Evaluation of ecotoxicity of carbon nanomaterials with polyoxometalates

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AbstractRecent research has shown the potential of nanomaterials in the environmental field, because their high specific surface area provides improved efficiency to several processes. Although their application in soil remediation and water treatment seems promising, information about their possible toxic effects in the aquatic ecosystems is still sparse. The toxicity of three polyoxometalates ($[PMo_{12}O_{40}]^{3-}$(PMo_{12}), $[PMo_{11}VO_{40}]^{4-}$($PMo_{11}V$),$[PMo_{10}V_2O_{40}]^{5-}$($PMo_{10}V_2$)), graphene flakes (GF) and the three polyoxometalates immobilized on GF (PMo_{12}@GF, $PMo_{11}V$@GF, $PMo_{10}V_2$@GF), which were developed to be used as catalysts on photo-oxidation processes, was evaluated towards the alga *Pseudokirchnerielasubcapittata*, a recommended species for ecotoxicity tests. The experimental evaluation was carried out according to the inhibition test for algae (EC Regulation 440/2008, which was based on the OCDE Guideline 201). This is based on determining the effect in growth inhibitionof the algae culture (*P. subcapitata*) exposed to the nanomaterial according to the exposure concentration of material, compared with a control culture. Determination of *in vivo* chlorophyll content by fluorescence, which is a fast and sensitive method, was used to estimate cell density. The most toxic material was the one with higher content of vanadium ($PMo_{10}V_2$@GF), for which 6.5 mg/L is the effective concentration that causes inhibition to 50% of the alga population.

Keywords: Ecotoxicity, growth inhibition, polyoxometalates,*Pseudokirchneriellasubcapitata*

1. Introduction

The presence of pharmaceuticals at trace amounts in theaquatic environment has being reported, showing the need and urgency to increase research in this area, mainly in the development of new treatment technologies(Santos *et al.*, 2010).Conventional domestic wastewater treatment systems do not achievethe necessary removal efficiency, so there is a need to develop efficient, sustainable and, if possible, economical treatment processes.

In the last decade of the twentieth century there was an amazing growth in the area of nanotechnology with application in several fields. Particles of the order of a few nanometers may have properties different from those observed on a macroscopic scale(Zarbin andOliveira, 2013). The reduction of particle size to nanometric dimensions increases the surface area of the material, improving the response time and efficiency in solid-liquid interface applications(Eder, 2010), such as in wastewater treatment.

Graphene based nanomaterials have interesting properties.Graphene is a single-layer solid withlamellar carbon structure where each carbon atom is attached to another three in a honeycomb lattice. This material has attracted wide interest in many fields from energy to sensing, owing to its outstanding electrical, optical, mechanical, thermal and electrochemical properties. Graphene is an ideal material in the electrochemistry field, owing to its large electrochemical potential window, its good electron-storage/-accepting properties, as well as its high specific surface area, and to the ability to act as an excellent matrix to anchor different molecular species. Amongst many other applications, graphene is a material that can be used in water treatment processes, namely as a photocatalyst in the decomposition of organic compounds(Zarbin andOliveira, 2013).

Polyoxometalates (POMs) are a class of inorganic compoundswith exceptional functionalities and applications in several fields such as catalysis, sensors, biochemistry, photochromic materials, among others(Luo andYang, 2011). Bulk ion agglomerates with a tetrahedral central phosphate (PO_4) structure surrounded by 12 molybdenum oxoanions form a closed structure, known as Keggin structure(Cuentas-Gallegos *et al.*, 2006). Phosphomolybdate ions (PMo_{12})with substitutions of one or two molybdenum atoms by vanadium ($PMo_{11}V$, $PMo_{10}V_2$) show favorable electrochemical properties for the application in oxidation-reduction reactions. One of its properties is the ability to undergo multi-reversible valence reductions, leading to the formation of mixed valence species, i.e. favorable in terms of electrocatalytic properties(Fernandes andFreire, 2015), consistent with the aforementioned need for oxidation of organic compounds.On the other hand,nanocomposites based on the immobilization of these POMs in graphene flakes (POM@GF) are even more stable materials, with better electrochemical properties than the free POMs. In addition, POM@GF are more sensitive to photoreduction too. Electrocatalytic properties were evaluated in the oxidation of ascorbic acid, with POM and POM@GF electrodes (Fernandes andFreire, 2015)suggesting the feasibility of using these nanocompositesas photocatalystsfor removalof organic compounds from wastewaters. The application of

nanomaterials in the field of water treatment raises environmental concerns, since theymay cause adverseeffectson the environment. Since there is no specific legislation regarding their use, their acute and chronic toxicity should be evaluated in order to determine their interaction with living organisms, before being produced for commercial use, as recommended by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (UN, 2015).

Standardized toxicity tests are a way of projecting the hazards to which a non-target organism may be subject to a particular substance or group of substances.*Pseudokirchneriella subcapitata*is a microalgae highly sensitive to the contamination of aquatic environments. In this way, it is recommended as a preferred organism in ecotoxicity tests by United States Environmental Protection Agency (EPA) and Organization for Economic Co-operation and Development (OECD). The main goal of this work was the evaluation of chronic ecotoxicity of seven nanomaterials, three free polyoxometalatesand immobilized on graphene flakes and also graphene itself, towards the microalgae *P. subcapitata*.

2. Materials and methods

The test substances, graphene flakes, POMs ($PMo_{10}V_2$, $PMo_{11}V$, PMo_{12}) used in the form of tetrabutylammonium salts, as well as the nanocomposite materials ($PMo_{10}V_2$@GF, $PMo_{11}V$@GF, PMo_{12}@GF) were prepared and characterized as described elsewhere(Fernandes and Freire, 2015).

The culture of *P. subcapitata*, strain 278/4, was obtained in theCulture Centre of Algae and Protozoa (United Kingdom).

The tests were carried out according to the algae inhibition test of the Commission Regulation EC 440/2008, which is based on the guideline OECD, test nº 201 Guidelines for the Testing of Chemical, Freshwater Alga and Cyanobacteria Growth Inhibition Test (OECD, 2011). The document Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA, 2002) was also used as guidance.

The temperature was controlled to 22± 2 °C andthe required light intensity was provided by four universal white light fluorescent lamps (Osram, L36W/865) at a distance of 0.35 m from algae cultures. All the solutions were prepared with deionized water (conductivity bellow 5 μScm^{-1}) obtained in adeionizer Millipore; model Helix 3 (France).All substances used in the preparation of the culture medium were of high purity grade (≥ 95%).The preparation of the culture medium, stock cultures and assay solutions was carried out in aseptic conditions, using a laminar flow chamber (Faster, model 30, Italy).

All the materials used were previously autoclaved (AJC, model uniclave 88, Portugal) at 121 °C for 20 min, including nanomaterials. In all the experiments an approximate cell density of 10^5cells/mL was used, for a total volume of 50 mL.A test with a reference substance, potassium dichromate, was performed in order to validate the test conditions.For each concentration of the nanomaterial assaysand controls (without test substance) were made in triplicate.Blank tests(without alga) were alsoperformed. The tests were carried out for 72 h. The shaking of the flasks, fashioned to keep the microalgae in suspension was performed by an orbital shaker (Bunsen AO-400), at a rate of 100 cycles per minute (EPA, 2002). The validation criteria foreseen in the method werethe pH variation (less than 1.5) and the test substance concentration (variation less than 20%), which was evaluated by conductivityusing a multiparameteranalyzer Consort C861 (Belgium).The pH was determined using a combined glass electrode, connected to the potentiometer Crison, micropH 2002 (Spain).

The growth of the biomass of the cultures was evaluated by determining the variation of the fluorescence.The increase in cell density (number of cells/mL= 1.70E+3*fluorescence-2.53E+5)was determined by measuring chlorophyll fluorescence *in vivo* at the beginning and end of test.Determination of the *in vivo* chlorophyll content by fluorescence is a measure recommended by EPA (2002) for its sensitivity.Fluorescence measurements were done ina Biotek Synergy HT microplate reader with Gen5 software version 2.0.18 (USA), usingan excitation/emission wavelength pair of 485/645 nm. Fluorescence determinations were performed for all experiments ona suspension volume of 0.25 mL and after 10 seconds of shaking to suspend the microalgae and the nanomaterials.

The ecotoxicityendpointsestimated were effective concentration of 50, 20 and 10% growth inhibition (EC_{50}, EC_{20}, EC_{10}).

3. Results and Discussion

Figure 1 illustrates the reduction of fluorescence of the microalgae when exposed to each of the nanomaterials.The effective concentrations EC_{50}, EC_{20} and EC_{10}were estimated for all the tested nanomaterials.Table 1 shows the obtained results.For the range of concentrations tested, no growth inhibitions below 10% were obtained for GF, $PMo_{10}V_2$@GF,$PMo_{11}V$@GF and PMo_{12}@GF, however EC_{10} were estimated by extrapolation, since the results obtained were close to 10% of growth inhibition.Nor were obtained growth inhibition results below 20% for $PMo_{10}V_2$@GF, and therefore EC_{20} and EC_{10} values wereextrapolated.

The absence of conductivity variation during the test indicated that there was no leaching of components from the nanomaterials. However their different composition affected the alga culture in different ways.

It wasfound that the most toxic materials werethose with two vanadium atoms, $PMo_{10}V_2$ and $PMo_{10}V_2$@GF. It wasalso observed a decrease in the toxicity of the nanomaterials as the number of atoms of vanadium in the material decrease. Therefore, the effect of the nanomaterials in the growth of the microalgae *P. subcapitata* increased in the following order: PMo_{12}<$PMo_{11}V$ <$PMo_{10}V_2$. The same behavior was verified for the POMs immobilized on graphene. In the comparison of POM with the corresponding POM@GF, the toxicity increases when immobilized on GF, for the POMs with vanadium atoms. However, for $PMo_{12}$the toxicity decreases after immobilization (PMo_{12}@GF).

From all the nanomaterials tested,graphene is the material that has the lowest toxicity, showing an EC_{50} of 262 mg/L., almost twice the least toxic POM ($PMo_{12}V$@GF) with an EC_{50} of 142 mg/L.

Table 1: Ecotoxicity parameters of the studied nanomaterials

Compound	EC_{10}(mg/L)	EC_{20}(mg/L)	EC_{50}(mg/L)
GF	200*	214	262
$PMo_{10}V_2$	3.0	4.0	9.9
$PMo_{11}V$	13.1	19.0	57.8
PMo_{12}	28.6	40.2	111
$PMo_{10}V_2$@GF	2.0*	2.7*	6.5
$PMo_{11}V$@GF	4.4	11.8	51.1
PMo_{12}@GF	43.4*	58.3	142

* Extrapolated values

From the results it can be concluded that the toxicity towardsthe alga *Pseudokirchnerielasubcapittata,*increased for POMs with vanadium and for the corresponding graphene-based nanomaterials, which suggests that vanadium has a strong contribution to the toxicity of POMs and POM@GF nanomaterials tested. Moreover, according to the National Institute for Public Health and the Environment of the Netherlands, the presence of vanadium in water poses environmental risks,which is leading to a revision of the water quality standards in accordance with the Water Framework Directive(Smit, 2012).

Therefore, in the future, other metals should be used in the synthesis of polyoxometalates, in order to reduce their ecotoxicity.

4. Conclusion

The highest toxicity observed corresponded to the $PMo_{10}V_2$@GF, with an EC_{50} value of 6.5 mg/L and an EC_{10} of 2.0 mg/L. It was also verified that, with the decrease of the number of vanadium atoms in the composition, the toxicity of the nanomaterialalso decreased in both the free POM and the POM@GF.

In accordance with the GHS classification the results of chronic toxicity obtained suggest that: $PMo_{10}V_2$, and $PMo_{10}V_2$@GF are toxic to aquatic organisms; PMo_{11} and $PMo_{11}V$@GF are harmful to aquatic organisms; PMo_{12},PMo_{12}@GF and GF may cause possible harmful effects in aquatic organisms, being GF the least toxic.

If applied for the removal of organic compounds from wastewaters, the problem of theremoval of the nanomaterials after treatment should be a matter of concern. The concentrations of these materials in the discharge of the treated wastewater may not exceed the values of EC_{10} in the aquatic environment. Besides the chronic toxicity for microalgae, it would be advisable to perform ecotoxicity tests with other trophic levels, in order to better understand the impact of this kind of nanomaterials in the aquatic ecosystems.

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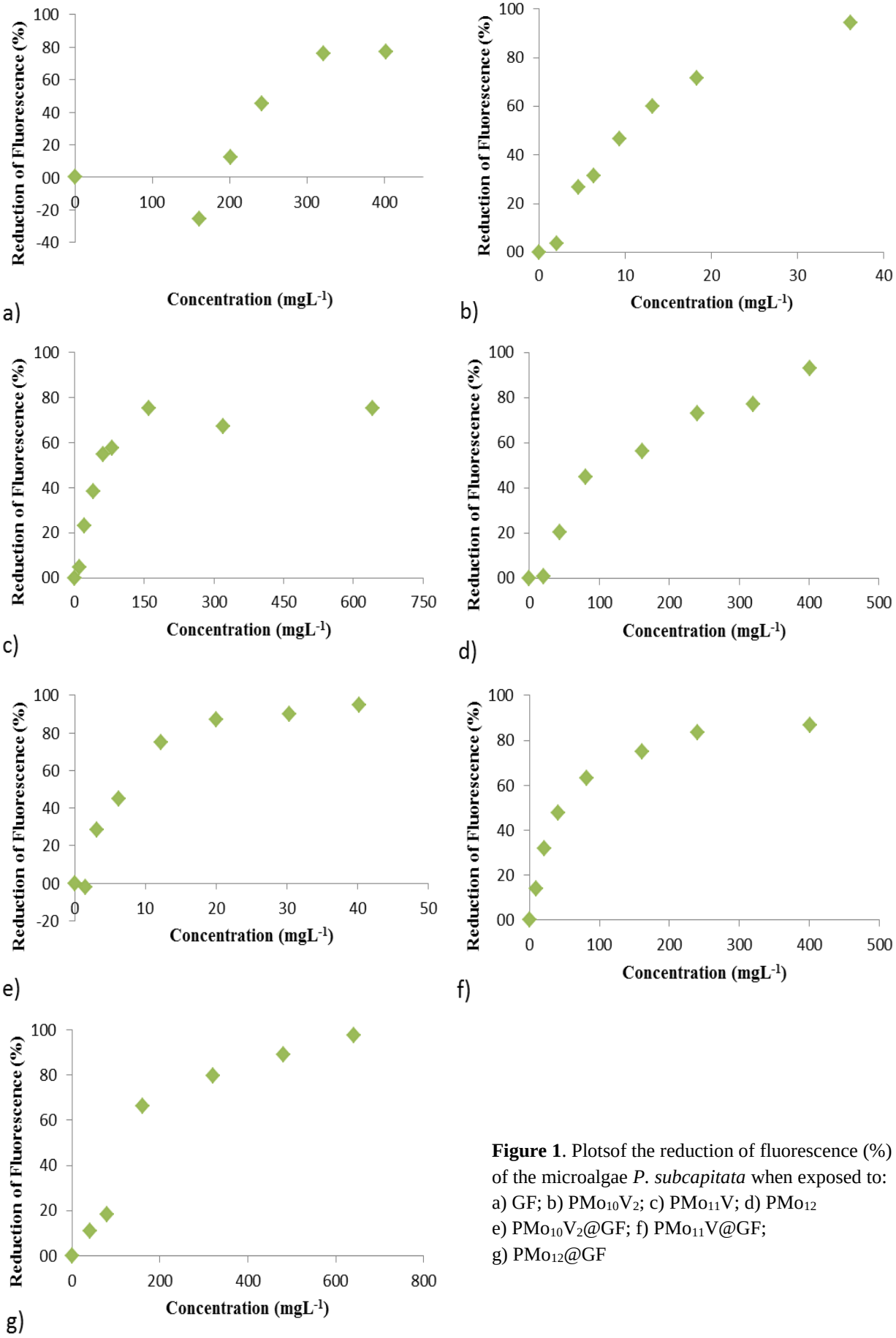

Figure 1. Plotsof the reduction of fluorescence (%) of the microalgae *P. subcapitata* when exposed to: a) GF; b) $PMo_{10}V_2$; c) $PMo_{11}V$; d) PMo_{12} e) $PMo_{10}V_2$@GF; f) $PMo_{11}V$@GF; g) PMo_{12}@GF

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