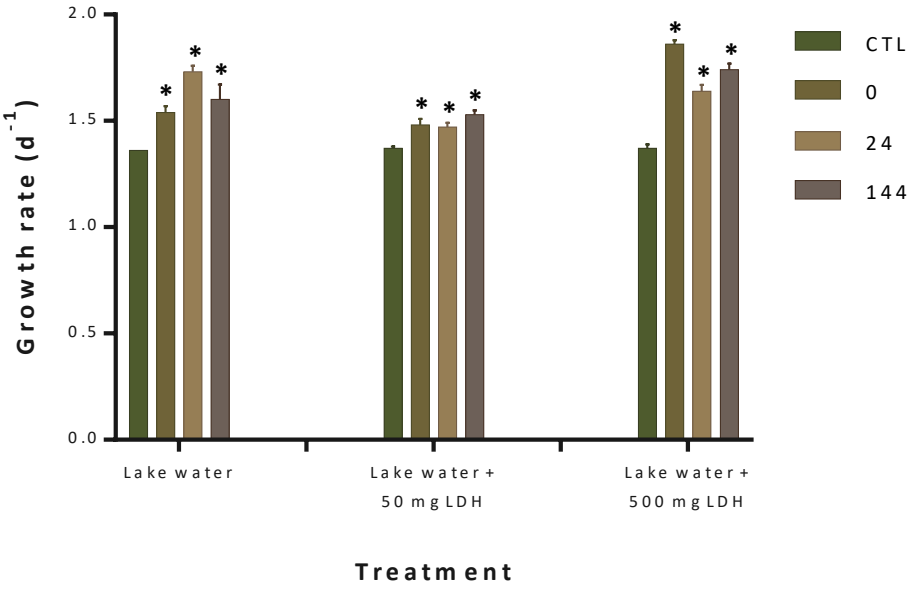

Figure 49 | Effects on the microalgae *Raphidocelis subcapitata* growth rate upon 72 h of exposure to phosphates-rich water (lake water) and the same solution remediated with 50 and 500 mg/L of Zn-Al layered double hydroxides (LDH; stabilized with nitrates) for a period of one week. CTL – only culture media; 0, 24 and 144 – sample collected at time 0, 24 or 144 h, respectively, from the P-rich solution ("Lake water") or from P-rich solution remediated with LDH ("Lake water + 50 or 500 mg LDH").

3.2.1.3.3. XRD

For the lake water tests, XRD analysis were performed only for the non-calcined Zn-Al LDH-NO_3 remained from the 500 mg assay. In all the replicates studied the results were similar, so only the XRD corresponding to one of them is presented (Figure 50). The results are similar to those carried out with the 10 mg/L phosphate solution: the new LDH phase formed is consistent with intercalation of hydroxides or carbonates. However, in this case the exchange with nitrates seems to have occurred in a larger extent as the shoulder around 10º is less marked. However, to be fully sure one would have to perform quantitative analysis of XRD, which was beyond the scope of the present thesis. In addition, the peaks are rather asymmetric. This can be better visualized in the (006) peak, with a shoulder around 22.5º, which may be due to the existence of secondary phase of LDHs intercalated with chlorides (the same asymmetry is seen in the (003) peak at 11.3º).

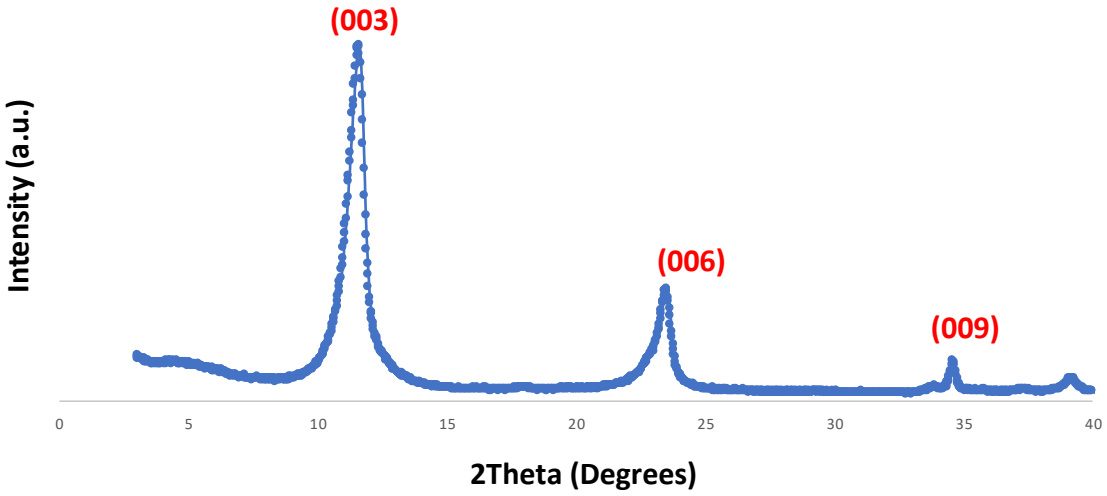

Figure 50 | XRD pattern of Zn-Al LDHs after exposure to the lake water.

3.2.2. Adsorption isotherms

The Langmuir and Freundlich isotherms were used to describe the adsorption behavior of the Zn-Al LDH. The experimental data used were obtained from the release profiles presented in previous sections. Equilibrium concentrations were assumed to be

reached after 144 hours, when the concentration of phosphate in solution remained unchanged. According to the R^2 values, the Freundlich model presented better values compared to Langmuir model (Table 6). This implies that a model based on a simple monolayer adsorption (Gimbert *et al*., 2008) of phosphates do not explain the adsorption of phosphates in LDHs. On the other hand, Freundlich adsorption isotherm, which is an empirical model for adsorption on heterogeneous surfaces, describing non-ideal reversible and multilayer energetic surface heterogeneity, seems to be a more suitable model (Chen, 2015).

Table 6 | Isothermal parameters of the composite Zn-Al LDH adjusted by the Langmuir and Freundlich models.

Langmuir model	
K_L (L/mg)	2.81 x 10^{-3}
$\frac{1}{q_m}$ (kg/mg)	72.173
R^2	0.2392

Freundlich model	
K_F	2.24 x 10^{-5}
$\frac{1}{n}$	2.3317
R^2	0.8307

3.2.3. Reference nanomaterial: calcined Zn-Al LDH

3.2.3.1. Remediation of a solution of 10 mg phosphates/L

3.2.3.1.1. Chemical assessment of remediated water

3.2.3.1.1.1. Phosphates uptake studies

When 250 mg of Zn-Al LDH was added, a decrease of 2.66 mg PO_4^{3-}/L in the concentration of phosphates was immediately noticed (up to 3 h). After 144 h, the quantity of phosphates present in the solution was 0.45 mg PO_4^{3-}/L (Figure 51).

By adding 2500 mg of Zn-Al LDH, phosphates decreased faster and in the end of the test, the concentration was close to 0 mg PO_4^{3-}/L (Figure 52).

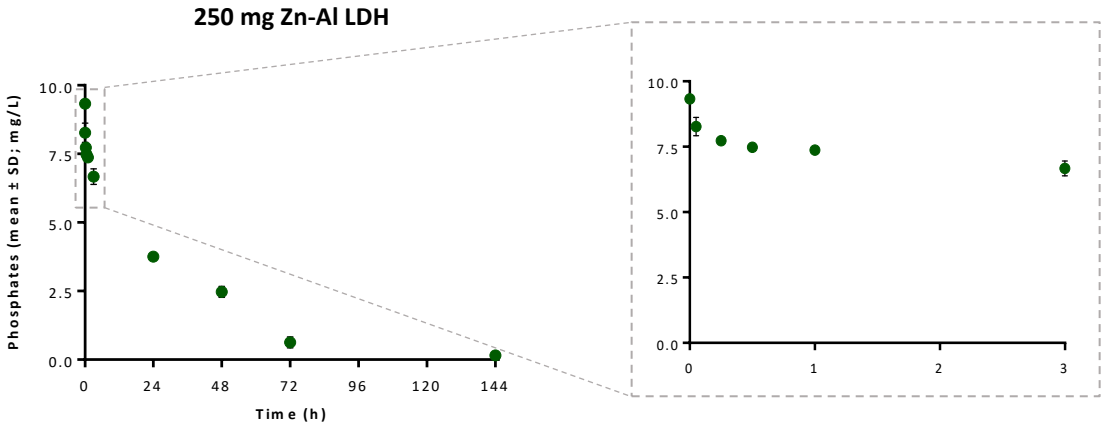

Figure 51 | Phosphates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 250 mg of calcined Zn-Al LDH.

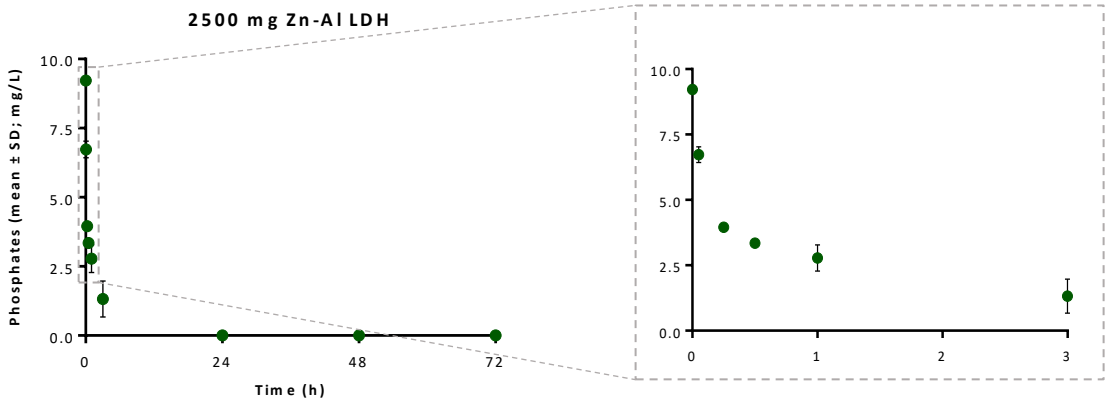

Figure 52 | Phosphates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 2500 mg of calcined Zn-Al LDH.

3.2.3.1.1.2. Phosphates removal efficacy

When 250 and 2500 mg of Zn-Al LDH were added, the removal rate was very high (between 98 and 100%) (Figures 53 and 54).

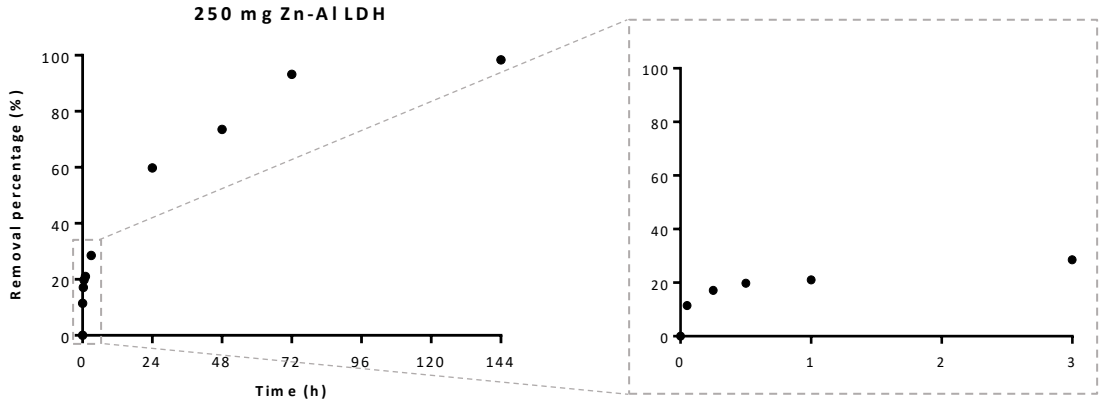

Figure 53 | Percentage of phosphates removed during the test with 10 mg PO_4^{3-}/L of phosphates and 250 mg of calcined Zn-Al LDH.

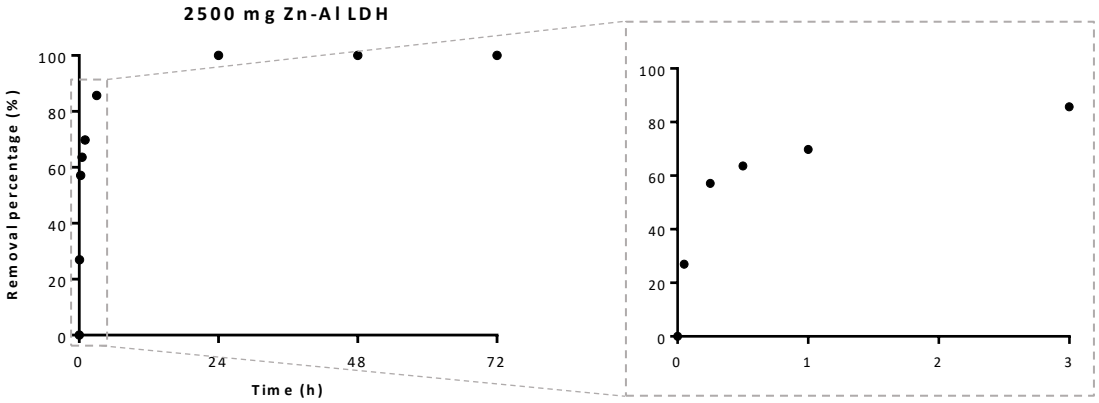

Figure 54 | Percentage of phosphates removed during the test with 10 mg PO_4^{3-}/L of phosphates and 2500 mg of calcined Zn-Al LDH.

3.2.3.1.1.3. Nitrates release studies

The addition of 250 mg/L of Zn-Al LDH caused an increase of 4.3 mg nitrates/L at 144 hours (Figure 55).

When 2500 mg of Zn-Al LDH was added, an increase of 19.8 mg nitrates/L at 144 hours was listed (Figure 56).

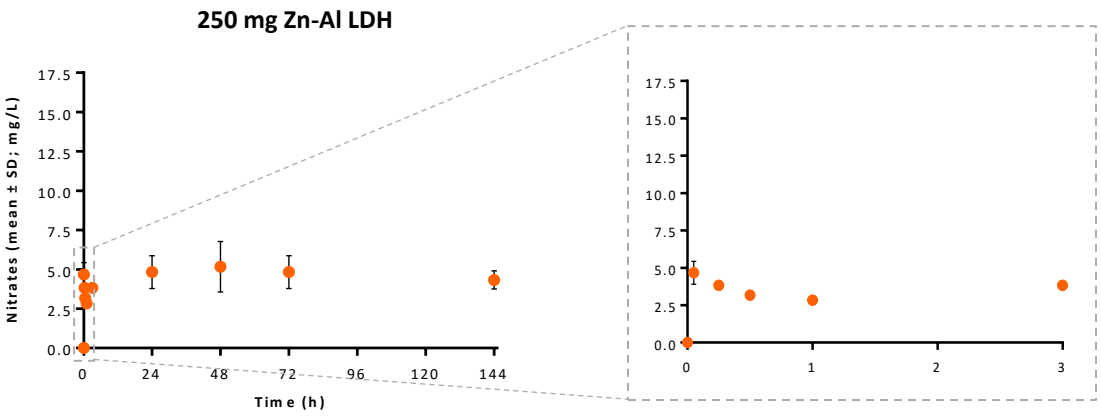

Figure 55 | Nitrates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 250 mg of calcined Zn-Al LDH.

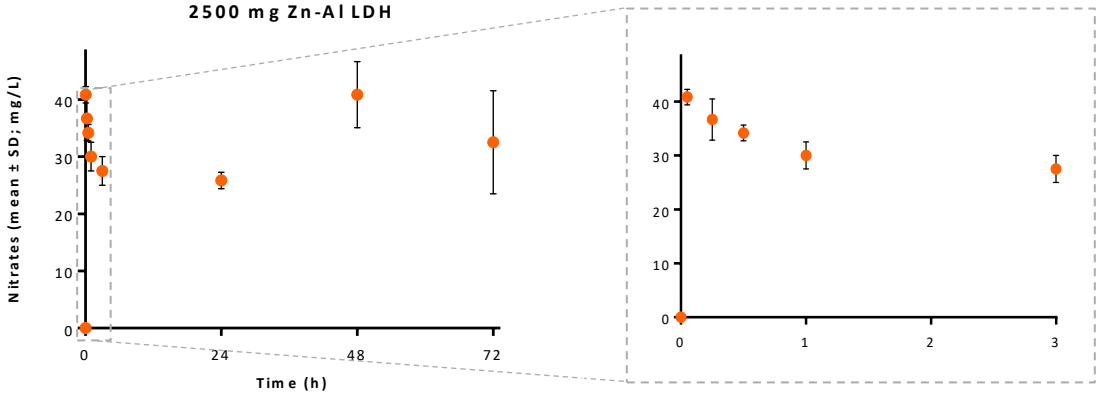

Figure 56 | Nitrates concentration during the test with 10 mg PO_4^{3-}/L of phosphates and 2500 mg of calcined Zn-Al LDH.

3.2.3.1.2. Ecotoxicological assessment of remediated water

Non-treated solution (just 10 mg PO_4^{3-}/L), along the different timepoints, caused no significant effects on the microalgae growth. Remediated water with 250 and 2500 mg Zn-Al LDH along the different timepoints, caused no significant effects on the microalgae growth (Figure 57).

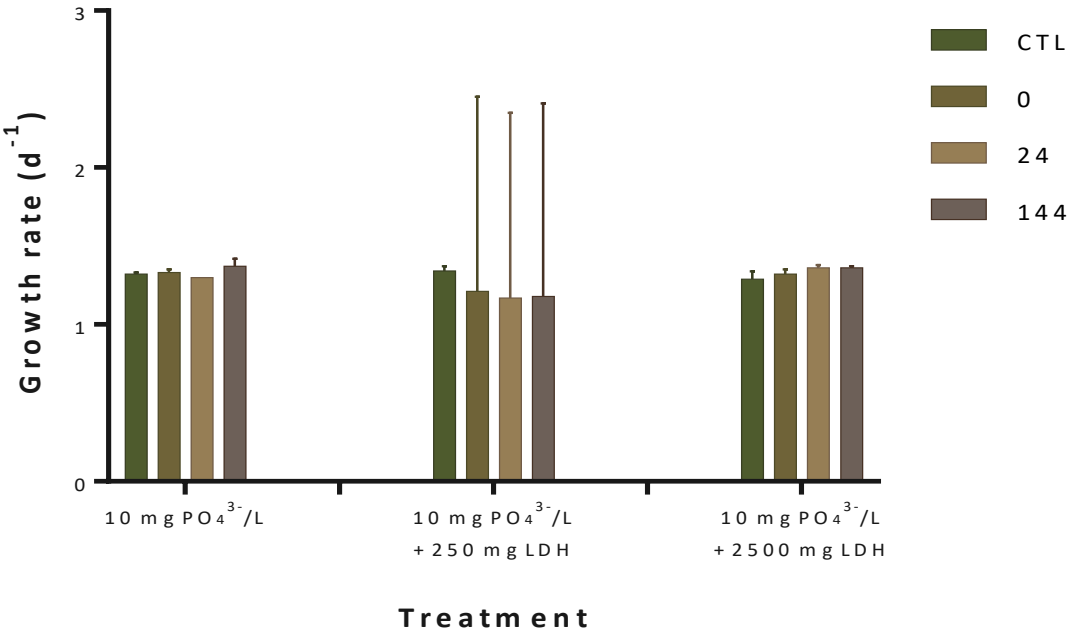

Figure 57 | Effects on the microalgae *Raphidocelis subcapitata* growth rate upon 72 h of exposure to phosphates-rich water (10 mg PO_4^{3-}/L) and the same solution remediated with 250 and 2500 mg/L of Zn-Al layered double hydroxides (reference nanomaterial: calcined Zn-AL LDH) for a period of one week. CTL – only culture media; 0, 24 and 144 – sample collected at time 0, 24 or 144 h, respectively, from the P-rich solution ("10 mg PO_4^{3-}/L") or from P-rich solution remediated with LDH ("10 PO_4^{3-}/L + 250 or 2500 mg LDH").

3.2.3.1.3. XRD

For the 10 mg/L of phosphate tests, XRD analysis were performed only with the calcined Zn-Al LDH remained from the 2500 mg assay. In all the replicates studied the results were similar, so only the XRD corresponding to one of them is presented (Figure 58). The XRD pattern reveals that after calcination, the LDHs so-called "memory effect" took place, with LDHs restoring part of it structure upon rehydration. Proof of this is the appearance of peaks at low 2theta angles which were not present right after calcination (cf. Figure 14). In addition, the position of these peaks is consistent with the formation of LDHs with intercalated hydroxides and/or carbonates.

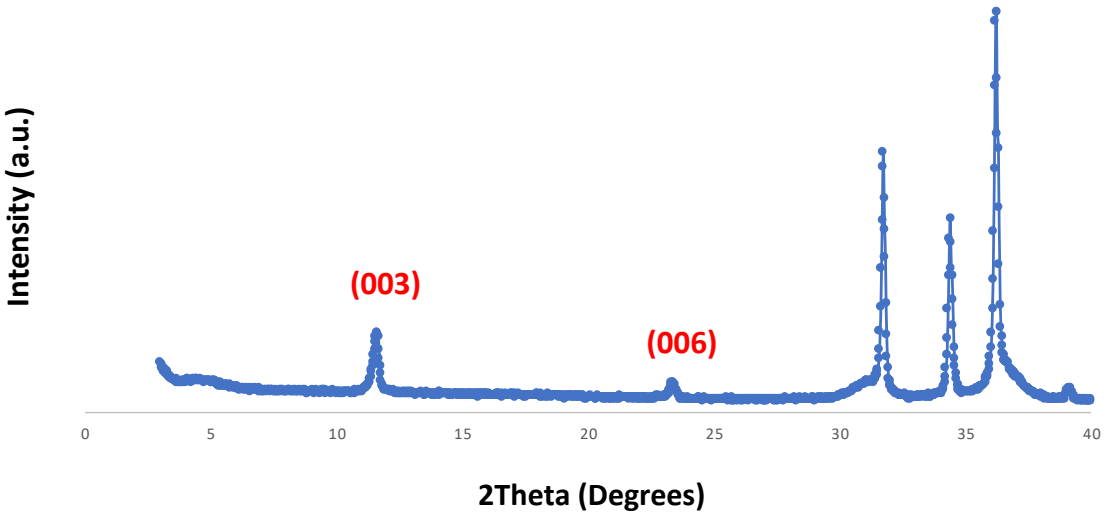

Figure 58 | XRD pattern of calcined Zn-Al LDHs after exposure to 10 mg/L phosphate solution.

3.2.3.2. Remediation of a solution of lake water

3.2.3.2.1. Chemical assessment of remediated water

3.2.3.2.1.1. Phosphates uptake studies

When 250 mg of Zn-Al LDH was added, a decrease of 5.28 mg PO_4^{3-}/L in the concentration of phosphates was immediately noticed (up to 3 h). After 144 h, the quantity of phosphates present in the solution was 0.45 mg PO_4^{3-}/L (Figure 59).

By adding 2500 mg of Zn-Al LDH, phosphates decreased much faster and in the end of the test, the concentration was close to 0 mg PO_4^{3-}/L (Figure 60).

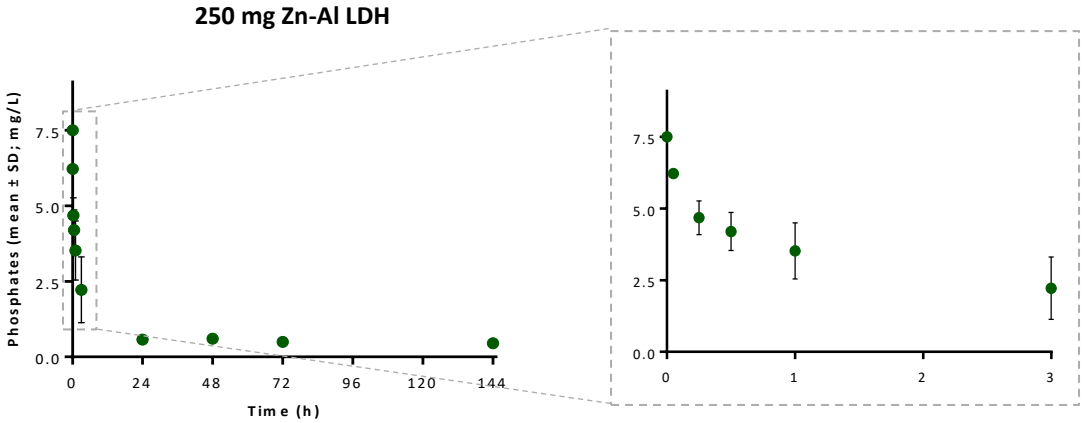

Figure 59 | Phosphates concentration during the test with lake water and 250 mg of calcined Zn-Al LDH.

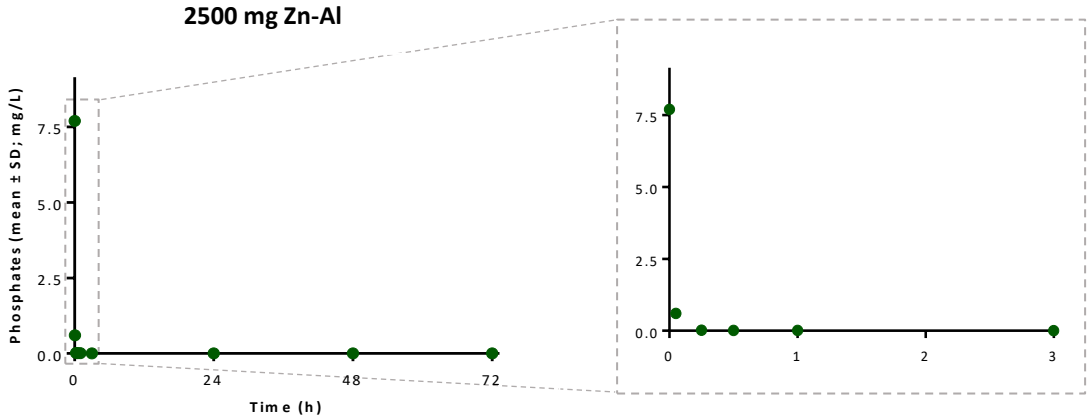

Figure 60 | Phosphates concentration during the test with lake water and 2500 mg of calcined Zn-Al LDH.

3.2.3.2.1.2. Phosphates removal efficacy

When 250 and 2500 mg of Zn-Al LDH were added, the removal rate was very high (between 94 and 100%) (Figures 61 and 62).

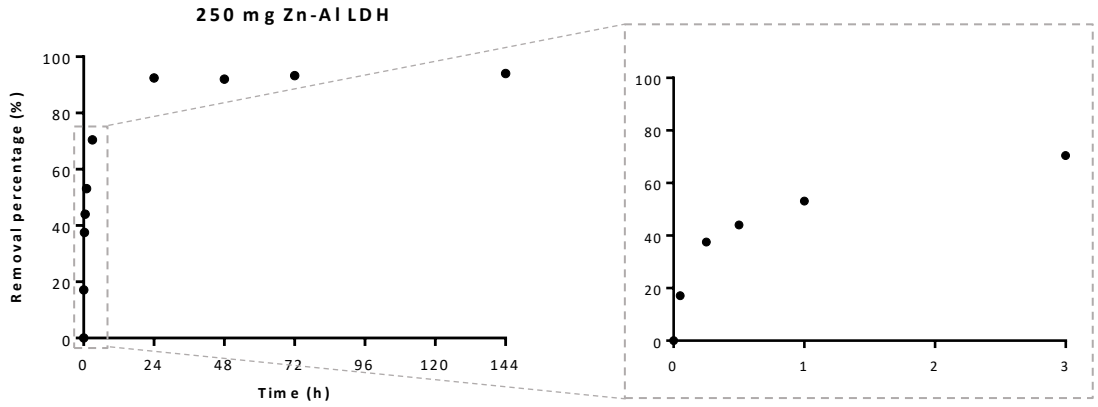

Figure 61 | Percentage of phosphates removed during the test with lake water and 250 mg of calcined Zn-Al LDH.

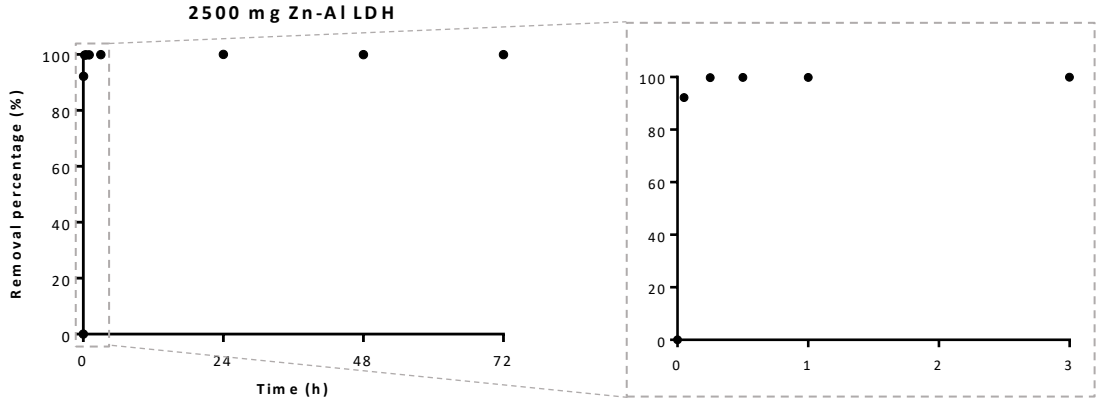

Figure 62 | Percentage of phosphates removed during the test with lake water and 2500 mg of calcined Zn-Al LDH.

3.2.3.2.1.3. Nitrates release studies

The addition of 250 mg/L of Zn-Al LDH caused an immediate increase of nitrates (4.2 mg/L) increasing up to 5.5 mg/L at 144 hours (Figure 63).

When 2500 mg of Zn-Al LDH was added, an increase of 60.8 mg nitrates/L at 144 hours was listed (Figure 64).

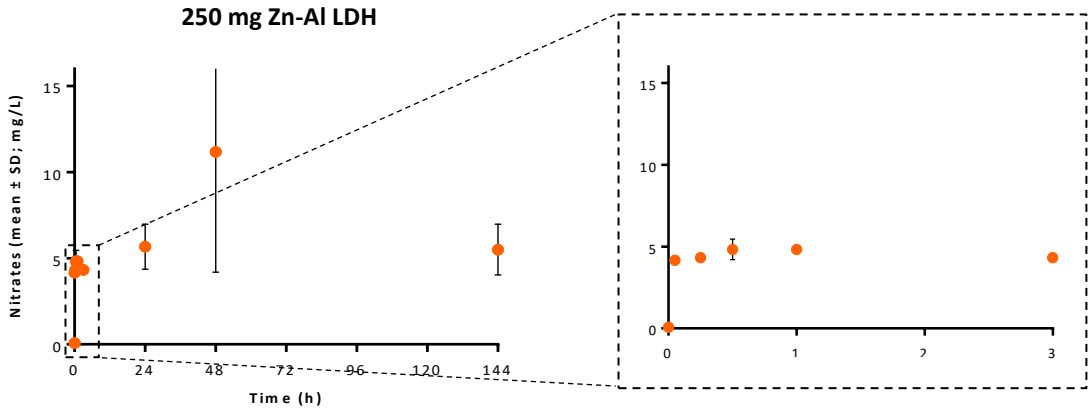

Figure 63 | Nitrates concentration during the test with lake water and 250 mg of calcined Zn-Al LDH.

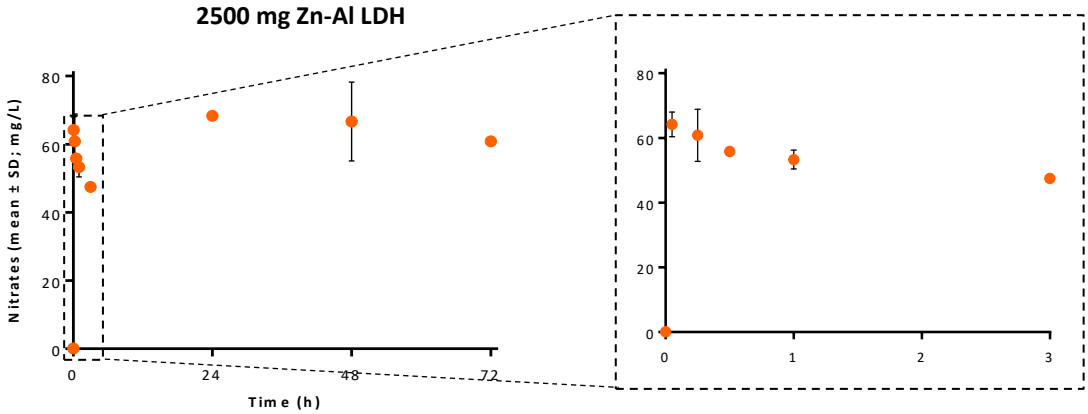

Figure 64 | Nitrates concentration during the test with lake water and 2500 mg of calcined Zn-Al LDH.

3.2.3.2.2. Ecotoxicological assessment of remediated water

Non-treated solution (just lake water), along the different timepoints, caused an increase on the microalgae growth. Samples treated with 250 and 2500 mg/L of Zn-Al LDH caused no significant growth inhibition effects on *R. subcapitata* except the water samples treated with 250 mg/L at time 144 which caused significant differences (Figure 65).

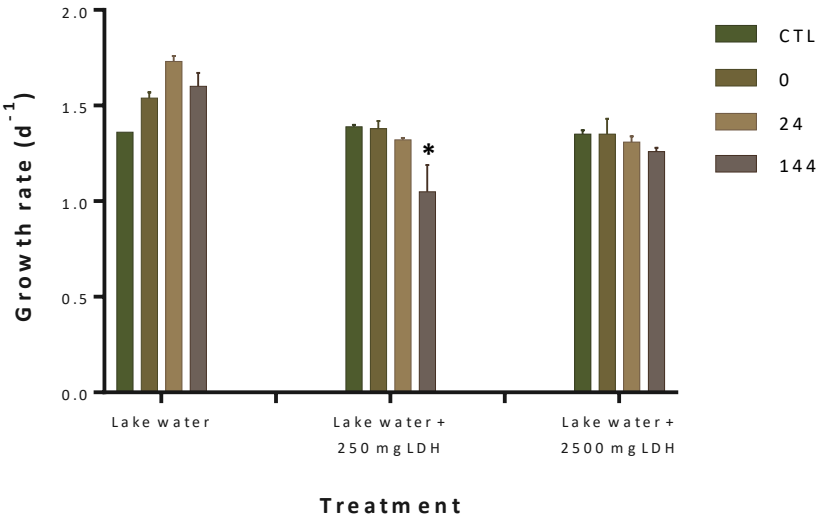

Figure 65 | Effects on the microalgae *Raphidocelis subcapitata* growth rate upon 72 h of exposure to phosphates-rich water (lake water) and the same solution remediated with 250 and 2500 mg/L of Zn-Al layered double hydroxides (reference nanomaterial: calcined Zn-AL LDH) for a period of one week. CTL – only culture media; 0, 24 and 144 – sample collected at time 0, 24 or 144 h, respectively, from the P-rich solution ("l5ke water") or from P-rich solution remediated with LDH ("lake water + 250 or 2500 mg LDH").

3.2.3.2.3. XRD

For the lake water tests, XRD analysis were performed only with the calcined Zn-Al LDH remained from the 2500 mg assay. In all the replicates studied the results were similar, so only the XRD corresponding to one of them is presented (Figure 66). Similar to what was observed using the 10 mg/L phosphate solution, the XRD pattern reveals that after calcination part of the LDH structure was restored upon rehydration. Moreover, the position of these peaks is consistent with the formation of LDHs with intercalated hydroxides and/or carbonates.

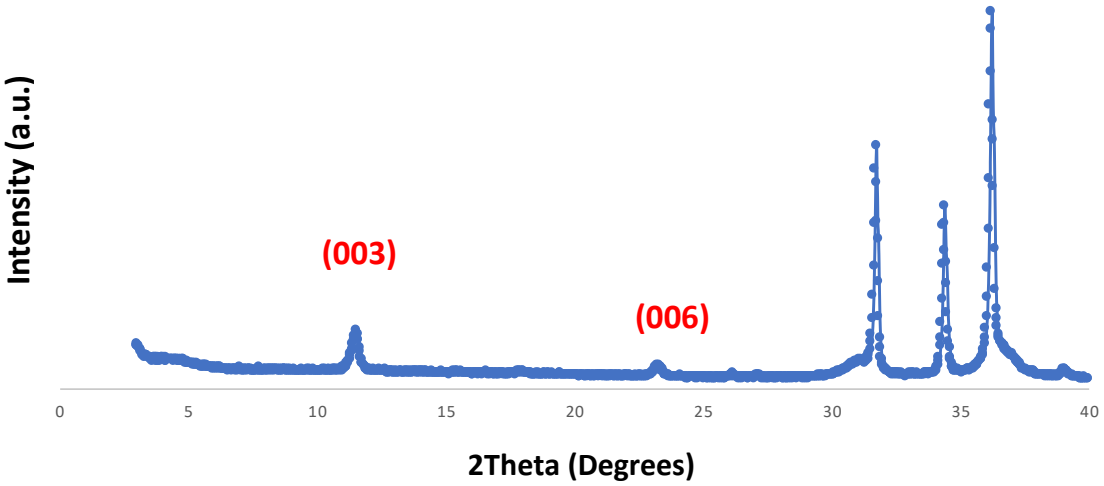

Figure 66 | XRD pattern of calcined Zn-Al LDHs after exposure to lake water solution.

CHAPTER 4

DISCUSSION

The present study showed that Zn-Al LDH-NO_3 proved to be very efficient and eco-friendly nanomaterials for the remediation of P-rich water bodies. The present findings proved that this nanomaterial is no/low toxic against the freshwater microalgae, causing only significant effects on the microalgae growth at very high exposure concentrations (500 mg/L for non-calcined form and 250 mg/L for the calcined form). In literature, it is only possible to find, at this moment, a study regarding the ecotoxicity of LDH in freshwater organisms. Ding *et al.*, 2018 tested green freshwater algae *Scenedesmus quadricauda* exposed to Cu-Mg-Fe LDH (EC_{50} = 8.22 mg/L). However, the tested LDH in this study is chemically different having Zn and Al as cations in the structure. In opposition to the freshwater environment, there are several studies that demonstrate that Zn-Al LDH-NO_3 causes low or no toxicity on marine organisms (Avelelas *et al*., 2017; Martins *et al*., 2017; Gutner-Hoch *et al*., 2018; Gutner-Hoch *et al*., 2019). Despite not comparable, these findings are a good indicator of the environmentally friendly properties of LDHs, in both major ecosystems. Other studies including different species and endpoints should validate these findings.

The phosphate removal capacity by Zn-Al LDH was carefully and thoroughly analyzed in the present study. This nanomaterial demonstrated (as it will be discussed), in all tested media, great qualities in the removal of phosphate. Different concentrations of non-calcined Zn-Al LDH were tested in media with different concentrations of phosphate. The addition of 50 and 500 mg of Zn-Al LDH-NO_3 were enough to remove more than 95% of the phosphates present in the solution of 0.4 mg PO_4^{3-}/L during the first minutes while the addition of 500 mg of Zn-Al LDH-NO_3, 250 and 2500 mg of calcined LDH were enough to reach a removal rate of phosphate higher than 90% (up to ≈ 100%) from the lake water and 10 mg PO_4^{3-}/L solution.

It is of utmost importance to mention that such results were obtained in the first hours of testing which is in agreement with the literature. It is known that initial adsorption is fast decreasing over time (Ferreira *et al*., 2006; Goh, Lim and Dong, 2008). This fast initial adsorption may occur due to the high surface area available to be uptake by the phosphates (Khitous, Salem and Halliche, 2015). Yang *et al.* (2014) demonstrated that the ratio of phosphate adsorbed by calcined Zn-Al LDH was above 90%, which is consistent with the results of the present study. Das e*t al*. (2006) showed that phosphate removal rates of calcined Mg-Al LDH were between 80 and 90%, demonstrating that

calcined Zn-Al LDH, which was used as reference material, was even more effective. The minimal differences between calcined and non-calcined Zn-Al LDHs forms demonstrate that the calcination process which is energy and time-consuming is not needed and the original Zn-Al LDH-NO_3 is industrially and environmentally promising.

The results obtained from XRD analysis show that Zn-Al LDH-NO_3 can intercalate other anions and that calcined LDHs can reconstruct their structure upon rehydration. These findings are fully in agreement with works reported in the literature (Tedim *et al.*, 2010; Abderrazek, Najoua and Srasra, 2016; Kuznetsova, 2020). However, despite the release and adsorption studies confirmed the phosphates uptake, the structural changes occurring within LDHs showed that phosphates did not enter the LDH galleries. Similar findings were found by Dolganov (2019), in a recent project work from the Master in Materials Engineering from the University of Aveiro, where Zn-Al LDH-NO_3 were exposed to solutions with increasing concentration of phosphates ($5x10^{-3}$ up to $5x10^{-1}$ M). Dolganov (2019) showed that the LDHs were able to capture phosphates in all the concentrations, but at the lowest concentration ($5x10^{-3}$ M), the XRD did not reveal any LDH-HPO_4 phase, although they were detected in the LDH samples by a spectrophotometric assay. Therefore, we can assume that in the present study, phosphate was most probably adsorbed in the LDH phase and, on high phosphate concentrations these anions may replace nitrates inside the galleries. In addition, two reasons may explain why carbonates (and/or hydroxides) may have been preferentially intercalated in the presence of phosphates: selectivity and concentration. Carbonates and hydroxides are among the species which form more stable LDH phases (Newman and Jones, 1998), so even in the presence of small amounts of carbonates and hydroxides in solution anion exchange with these species may occur. On the other hand, concentration plays an important role. Ion-exchange reaction is governed by equilibrium, so if one wants to "force" intercalation of anions with low equilibrium constant associated, higher concentrations of this anion has to be used. In fact, in the present work, tested phosphate concentration was too low to cause structural changes ($4.2x10^{-6}$ M, $1.1x10^{-4}$ M and $8.1x10^{-5}$ M), which is consistent with results obtained by Dolganov (2019). This is particularly relevant in the presence of carbonates (CO_2 (aq) is present every time water is exposed to atmosphere, speciating in carbonates, which depends on pH of the solution (Hanrahan, 2012)).

Analyzing the XRD patterns of calcined LDH it is possible to notice two obvious reflections that suggest an almost total decomposition of the original LDH and, because of that, it is also possible to prove that calcination and posterior rehydration of the nanomaterial were well succeed (Das *et al*., 2006; Laipan *et al*., 2015). As it is known, LDH has a feature known as memory effect. Such feature consists in the fact that, at 450 - 500 °C LDHs lose their form of interlayer, thus forming highly active composite metal oxides with high stability, large surface area, small crystal size and high thermal stability under extreme conditions (Li *et al*., 2005). Nevertheless, the calcined nanomaterial returns to its original form when rehydrated in an aqueous environment. There are even studies that prove that calcined LDH is more effective in the adsorption of contaminants than non-calcined LDH, since such adsorption process will occur simultaneously with the rehydration of the nanomaterial which allows contaminants to more easily access the interlayered spaces (Crepaldi *et al*., 2002; Zhu *et al*., 2005; Lv *et al*., 2008).

So, regarding calcined LDHs, similar reasons justify the fact that LDHs do not intercalate phosphates upon rehydration. The rehydration process in water is accompanied by uptake of carbonates and hydroxides formed from the reaction of rehydration of calcined LDHs (oxides converted into hydroxides). Having said this, these results do not exclude the use of LDHs for phosphate fixation, quite the opposite: the LDHs can fixate phosphates which were not fully achieved under the experimental conditions tested. In other words, LDHs can fixate more phosphate per gram than the one that was attempted in this work.

It was also possible to conclude, through the isotherm equation tested, that Freundlich model was most appropriate to represent the uptake equilibrium data (based on the R^2 values). Also, as Cheng *et al*. (2013) showed, a multi-step pathway is followed in the uptake process of phosphates and, consequently, many mechanisms are involved in the uptake process. In Freundlich model, the strength of adsorption process is demonstrated by $\frac{1}{n}$ a ratio which varies between 0 and 1 (Chitrakar *et al*., 2005; Cheng *et al.*, 2013; Novillo *et al*., 2014; Hatami, Fotovat and Halajnia, 2018). However, it is extremely important to mention that these results are only preliminary, since the values obtained in the studies with 500 mg of the nanomaterial were excluded from the analysis, due to the difficulty in quantifying the amount of phosphate remaining in

solution, which is below the detection limit associated with the analytical method used in this work for phosphate quantification.

However, and although calcined LDH has many advantages, non-calcined LDH has also proved to be an excellent nanomaterial for phosphate removal in addition to have a key advantage since does not need to undergo heat treatment, which means less energetic costs during its manufacture with clear environmental and economic benefits. This is aligned with the United Nations Sustainable Development Goals for 2030 which seeks sustainable and accessible technologies for water remediation (SDG nr. 6) with low environmental impact for the aquatic (SDG nr. 14) and terrestrial ecosystems (SDG nr. 15), based on an innovative, knowledge-based and sustainable industrialization (SDG nr. 9) (United Nation, 2020).

CHAPTER 5

CONCLUSION

The present study used non-calcined Zn-Al LDH-NO_3 as a novel sorbent nanomaterial to remove phosphates from laboratorial phosphates-rich solutions and eutrophic water. Present findings were benchmarked against calcined LDHs. The nanomaterial was characterized by XRD before and after phosphate sorption demonstrating that it was not immobilized in the intra-lamellar space, but immobilized only on their external surface. But more important than all that was previously said, this study proved that Zn-Al LDH has a great adsorption capacity and showed solid results (and maybe even better results than all other documented phosphate sorbents) in the remediation of P-rich eutrophic water.

With these analyzes, it was possible to understand that this nanomaterial is very innovative and can certainly be a decisive factor in the remediation of eutrophic bodies of water. However, it is important to understand that there are still no studies on this nanomaterial for freshwater organisms, and this study is, therefore, a small step in filling the knowledge gap that still exists. Future works may also consider the development of strategies to immerse quickly nanomaterial and remove it. In this way, the phosphate present in the water would undoubtedly be adsorbed, but the nitrates would not be released in large quantities.

In conclusion, this study suggest that Zn-Al LDH is a technological and environmentally friendly promising solution for phosphate remediation. So, an integrative ecotoxicological assessment in freshwater ecosystems are also widely recommended in order to anticipate eventual negative impacts in the surrounding biota caused by the introduction of Zn-Al LDHs as an additive to remove excess of phosphates in the freshwater water bodies.

CHAPTER 6

REFERENCES

Abderrazek K, Najoua FS and Srasra E. 2016. Synthesis and characterization of [Zn–Al] LDH: Study of the effect of calcination on the photocatalytic activity. Applied Clay Science. 119: 229 – 235.

Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil C, Kudela R, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL and Vargo G. 2008. Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae. 8(1): 39 – 53.

Aryal M and Liakopoulou-Kyriakides M. 2011. Equilibrium, kinetics and thermodynamic studies on phosphate biosorption from aqueous solutions by Fe(III)-treated *Staphylococcus xylosus* biomass: Common ion effect. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 387 (1 – 3): 43 – 49.

Avelelas F, Martins R, Oliveira T, Maia F, Malheiro E, Soares AMVM, Loureiro S and Tedim J. 2017. Efficacy and Ecotoxicity of Novel Anti-Fouling Nanomaterials in Target and Non-Target Marine Species. Marine Biotechnology. 19: 164 – 174.

Bergaya F and Lagaly G (Eds). 2013. Handbook of Clay Science. 5: 813 pp.

Bradder P, Ling SK, Wang S and Liu S. 2011. Dye Adsorption on Layered Graphite Oxide. Journal of Chemical Engineering Data. 56 (1): 138 − 141.

Bunce JT, Ndam E, Ofiteru ID, Moore A and Graham DW. 2018. A Review of Phosphorus Removal Technologies and Their Applicability to Small-Scale Domestic Wastewater Treatment Systems. Frontiers in Environmental Science. 6 (8): 15 pp.

Cardoso AC, Duchemin J, Magoarou P and Premazzi G. 2001. Criteria for the Identification of Freshwaters Subject to Eutrophication – Their Use for the Implementation of the “Nitrates” and Urban Waste Water Treatment Directives. Joint Research Centre – European Commission. 96 pp.

Carillo AC and Griego DA (Eds). 2012. Hydroxides: Synthesis, Types and Applications. Nova Science Publishers, Inc. 300 pp.

Carmichael WW. 1991. Toxic freshwater blue-green algae (cyanobacteria): An overlooked health threat. Health & Environment Digest. 5(6): 4 pp.

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN and Smith VH. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications. 8(3): 559 – 568.

Chen X. 2015. Modeling of Experimental Adsorption Isotherm Data. Information. 14 – 22.

Cheng X, Wang Z, Sun D and Wang A. 2013. Pathways of phosphate uptake from aqueous solution by ZnAl layered double hydroxides. Water Science & Technology. 67 (8): 1757 – 1763.

Chitrakar R, Tezuka S, Sonoda A, Sakane K, Ooi K, Hirotsu T. 2005. Adsorption of phosphate from seawater on calcined MgMn-layered double hydroxides. Journal of Colloid and Interface Science. 290 (1): 45 – 51.

Comissão Europeia. 2017. Comunicação da Comissão ao Parlamento Europeu, ao Conselho, ao Comité Económico e Social Europeu e ao Comité das Regiões relativa à lista de 2017 das matérias primas essenciais para a EU.

Cooke GD, Welch EB, Peterson SA and Nichols SA (Eds). 2005. Restoration and Management of Lakes and Reservoirs. 3rd Edition. CRC Press. 616 pp.

Cornejo J, Celis R, Pavlovic I and Ulibarri MA. 2008. Interactions of pesticides with clays and layered double hydroxides: a review. Clay Minerals. 43(2): 155 – 176.

Crepaldi EL, Tronto J, Cardoso LP and Valim JB. 2002. Sorption of terephthalate anions by calcined and uncalcined hydrotalcite-like compounds. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 211 (2 – 3): 103 – 114.

Dai R, Liu H, Qu J, Ru J and Hou Y. 2008. Cyanobacteria and their toxins in Guanting Reservoir of Beijing, China. Journal of Hazardous Materials. 153(1 – 2): 470 – 477.

Das J, Patra BS, Baliarsingh N and Parida KM. 2006. Adsorption of phosphate by layered double hydroxides in aqueous solutions. Applied Clay Science. 32: 252 – 260.

da Silva MB, Abrantes N, Nogueira V, Gonçalves F and Pereira R. 2016. TiO_2 nanoparticles for the remediation of eutrophic shallow freshwater systems: Efficiency and impacts on aquatic biota under a microcosm experiment. Aquatic Toxicology. 178: 58 – 71.

Decreto-Lei nº 236/98 de 1 de Agosto de 1998. Diário da República nº176/1998 - Série I-A. Ministério do Ambiente, Lisboa. 47 pp.

de Figueiredo DR, Reboleira ASSP, Antunes SC, Abrantes N, Azeiteiro U, Gonçalves F and Pereira MJ. 2006. The effect of environmental parameters and cyanobacterial

blooms on phytoplankton dynamics of a Portuguese temperate lake. Hydrobiologia. 568(1): 145 – 157.

de Varennes A. 2003. Produtividade dos Solos e Ambiente. Escolar Editora. First Edition. Lisboa. 490 pp.

Dias JM, Alvim-Ferraz MCM, Almeida MF, Rivera-Utrilla J and Sánchez-Polo M. 2007. Waste materials for activated carbon preparation and its use in aqueous-phase treatment: A review. Journal of Environmental Management. 85 (4): 833 − 846.

Diaz RJ and Rosenberg R. 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. Science. 321 (5891): 926 – 929.

Dodds WK, Bouska WW, Eitzmann JL, Pilger TJ, Pitts KL, Riley AJ, Schloesser JT and Thornbrugh DJ. 2009. Eutrophication of U.S. Freshwaters: Analysis of Potential Economic Damages. Environmental Science & Technology. 43(1): 12 – 9.

Donnert D, Berg U, Weidler PG, Nüesch R, Song Y, Salecker M, Kusche I, Bumiller W and Friedrich F. 2002. Phosphorus removal and recovery from waste water by crystallization. 1 – 21.

Eberhardt TL and Min SH. 2008. Biosorbents prepared from wood particles treated with anionic polymer and iron salt: Effect of particle size on phosphate adsorption. Bioresource Technology. 99 (3): 626 – 630.

Ferreira OP, de Moraes SG, Duran N, Cornejo L and Alves OL. 2006. Evaluation of boron removal from water by hydrotalcitelike compounds. Chemosphere. 62 (1): 80 – 88.

Freundlich H. 1906. Over the adsorption in solution. The Journal of Physical Chemistry 57: 358 – 471.

Geis SW, Leming KL, Korthals ET, Searle G, Reynolds L and Karner DA. 2000. Modifications to the algal growth inhibition test for use as a regulatory assay. Environmental Toxicology and Chemistry. 19(1): 36 – 41.

Gensemer RW and Playle RC. 1999. The Bioavailability and Toxicity of Aluminum in Aquatic Environments. Critical Reviews in Environmental Science and Technology. 29(4): 315 – 450.

Gibbs M and Özkundakci D. 2011. Effects of a modified zeolite on P and N processes and fluxes across the lake sediment-water interface using core incubations. Hydrobiologia. 661(1): 21 – 35.

Gimbert F, Crini NM, Renault F, Badot PM and Crini G. 2008. Adsorption isotherm models for dye removal by cationized starch-based material in a single component system: Error analysis. Journal of Hazardous Materials. 34 – 46.

Glasgow HB and Burkholder JM. 2000. Water quality trends and management implications from a five-year study of a eutrophic estuary. Ecological Applications. 10(4): 1024 – 1046.

Goh KH, Lim TT and Dong Z. 2008. Application of layered double hydroxides for removal of oxyanions: A review. Water Research. 42(6 – 7): 1343 – 1368.

Guerra FD, Mohamed EA, Whitehead DC and Alexis F. 2018. Nanotechnology for Environmental Remediation: Materials and Applications. Molecules. 23(7): 23 pp.

Gutner-Hoch E, Martins R, Maia F, Oliveira T, Shpigel M, Weis M, Tedim J and Benayahy. 2019. Toxicity of engineered micro- and nanomaterials with antifouling properties to the brine shrimp *Artemia salina* and embryonic stages of the sea urchin *Paracentrotus lividus*. Environmental Pollution. 251: 530 – 537.

Gutner-Hoch E, Martins R, Oliveira T, Maia F, Soares AMVM, Loureiro S, Piller C, Preiss I, Weis M, Larroze SB, Teixeira T, Tedim J and Benayahu Y. 2018. Antimacrofouling Efficacy of Innovative Inorganic Nanomaterials Loaded with Booster Biocides. Journal of Marine Science and Engineering. 6(1): 12 pp.

Hanrahan G. 2012. Key Concepts in Environmental Chemistry. Academic Press. 384 pp.

Hatami H, Fotovat A and Halajnia A. 2018. Comparison of adsorption and desorption of phosphate on synthesized Zn-Al LDH by two methods in a simulated soil solution. Applied Clay Science. 152: 333 – 341.

Hautier Y, Niklaus PA and Hector A. 2009. Competition for Light Causes Plant Biodiversity Loss after Eutrophication. Science. 324 (5927): 636 – 638.

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK and Suddleson M. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae. 8(1): 3 – 13.

Hickey CW and Gibbs MM. 2009. Lake sediment phosphorus release management—Decision support and risk assessment framework. New Zealand Journal of Marine and Freshwater Research. 43(3): 819 – 856.

Howarth R, Swaney DP, Butler TJ and Marino R. 2000. Climatic Control on Eutrophication of the Hudson River Estuary. Ecossistems. 3(2): 210 – 2015.

Iftekhar S, Küçük ME, Srivastava V, Repo E and Sillanpää. 2018. Application of zinc-aluminium layered double hydroxides for adsorptive removal of phosphate and sulfate: Equilibrium, kinetic and thermodynamic. Chemosphere. 209: 470 – 479.

Jenkins D, Fergurson JF and Menar AB. 1971. Chemical processes for phosphate removal. Water Research. 5: 369 – 389.

Kasprzyk-Hordern B. 2004. Chemistry of alumina, reactions in aqueous solution and its application in water treatment. Advances in Colloid and Interface Science. 110 (1 – 2): 19 – 48.

Khan AI and O'Hare D. 2002. Intercalation chemistry of layered double hydroxides: recent developments and applications. Journal of Materials Chemistry. 12(11): 8 pp.

Khithous M, Salem Z and Halliche D. 2015. Removal of phosphate from industrial wastewater using uncalcined MgAl-NO_3 layered double hydroxide: bath study and modeling. Desalination and Water Treatment. 1 – 12.

Klapper H. 2003. Technologies for lake restoration. Journal of Limnology. 62(1): 73 – 90.

Kumar KM, Mandal BK, Kumar KS, Reddy PS and Sreedhar B. 2013. Biobased green method to synthesise palladium and iron nanoparticles using *Terminalia chebula* aqueous extract. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 102: 128 – 133.

Kuznetsova A. 2020. Hidróxidos Duplos Lamelares (HDL) como nanoreservatórios versáteis para aplicação em revestimentos multifuncionais. Universidade de Aveiro.

Laipan M, Zhu R, Chen Q, Zhu J, Xi Y, Ayoko GA and He H. 2015. From spent Mg/Al layered double hydroxide to porous carbon materials. Journal of. Hazardous. Matererials. 300: 572 – 580.

Lai L, Xie Q, Chi L, Gu W, Wu D. 2016. Adsorption of phosphate from water by easily separable Fe_3O_4@SiO_2 core/shell magnetic nanoparticles functionalized with hydrous lanthanum oxide. Journal of Colloid and Interface Science. 465: 76 – 82.

Langmuir I. 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. Journal of the American Chemical Society. 40: 1361 – 1403.

Li F, Jiang XR, Evans DG and Duan X. 2005. Structure and basicity of mesoporous materials from Mg/Al/In layered double hydroxides prepared by separate nucleation and aging steps method. Journal of Porous Materials. 12 (1): 55 – 63.

Li HM, Tang HJ, Shi XY, Zhang CS and Wang XL. 2014. Increased nutrient loads from the Changjiang (Yangtze) River have led to increased Harmful Algal Blooms. Harmful Algae. 39: 92 – 101.

Lv L, Wang Y, Wei M and Cheng J. 2008. Bromide ion removal from contaminated water by calcined and uncalcined MgAl-CO_3 layered double hydroxides. Journal of Hazardous Materials. 152: 1130 – 1137.

Machado MD, Lopes AR and Soares EV. 2015. Responses of the alga *Pseudokirchneriella subcapitata* to long-term exposure to metal stress. Journal of Hazardous Materials. 296: 82 – 92.

Machado S, Pinto SL, Grosso JP, Nouws HPA, Albergaria JT and Delerue-Matos C. 2013. Green production of zero-valent iron nanoparticles using tree leaf extracts. Science of The Total Environment. 445 – 446: 1 – 8.

Martins R, Oliveira T, Santos C, Kuznetsova A, Ferreira V, Avelelas F, Caetano APF, Tedim J, Ferreira M, Freitas R, Soares AMVM and Loureiro S. 2017. Effects of a novel anticorrosion engineered nanomaterial on the bivalve *Ruditapes philippinarum*. Environmental Science-Nano. 4 (5): 1064 – 1076.

Mezenner NY and Bensmaili A. 2009. Kinetics and thermodynamic study of phosphate adsorption on iron hydroxide-eggshell waste. Chemical Engineering Journal. 147 (2 – 3): 87 – 96.

Misaelides P. 2011. Application of natural zeolites in environmental remediation: A short review. Microporous and Mesoporous Materials. 144(1 – 3): 15 – 18.

Morris R, Taylor EW, Brown DJA and Brown JA (Eds). 1989. Acid toxicity and aquatic animals. Cambridge University Press. 282 pp.

Morse GK, Brett SW, Guy JA and Lester JN. 1998. Review: Phosphorus removal and recovery technologies. The Science of the total environment. 212: 69 – 81.

Mortula M, Shabani S, Rumaithu KA, Nawaz W and Kashwani G. 2011. Removal of Phosphorus and BOD from Secondary Effluent using Coagulation. 4 pp.

Mubarak NM, Sahu JN, Abdullah EC and Jayakumar NS. 2014. Removal of Heavy Metals from Wastewater Using Carbon Nanotubes. Separation & Purification Reviews. 43 (4): 311 − 338.

Nalawade P, Aware B, Kadam VJ and Hirlekar RS. 2009. Layered double hydroxides: A review. Journal of Scientific & Industrial Research. 68: 267 – 272.

Namvari M and Namazi H. 2014. Synthesis of magnetic citric-acid-functionalized graphene oxide and its application in the removal of methylene blue from contaminated water. Polymer International. 63 (10): 1881 − 1888.

National Research Council. 2000. Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. National Academies Press. Washington, DC. 428 pp.

Newman SP and Jones W. 1998. Synthesis, characterization and applications of layered double hydroxides containing organic guests. New Journal of Chemistry. 22(2): 105 – 115.

Nolan BT, Ruddy BC, Hitt KJ and Helsel DR. 1997. Risk of Nitrate in Groundwaters of the United States – A National Perspective. Environmental Science and Technology. 31: 2229 – 2236.

Novillo C. Guaya D. Avendaño AAP. Armijos C. Cortina JL. Cota I. 2014. Evaluation of phosphate removal capacity of Mg/Al layered double hydroxides from aqueous solutions. Fuel. 138: 72 – 79.

Novell-Leruth G, Pérez-Sánchez G, Galvão TLP, Boiba D, Poznyak S, Carneiro J, Tedim J and Gomes JRB. 2020. Unveiling the local structure of 2-mercaptobenzothiazole intercalated in (Zn_2Al) layered double hydroxides. Applied Clay Science. 198: 13 pp.

OECD. 2011. OECD Guidelines for the testing of chemicals - Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Organisation for Economic Co-operation and Development. 1 – 22.

Othman A, Dumitrescu E, Andreescu D and Andreescu S. 2018. Nanoporous Sorbents for the Removal and Recovery of Phosphorus from Eutrophic Waters: Sustainability Challenges and Solutions. ACS Sustainable Chemistry & Engineering. 6(10): 20 pp.

Pilgrim KM, Huser BJ and Brezonik PL. 2007. A method for comparative evaluation of whole-lake and inflow alum treatment. Water Research. 41 (6): 1215 – 1224.

Quan Q, Shen B, Zhang Q and Ashraf MA. 2016. Research on Phosphorus Removal in Artificial Wetlands by Plants and Their Photosynthesis. Brazilian Archives of Biology and Technology. 59: 9 pp.

Razzaque MS. 2011. Phosphate toxicity: New insights into an old problem. Clinical Science. 120 (3): 91 – 97.

Roco MC. 2003. Nanotechnology: convergence with modern biology and medicine. Current Opinion in Biotechnology. 14(3): 337 – 346.

Santos MCR, Pacheco DM, Santana FJS and Rodrigues AMF. 2004. A Eutrofização das Lagoas das Sete-Cidades e Furnas (S. Miguel – Açores) - Análise evolutiva entre 1988 e 2002. 7º Congresso da Água. APRH – Associação Portuguesa dos Recursos Hídricos. 16 pp.

Schlesinger WH and Bernhardt E. 1991. Biogeochemistry: An Analysis of Global Change. Academic Press. Third Edition. San Diego. 688 pp.

Sipaúba-Tavares LH and Rocha O. 2003. Produção de Plâncton (Fitoplâncton e Zooplâncton) para Alimentação de Organismos Aquáticos. RiMa. 122 pp.

Skulberg OM, Codd GA and Carmichael WW. 1984. Toxic Blue-Green Algal Blooms in Europe: a Growing Problem. AMBIO - A Journal of the Human Environment. 13(4): 244 – 247.

Smeltzer E. 1990. A Successful Alum/Aluminate Treatment of Lake Morey, Vermont. Lake and Reservoir Management. 6(1): 9 – 19.

Smeltzer E, Kim RA and Fiske S. 1999. Long-Term Water Quality and Biological Effects of Alum Treatment of Lake Morey, Vermont. Lake and Reservoir Management. 15(3): 173 – 184.

Smuleac V, Varma R, Sikdar S and Bhattacharyya D. 2011. Green synthesis of Fe and Fe/Pd bimetallic nanoparticles in membranes for reductive degradation of chlorinated organics. Journal of Membrane Science. 379(1 – 2): 131 – 137.

Tchobanoglous G and Burton FL. 1991. Wastewater Engineering: Treatment Disposal Reuse. McGraw-Hill Inc. Third Edition. New York. 1334 pp.

Tedim J, Poznyak SK, Kuznetsova A, Raps D, HAck T, Zheludkevich ML and Ferreira MGS. 2010. Enhancement of Active Corrosion Protection via Combination of Inhibitor-Loaded Nanocontainers. ACS Applied Materials & Interfaces. 2(5): 1528 – 1535.

Thomann RV and Mueller JA. 1987. Principles of Surface Water Quality Modeling and Control. Pearson. First Edition. New York. 644 pp.

United Nation. 2020. Objetivos de desenvolvimento sustentável – 17 objetivos para transformar o nosso mundo. URL: https://unric.org/pt/objetivos-de-desenvolvimento-sustentavel/. Accessed on December 20, 2020.

Vaccari A. 1999. Clays and catalysis: a promising future. Applied Clay Science. 14(4): 161 – 198.

Vallet-Regí M (Eds). 2014. Bio-Ceramics with Clinical Applications. John Wiley & Sons, Ltd: Chichester, UK. 484 pp.

Valsami-Jones E (Eds). 2004. Phosphorous in Environmental Technologies – Principles and Application. IWA Publishing. UK. 93 – 119.

Vasconcelos VM, Sivonen K, Evans WR, Carmichael WW and Namikoshi M. 1996. Hepatotoxic microcystin diversity in cyanobacterial blooms collected in portuguese freshwaters. Water Research. 30(10): 2377 – 2384.

Vitousek PM, Aber J, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH and Tilman GD. 1997a. Human alteration of the global nitrogen cycle: causes and consequences. Ecological Applications. 7(3): 737 – 750.

Vitousek PM, Mooney HA, Lubchenko J and Melillo JM. 1997b. Human Domination of Earth's Ecosystems. Science. 277 (5325): 494 – 499.

Wang S, Sun H, Ang HM and Tadé M. O. 2013 Adsorptive remediation of environmental pollutants using novel graphene-based nanomaterials. Chemical Engineering Journal. 226: 336 − 347.

Wang T, Jin X, Chen Z, Megharaj M and Naidu R. 2014. Green synthesis of Fe nanoparticles using eucalyptus leaf extracts for treatment of eutrophic wastewater. Science of The Total Environment. 466 – 467: 210 – 2013.

Wang Y, Shen Z, Niu J and Liu R. 2009. Adsorption of phosphorus on sediments from the Three-Gorges Reservoir (China) and the relation with sediment compositions. Journal of Hazardous Materials. 162 (1): 92 – 98.

Wells PG, Lee K and Blaise C (Eds). 1998. Microscale Testing in Aquatic Toxicology - Advances, Techniques and Practice. CRC Press. 720 pp.

Whitley S. 2017. Eutrophication in Coastal New Zealand Lakes and the Mitigation Potential of Phosphorus Immobilisation Using Clay Based Amendments. Lincoln University. 49 pp.

Yang K, Yan LG, Yang YM, Yu SJ, Shan RR, Yu HQ, Zhu BC and Du B. 2014. Adsorptive removal of phosphate by Mg–Al and Zn–Al layered double hydroxides: Kinetics, isotherms and mechanisms. Separation and Purification Technology. 124: 36 – 42.

Yuan G and Wu L. 2007. Allophane nanoclay for the removal of phosphorus in water and wastewater. Science and Technology of Advanced Materials. 8(1 – 2): 60 – 62.

Zarrabi M, Soori MM, Sepehr MN, Amrane A, Borji S and Ghaffari HR. Removal of phosphorus by ion-exchange resins: Equilibrium, kinetic and thermodynamic studies. Environmental Engineering and Management Journal. 13 (4): 891 – 903.

Zheludkevich ML, Tedim J and Ferreira MGS. 2012. “Smart” coatings for active corrosion protection based on multi-functional micro and nanocontainers. Electrochimica Acta. 82: 314 – 323.

Zhu MX, Li YP, Xie M and Xin HZ. 2005. Sorption of an anionic dye by uncalcined and calcined layered double hydroxides: a case study. Journal of Hazardous Matererials. 120: 163 – 171.

SUPLEMENTARY MATERIAL

Non calcined Zn-Al LDH

0.4 mg/L of phosphate in mili-Q water

Table 7 | Phosphates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the 0.4 mg P/L test.

	(mg/L) PO_4^{3-} - P								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.40	0.40	0.41	0.39	0.39	0.39	0.38	0.38	0,.38
2	0.38	0.40	0.38	0.37	0.37	0.37	0.37	0.37	0.37
3	0.38	0.41	0.37	0.37	0.37	0.37	0.37	0.37	0.37

Table 8 | Nitrates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the 0.4 mg P/L test.

	(mg/L) NO_3^- - N								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 9 | Phosphates concentration (in mg/L) observed when added 5 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.38	0.35	0.36	0.36	0.31	0.35	0.28	0.22	0.21	0.18
2	0.38	0.23	0.21	0.25	0.21	0.22	0.20	0.10	0.17	0.15
3	0.38	0.36	0.38	0.38	0.37	0.32	0.31	0.20	0.23	0.18

Table 10 | Nitrates concentration (in mg/L) observed when added 5 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) NO_3 – N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	0.1	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.2
2	0.0	0.2	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.1
3	0.0	0.1	0.1	0.1	0.0	0.1	0.2	0.1	0.1	0.1

Table 11 | Phosphates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.40	0.01	0.03	0.04	0.01	0.02	0.01	---	---	0.01
2	0.39	0.01	0.06	0.01	0.02	0.01	0.01	---	---	0.01
3	0.40	0.01	0.03	0.01	0.01	0.01	0.01	---	---	0.01

Table 12 | Nitrates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) NO_3 – N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	1.0	1.0	1.1	0.9	1.0	1.3	---	---	1.8
2	0.0	1.1	1.1	1.1	1.4	1.2	1.6	---	---	1.7
3	0.0	0.9	1.0	1.0	0.8	1.0	1.3	---	---	1.3

Table 13 | Phosphates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.40	0.01	0.01	0.01	0.01	0.01	0.01	---	---	0.01
2	0.40	0.00	0.01	0.04	0.01	0.04	0.02	---	---	0.01
3	0.49	0.00	0.01	0.03	0.01	0.01	0.01	---	---	0.01

Table 14 | Nitrates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 0.4 P/L test during 144 hours.

	(mg/L) NO_3 – N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	3.0	6.5	6.5	7.5	9.0	13.5	---	---	16.0
2	0.0	3.4	7.2	7.0	7.0	10.0	12.0	---	---	14.5
3	0.0	3.5	6.2	7.0	8.5	11.0	13.0	---	---	14.0

10 mg/L of phosphate in mili-Q water

Table 15 | Phosphates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the 10 mg P/L test.

	(mg/L) PO_4^{3-} - P								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	10.20	9.64	10.00	9.50	9.50	9.50	9.50	9.50	9.55
2	9.45	9.80	9.65	9.60	10.00	9.60	9.50	9.80	10.05
3	9.90	9.70	10.00	9.55	9.45	9.45	9.45	9.40	9.40

Table 16 | Nitrates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the 10 mg P/L test.

	(mg/L) NO_3 – N								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
CTL1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CTL2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CTL3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 17 | Phosphates concentration (in mg/L) observed when added 5 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	9.40	8.30	8.40	7.80	7.90	7.75	8.35	7.95	8.10	8.00
2	9.60	9.00	9.10	8.85	8.75	8.75	8.85	8.55	8.75	8.70
3	8.55	7.85	7.95	7.90	7.90	7.70	7.90	7.90	7.90	7.90

Table 18 | Nitrates concentration (in mg/L) observed when added 5 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) NO_3 – N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
2	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
3	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 19 | Phosphates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	10.05	7.45	6.55	6.45	6.30	6.35	6.35	5.85	5.85	5.85
2	9.65	7.25	6.10	6.00	5.95	5.70	5.50	5.30	5.40	5.15
3	9.40	6.40	6.15	6.15	6.15	6.00	5.50	5.30	5.45	5.10

Table 20 | Nitrates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) NO_3 – N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
2	0.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
3	0.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Table 21 | Phosphates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	9.40	0.01	0.01	0.00	0.01	0.01	0.03	---	---	0.01
2	9.60	0.01	0.01	0.01	0.01	0.01	0.00	---	---	0.01
3	9.20	0.01	0.01	0.01	0.01	0.01	0.01	---	---	0.01

Table 22 | Nitrates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) NO_3 - N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	5.0	10.0	10.5	9.5	12.0	13.5	---	---	17.0
2	0.0	6.5	10.0	11.0	13.5	13.5	14.5	---	---	18.0
3	0.0	6.5	10.0	11.5	10.0	13.0	12.5	---	---	15.5

Lake water

Table 23 | Phosphates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the lake water test.

	(mg/L) PO_4^{3-} - P								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	7.55	7.85	7.65	7.75	7.95	7.60	7.70	8.30	7.60
2	7.80	7.75	7.80	8.05	7.80	7.50	7.75	9.05	7.70
3	7.70	7.70	7.75	7.90	7.65	7.50	7.80	7.60	7.65

Table 24 | Nitrates concentration (in mg/L) observed in the control solutions (1, 2 and 3) during 144 hours of the lake water test.

	(mg/L) NO_3 - N								
	0*	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1

Table 25 | Phosphates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	8.05	7.15	6.45	6.35	6.15	5.60	5.00	4.90	4.75	4.65
2	7.90	7.25	6.60	6.10	6.10	5.75	5.05	5.05	4.65	4.95
3	7.80	7.00	6.35	6.55	6.15	6.40	5.10	5.25	4.75	4.95

Table 26 | Nitrates concentration (in mg/L) observed when added 50 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) NO_3 - N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	4.5	2.0	1.5	2.0	2.0	4.0	4.5	7.5	3.5
2	0.1	1.0	1.0	1.5	1.5	1.5	1.5	2.0	2.5	2.5
3	0.1	1.5	1.5	1.0	2.0	2.0	2.0	2.0	2.5	2.5

Table 27 | Phosphates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	7.55	4.20	2.05	1.20	0.85	0.60	0.30	0.40	0.45	0.40
2	7.65	4.20	1.70	1.20	0.90	0.60	0.35	0.45	0.50	0.50
3	7.55	3.90	2.30	1.55	1.05	0.60	0.60	0.55	0.60	0.65

Table 28 | Nitrates concentration (in mg/L) observed when added 500 mg of non-calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) NO_3 - N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	9.0	10.0	10.0	10.5	10.5	15.0	14.5	12.5	16.0
2	0.0	8.5	9.5	9.5	9.5	10.5	12.0	13.0	13.5	14.0
3	0.0	8.0	9.5	9.0	9.5	9.5	12.5	12.0	12.5	13.0

Calcined Zn-Al LDH

10 mg/L of phosphate in mili-Q water

Table 29 | Phosphates concentration (in mg/L) observed when added 250 mg of calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	9.50	7.90	7.90	7.60	7.40	6.90	3.85	2.56	0.85	0.30
2	9.20	8.30	7.55	7.30	7.30	6.35	3.70	2.25	0.60	0.10
3	9.30	8.60	7.75	7.55	7.40	6.75	3.70	2.50	0.45	0.05

Table 30 | Nitrates concentration (in mg/L) observed when added 250 mg of calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) NO_3 - N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.0	5.5	4.0	3.5	3.0	4.0	6.0	7.0	6.0	5.0
2	0.0	4.5	4.0	3.0	3.0	3.5	4.5	4.5	4.5	4.0
3	0.0	4.0	3.5	3.0	2.5	4.0	4.0	4.0	4.0	4.0

Table 31 | Phosphates concentration (in mg/L) observed when added 2500 mg of calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) PO_4^{3-} - P								
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**
1	9.15	7.05	3.85	3.50	3.30	2.05	0.00	0.00	0.00
2	9.35	6.70	4.05	3.40	2.75	0.80	0.00	0.00	0.00
3	9.15	6.45	3.95	3.15	2.30	1.10	0.00	0.00	0.00

Table 32 | Nitrates concentration (in mg/L) observed when added 2500 mg of calcined Zn-Al LDH (1, 2 and 3) to the 10 P/L test during 144 hours.

	(mg/L) NO_3 - N								
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**
1	0.0	42.5	40.0	35.0	30.0	30.0	27.5	47.5	42.5
2	0.0	40.0	32.5	32.5	27.5	27.5	25.0	37.5	30.0
3	0.0	40.0	37.5	35.0	32.5	25.0	25.0	37.5	25.0

Lake water

Table 33 | Phosphates concentration (in mg/L) observed when added 250 mg of calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) PO_4^{3-} - P									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	7.45	6.10	5.35	4.95	4.60	3.45	0.60	0.50	0.50	0.45
2	7.55	6.25	4.45	3.95	3.25	1.80	0.65	0.80	0.55	0.55
3	7.50	6.30	4.25	3.70	2.70	1.40	0.45	0.50	0.45	0.35

Table 34 | Nitrates concentration (in mg/L) observed when added 250 mg of calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) NO_3 - N									
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**	**144**
1	0.1	4.0	4.5	5.0	5.0	4.0	7.5	21.0	55.0	7.5
2	0.0	4.0	4.0	4.0	4.5	4.5	4.5	7.0	10.0	4.0
3	0.1	4.5	4.5	5.5	5.0	4.5	5.0	5.5	6.0	5.0

Table 35 | Phosphates concentration (in mg/L) observed when added 2500 mg of calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) PO_4^{3-} - P								
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**
1	7.75	0.70	0.01	0.01	0.00	0.00	0.00	0.00	0.01
2	7.70	0.70	0.01	0.00	0.02	0.01	0.00	0.01	0.00
3	7.65	0.40	0.03	0.01	0.00	0.00	0.00	0.00	0.00

Table 36 | Nitrates concentration (in mg/L) observed when added 2500 mg of calcined Zn-Al LDH (1, 2 and 3) to the lake water test during 144 hours.

	(mg/L) NO_3 – N								
	0*	**0**	**0.25**	**0.50**	**1**	**3**	**24**	**48**	**72**
1	0.1	65.0	55.0	55.0	55.0	47.5	67.5	80.0	60.0
2	0.1	60.0	57.5	57.5	50.0	47.5	67.5	60.0	60.0
3	0.1	67.5	70.0	55.0	55.0	47.5	70.0	60.0	62.5

Table 37 | Removal percentage (%) when added 5, 50 and 500 mg of non-calcined Zn-Al LDH (respectively) to the 0.4 mg P/L solution.

Time (h)	Removal (%)
0*	0.0
0	21.7
0.25	20.8
0.5	17.5
1	25.8
3	25.8
24	34.2
48	56.7
72	49.7
144	57.5

Time (h)	Removal (%)
0*	0.0
0	97.5
0.25	90.0
0.5	95.0
1	96.7
3	96.7
24	97.5
144	97.5

Time (h)	Removal (%)
0*	0.0
0	99.2
0.25	97.5
0.5	93.2
1	97.5
3	95.5
24	96.7
144	97.5

Table 38 | Removal percentage (%) when added 5, 50 and 500 mg of non-calcined Zn-Al LDH (respectively) to the 10 mg P/L solution.

Time (h)	Removal (%)
0*	0.0
0	8.7
0.25	7.6
0.5	10.9
1	10.9
3	12.2
24	8.9
48	11.4
72	10.2
144	10.7

Time (h)	Removal (%)
0*	0.0
0	27.5
0.25	35.4
0.5	36.1
1	36.8
3	38.0
24	40.4
48	43.5
72	42.7
144	44.7

Time (h)	Removal (%)
0*	0.0
0	99.9
0.25	99.9
0.5	99.9
1	99.9
3	99.9
24	99.9
144	99.9

Table 39 | Removal percentage (%) when added 50 and 500 mg of non-calcined Zn-Al LDH (respectively) to the lake water solution.

Time (h)	Removal (%)
0*	0.0
0	9.9
0.25	18.3
0.5	20.0
1	22.5
3	25.3
24	36.2
48	36.0
72	40.4
144	38.7

Time (h)	Removal (%)
0*	0.0
0	45.9
0.25	73.4
0.5	82.6
1	87.7
3	92.1
24	94.5
48	93.9
72	93.2
144	93.2

Table 40 | Removal percentage (%) when added 250 and 2500 mg of calcined Zn-Al LDH (respectively) to the 10 mg P/L solution.

Time (h)	Removal (%)
0*	0.0
0	11.4
0.25	17.1
0.5	19.8
1	21.1
3	28.6
24	59.8
48	73.6
72	93.2
144	98.4

Time (h)	Removal (%)
0*	0.0
0	26.9
0.25	57.1
0.5	63.7
1	69.8
3	85.7
24	100.0
48	100.0
72	100.0

Table 41 | Removal percentage (%) when added 250 and 2500 mg of calcined Zn-Al LDH (respectively) to the lake water solution.

Time (h)	Removal (%)
0*	0.0
0	17.1
0.25	37.6
0.5	44.0
1	53.1
3	70.4
24	92.4
48	92.0
72	93.3
144	94.0

Time (h)	Removal (%)
0*	0.0
0	92.2
0.25	99.8
0.5	99.9
1	99.9
3	100.0
24	100.0
48	100.0
72	100.0