Ecotoxicological evaluation of magnetic ionic liquids

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ABSTRACT

Although magnetic ionic liquids (MILs) are not yet industrially applied, their continued development and eventual commercial use may lead to their appearance into the aquatic ecosystem through accidental spills or effluents, consequently promoting aquatic contaminations. Furthermore, the deficient information and uncertainty surrounding the environmental impact of MILs could be a major barrier to their widespread industrial application and international registration. Thus, in the present work, a range of cholinium salt derivatives with magnetic properties was synthesized and their ecotoxicity was evaluated towards the luminescent bacteria *Vibrio fischeri*. The results suggest that all MILs structures tested are moderately toxic, or even toxic, to the bacteria. Furthermore, their toxicity is highly dependent on the structural modifications of the cation, namely the alkyl side chain length and the number of hydroxyethyl groups, as well as the atomic number of the metal anion. Finally, from the magnetic anions evaluated, the $[MnCl_4]^{2-}$ is the less toxic. In order to improve the knowledge for the prospective design of environmentally safer MILs, it is important to expand this study to other aquatic organisms at different trophic levels.

1. Introduction

The increasing attention that ionic liquids (ILs) are attracting is justified by their unique properties, such as their negligible vapor pressure, high chemical and thermal stability, their non-flammability, high ionic conductivity, wide electrochemical potential window and high solvation ability (Rogers and Seddon, 2003). Furthermore, these ionic compounds are considered as "designer solvents" due to their tunable properties, which means that they can be designed for a specific application by the proper combination of the adequate cation and anion (Rogers and Seddon, 2003). Some reviews have highlighted the industrial applications of ILs as an innovative approach to "Green Chemistry" and sustainability (Rogers et al., 2002). However, the fact that they have a negligible vapor pressure is not enough to assure that these compounds can be considered as environmentally harmless solvents. Actually, even the most hydrophobic ILs present some solubility in water and thus, they may constitute an environmental risk to aquatic ecosystems (Nacham et al., 2015). A broad range of testing models (bacteria, fungi, algae, aquatic plants, crustaceans, mammalian cell lines and vertebrates) has been used to evaluate the toxicity of distinct ILs (Amde et al., 2015; Cvjetko Bubalo et al., 2014; Petkovic et al., 2011; Santos et al., 2015). In what concerns their aquatic toxicity,

the most tested trophic levels are decomposers represented by marine bacteria (e Silva et al., 2014; Ventura et al., 2012), producers represented by microalgae (Samorì et al., 2015; Ventura et al., 2012) and primary consumers represented by cladocerans (Samorì et al., 2015; Ventura et al., 2012). Magnetic ionic liquids (MILs) appear as an emerging class of ILs that are inherently paramagnetic, being able to respond to an external magnetic field. These magnetic compounds favorably combine the advantageous properties of ILs with magnetic characteristics, which can be interesting for applications in process and product engineering as an expanding field full of opportunities to create devices, processes and products (Clark et al., 2016; Santos et al., 2014a). In fact, MILs have been studied in fluid-fluid separations (Zhao et al., 2016), in the extraction of nucleic acids like DNA (Clark et al., 2015), in chemical reactions, namely as catalysts (Bica and Gaertner, 2008), reaction medium (Mohammad Fauzi et al., 2014) and solvents (Zakrzewska et al., 2013), in polymer chemistry (Dobbelin et al., 2011), in electrochemical devices (Branco et al., 2011) and as magnetic fluids (Guerrero-Sanchez et al., 2007). The paramagnetic properties of MILs may be located on the anion, cation or both. Their synthesis is normally based on the use of transition metals or lanthanide complexes in the anion structure (Santos et al., 2014a). The first MIL, namely the 1-butyl-3-methylimidazolium tetrachloroferrate, [C₄mim]

[FeCl$_4$] was reported in 2004 (Hayashi and Hamaguchi, 2004). Since then, other MILs based on transition-metal coordination complexes such as Fe(III) (Del Sesto et al., 2008; Santos et al., 2014b; Yoshida and Saito, 2006), Co(II) (Del Sesto et al., 2008; Pitula and Mudring, 2010; Santos et al., 2014b) and Mn(II) (Del Sesto et al., 2008; Pitula and Mudring, 2010) have been reported. Furthermore, lanthanide complexes, like gadolinium (Gd), neodymium (Nd) and dysprosium (Dy), have also emerged due to their strong response to an external magnetic field and, in some cases, luminescent properties (Mallick et al., 2008; Santos et al., 2014b). In general, these magnetic anions have been combined with imidazolium- (Yoshida and Saito, 2006), phosphonium- (Del Sesto et al., 2008; Pitula and Mudring, 2010; Santos et al., 2014b), cholinium- (Frade et al., 2013; Zakrzewska et al., 2013) and ammonium-based cations (Zhang et al., 2012). Recently, the first magnetic chiral ILs, which simultaneously contain chiral and magnetic properties, have been synthesized by the simple combination of a chiral cation with the tetrachloroferrate magnetic anion (Li et al., 2009). Considering not only the variability of structures and properties (including the magnetic ones) and the importance of MILs in different applications using water as the principal media, it seems to be crucial to analyze and evaluate their (eco)toxicity. In this context, Luis et al. (2011) have evaluated the ecotoxicity of some MILs towards the *Vibrio fischeri* (*V. fischeri*) bioluminescent marine bacteria. In addition, a Quantitative Structure-Activity Relationship (QSAR) based on group contribution methods was applied to describe the influence of the molecular structure of common ILs and MILs on the (eco)toxicity (Luis et al., 2011). The authors have shown that the presence of iron in the molecular structure of imidazolium-based ILs leads to an increase in ecotoxicity (Luis et al., 2011). Alvarez-Guerra and Irabien (2011) reported a new approach for estimating the (eco)toxicity of ILs, including MILs, using a Partial Least Squares-Discriminant Analysis (PLS-DA). In this study, the [FeCl$_4$]$^-$ anion had the most severe influence on the PLS-DA model as positive discriminator, being more toxic than toluene, taken as reference for common volatile solvents. Despite the initial efforts carried to offer a preliminary insight into the environmental behavior of MILs, data on their ecotoxicity is still scarce, and needs to be expanded to improve the knowledge for the adequate design of safer MILs. Moreover, the (eco)toxicological hazard profile of ILs with industrial potential must address a set of different rules, including those related with the regulatory demands defined by the European Union regulation for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) (EC, 2007). In this context, the present study proposes the ecotoxicological assessment of a series of MILs based on the cholinium derivative cation in combination with [FeCl$_4$]$^-$, [MnCl$_4$]$^{2-}$, [CoCl$_4$]$^{2-}$ and [GdCl$_6$]$^{3-}$ anions, towards the *V. fischeri* marine bacteria. This work is part of an integrated study being performed by our group in which different cholinium IL structures have been analyzed. The purpose is not only to evaluate the effect of different anions conjugated with the cholinium cation but also to infer about the benign toxicological character normally claimed for cholinium-based ILs and derivatives when this structure is conjugated with magnetic anions.

2. Experimental

2.1. Materials

All solvents were distilled prior to use. All chemicals were purchased from Sigma-Aldrich.

2.2. General method for the synthesis of MILs

2.2.1. General method for cholinium chloride derivative based ILs

Synthesis of [N$_{x,x,x,x}$]Cl (x = alkyl or ethanol) was adapted from (e Silva et al., 2014; Rosatella et al., 2016). In a closed vessel at room temperature, it was added *N*-methyldiethanolamine (143 mmol), or *N*-dimethylethanolamine (143 mmol) to the correspondent alkyl chloride (1–4 mol eq.), and sodium iodide (10–30 mol eq.). In some procedures acetonitrile was used as a solvent. The solution was heated at 60–80 °C for 1–7 days. The solvent was removed on a rotary evaporator. The resulted salt was dissolved in dichloromethane and the sodium halide removed by filtration. The obtained salt was dried under vacuum and used in the next step without further purification. For more details see the Supporting Information.

2.2.2. General method for magnetic ILs [N$_{1,1,n,2(OH)}$][MCl$_y$] and [N$_{1,n,2(OH),2(OH)}$][MCl$_y$]

The MILs were prepared using the reported procedures with some modifications (Del Sesto et al., 2008; Frade et al., 2013; Rosatella et al., 2016). The metal chloride hydrated salt MCl$_y$·H$_2$O (1 equiv. for FeCl$_3$·6H$_2$O; 0.5 equiv. for CoCl$_2$·6H$_2$O and MnCl$_2$·4H$_2$O, and 0.3 equiv. for GdCl$_3$·6H$_2$O) was added to a solution of cholinium derivative chloride salt (10 mmol) in methanol (20 mL). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated on a rotary evaporator at 50 °C, and then kept under vacuum for 48 h at 1–4 × 10^{-2} mbar (rotatory pump) and for 48 h at 6 × 10^{-5} mbar with stirring at 50 °C. More detailed information about the synthesis of MILs is reported in the Supporting Information, namely in Table S1.

2.3. Standard Microtox® liquid-phase assays

In order to evaluate the ecotoxicity of the MILs prepared, the Standard Microtox® liquid-phase assay was applied in this work. The Microtox® test is based on the evaluation of the bioluminescence inhibition considering the bacteria *V. fischeri* (strain NRRLB-11177) tested at 15 °C. The bacteria was exposed to a range of diluted aqueous solutions (from 0 to 81.9 wt%) of each tested compound, where 100% corresponds to the stock solution concentration. The light output of *V. fischeri* was measured after 5, 15 and 30 min of exposure to each MIL, and compared with the light output obtained for the blank control sample, an aqueous solution without the presence of any MIL (Figs. 1–4). Then, the corresponding 15 min- and 30 min-EC$_{50}$ values considered as the most reliable parameters to take into account (concentration of MIL which promotes 50% of bacteria luminescence inhibition), plus the corresponding 95% confidence intervals, were estimated for each compound tested by a non-linear regression, and using the least-squares method to fit the data to the logistic equation. Considering that the luminescence changes as a direct function of the bacteria's overall viability, the lower the EC$_{50}$ value, the higher the MIL toxicity. In order to obtain the correct EC$_{50}$ values, prior to Microtox® testing, the water content of each MIL was determined by Karl Fischer (KF) titration using a Metrohm 831 KF coulometric titrator.

3. Results and discussion

Distinct cholinium and cholinium derivatives, with different number of hydroxyethyl groups and lengths of the alky side chain, were combined with the magnetic anions [FeCl$_4$]$^-$, [MnCl$_4$]$^{2-}$, [CoCl$_4$]$^{2-}$, [GdCl$_6$]$^{3-}$ in order to obtain 24 MILs, whose toxicities were tested against the marine bacteria (Fig. S1). All MILs were soluble in water for the range of concentrations used. The ecotoxicological impact of these MILs was evaluated using the standard Microtox® acute assay. Although this is not a standard toxicity test defined in the European Union legislation and by REACH, it is nevertheless a quick and cost-efficient first-approach to evaluate the toxicity of various compounds. Moreover, this is a methodology well accept in the development of QSAR models for the prediction of ILs toxicity (Das et al., 2016, 2015; Luis et al., 2007), and the QSAR approach for toxicity predictions is encouraged by REACH legislation of the European Union. This assay has been widely used to evaluate the toxicity of ILs, including cholinium derivatives (e Silva et al., 2014; Ventura et al., 2012). This test is based on the bioluminescent bacteria *V. fischeri*, with the luminescence being a
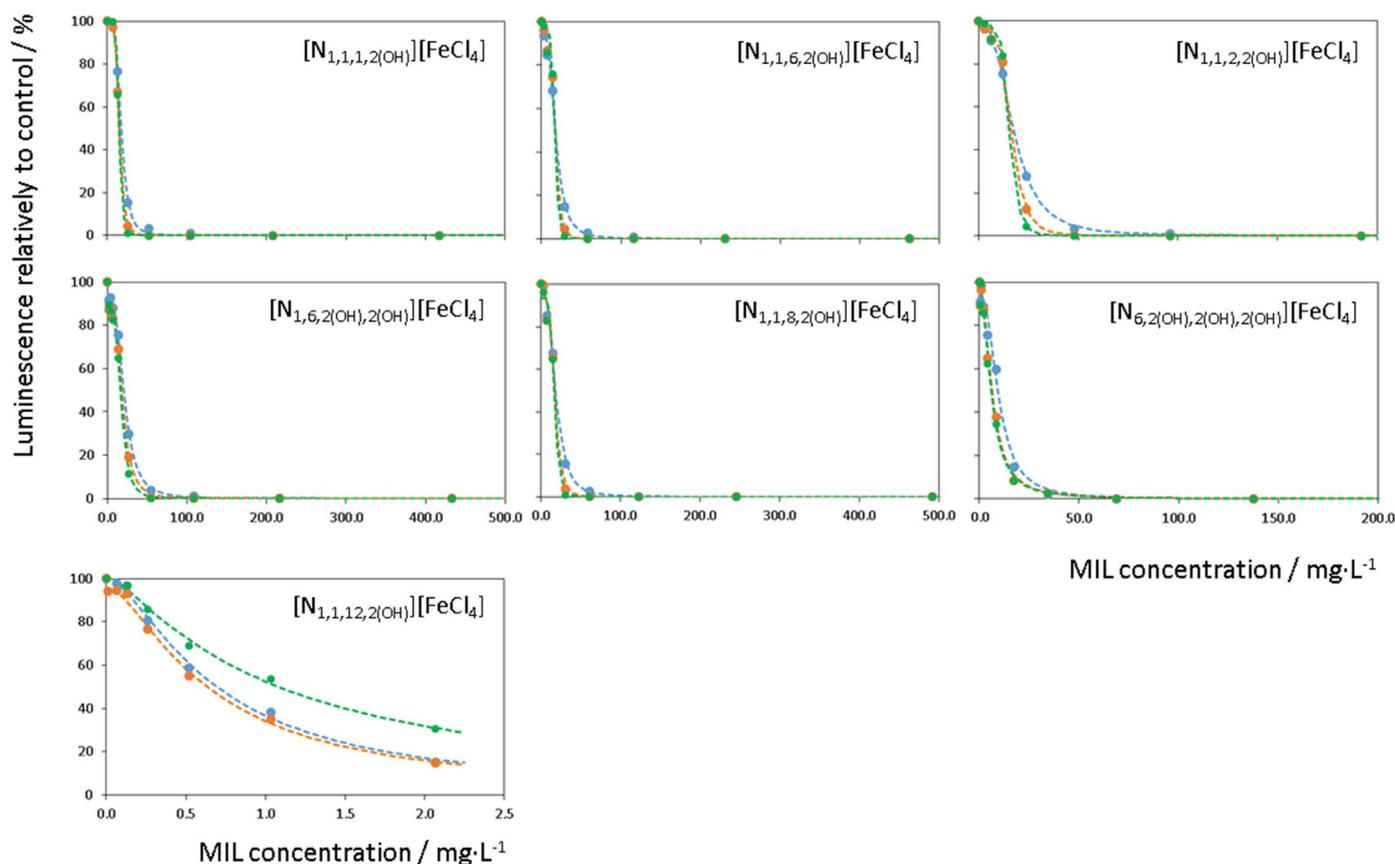
Fig. 1. Concentration response plot for [FeCl$_4$]-based MILs after (⬤) 5, (⬤) 15 and (⬤) 30 min of exposure to the luminescent marine bacteria *V. fischeri*. The standard error associated to these results is lower than 10%.

natural part of its metabolism. When exposed to a toxic compound, the respiratory process of the bacteria is disturbed, reducing the light output, causing the bioluminescence inhibition correlated with the toxicity. Thus, EC$_{50}$ values (mg. L^{-1}), the estimated concentration yielding a 50% reduction in the luminescence, were determined for the synthetized MILs after 15 and 30 min of exposure to the marine bacteria (Table 1 and S2). The results obtained for the MILs composed of [CoCl$_4$]$^{2-}$ and [GdCl$_6$]$^{3-}$ anions, [N$_{1,1,1,2(OH)}$]$_2$[MnCl$_4$] and [N$_{1,6,2(OH),2(OH)}$]$_2$[MnCl$_4$] show a decreasing order of the EC$_{50}$ values with the increase of the exposure time from 15 to 30 min. This behavior can be justified by their toxicokinetics (see Ventura et al., 2014), that means the passage of the MILs troughout the cell wall increased over the time and/or the sorption of the MILs to the bacterial surface increased over the time, both disturbing the physiological process of the organism. This relationship was not observed for the other compounds studied, namely those based in the [FeCl$_4$]$^-$ and [MnCl$_4$]$^{2-}$ anions, probably because the permeability ratios of Gram-negative cell wall channels were rather high in favor of the cations, being the number of charges important in this process. On the other hand, the detoxication phenomenon, described as the momentary physiological adaptation of the microorganism to the toxicant effect, which is identified during the period of exposure (Denich et al., 2003), can contribute to explain our results. The outer membrane of Gram-negative bacteria (like *V. fischeri*) plays an important role controlling the binding process (or passage) of some substances that can impair the physiology of these organisms. Among the factors that may shape this process, one can identify the charge, the chain length of the substances, the selectivity of cell wall channel, and the presence of the specific binding sites, which can contribute to fully explain our results.

In order to easily understand the impact of the chemical structure of MILs studied on their ecotoxicity, the EC$_{50}$ values obtained after 30 min of exposure were considered for further discussion, since this is the time

recommended for the toxicity analysis of chemical compounds using Microtox® assay (Denich et al., 2003). Thus, according to these values, it is possible to categorize these compounds as moderately toxic (meaning 10 mg L^{-1} < EC$_{50}$ < 100 mg L^{-1}) except for [N$_{1,1,1,2(OH)}$]$_2$[CoCl$_4$], [N$_{1,1,12,2(OH)}$][FeCl$_4$], [N$_{1,1,12,2(OH)}$]$_2$[MnCl$_4$], [N$_{1,1,12,2(OH)}$]$_2$[CoCl$_4$] and [N$_{6,2(OH),2(OH),2(OH)}$][FeCl$_4$], that are considered as toxic substances, with 1 mg L^{-1} < EC$_{50}$ < 10 mg L^{-1}. Curiously, among the MILs studied, the compounds presenting the lowest and highest toxicity against *V. fischeri* are MILs based on the [MnCl$_4$]$^{2-}$ anion, the [N$_{1,6,2(OH),2(OH)}$]$_2$[MnCl$_4$] and [N$_{1,1,12,2(OH)}$]$_2$[MnCl$_4$]. This suggests that part of the toxicity observed is derived from the cation and not the anion (see above), further supporting previous results suggesting that the cholinium derivatives are not innocuous (Santos et al., 2015; Ventura et al., 2014) contrary to several studies (Li et al., 2012; Petkovic et al., 2011).

The range of MILs studied allows the assessment of the impact of several structural features on their ecotoxicity, such as the increase in the alkyl chain length and the addition of hydroxyethyl groups to the cation, and the impact of various magnetic anions. Fig. 5A depicts the results of toxicity towards the marine bacteria considering the series with one hydroxyethyl group and the various metal anions, with the exception of ILs based in the [GdCl$_6$]$^{3-}$, due to the absence of experimental data (for more details see Table 1). The results reported in Fig. 5A display a trend of increasing toxicity with the cation alkyl chain's length for the series [N$_{1,1,n,2(OH)}$]$_2$[MnCl$_4$] (n = 1, 6, 8, 12), as well-reported in literature for a variety of ILs (e Silva et al., 2014). This phenomenon has been justified by the enhancement of the ILs hydrophobic/lipophilic character, defined in several woks by the octanolwater partition coefficients (Montalbán et al., 2016; Ropel et al., 2005). Thus, the increase in the length of the alkyl chain makes possible their interaction with the membrane phospholipids and/or the hydrophobic domains of the membrane proteins, leading to the disruption of the
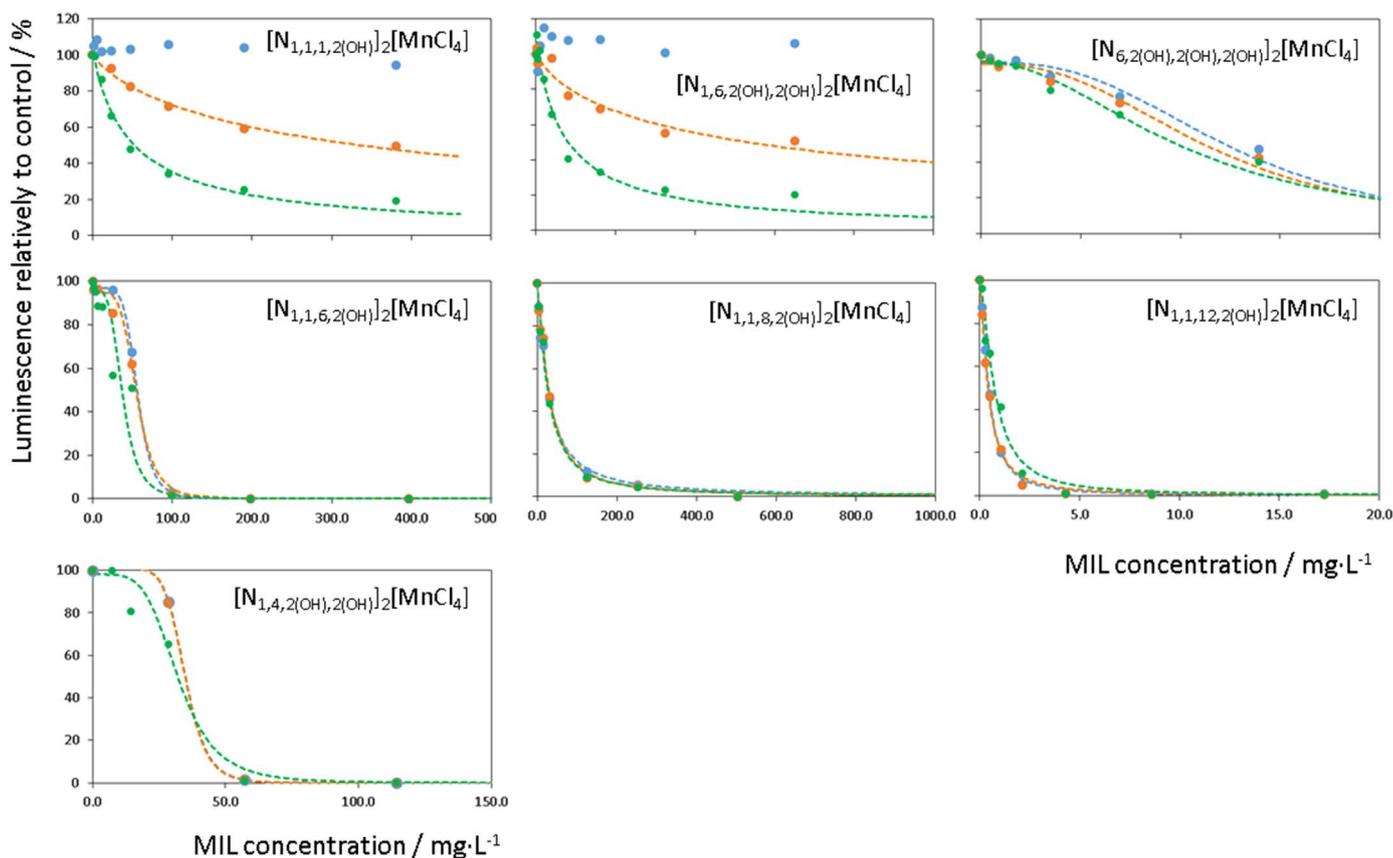
Fig. 2. Concentration response plot for $[MnCl_4]$-based MILs after (🔵) 5, (🟠) 15 and (🟢) 30 min of exposure to the luminescent marine bacteria *V. fischeri*. The standard error associated to these results is lower than 10%.

membrane physiological functions and, consequently, to cell death (Cvjetko Bubalo et al., 2014; Stolte et al., 2007). However, this dependency is less significant for the series $[N_{1,1,n,2(OH)}][FeCl_4]$ (n = 1, 2, 6, 8, 12) and $[N_{1,1,n,2(OH)}]_2[CoCl_4]$ (n =1, 8, 12), except in the transition from $[N_{1,1,8,2(OH)}]_2[CoCl_4]$ to $[N_{1,1,12,2(OH)}]_2[CoCl_4]$. Furthermore, when the alkyl chain increases from 4 to 6 carbons in the series $[N_{1,n,2(OH),2(OH)}][M]$ (M = metal anion), the EC_{50} (μM) is practically constant, if considering the confidence limits, as present in Fig. 5B. In a

previous work, the toxicity of similar series based on the bromide anion, $[N_{1,1,n,2(OH)}]Br$ (n = 2, 3, 4, 5, 6, 8, 12) and $[N_{1,n,2(OH),2(OH)}]Br$ (n = 3, 6, 12), was also assessed using the luminescent bacteria *V. fischeri* (e Silva et al., 2014). For these series based on the bromide anion, the effect of the alkyl chain length was pronounced. The EC_{50} values for the $[N_{1,1,n,2(OH)}]Br$ are also presented in Fig. 5A. These results highlight the impact of the replacement of an halide (bromide) by a metal anion, leading to a very significant increase in the toxicity against
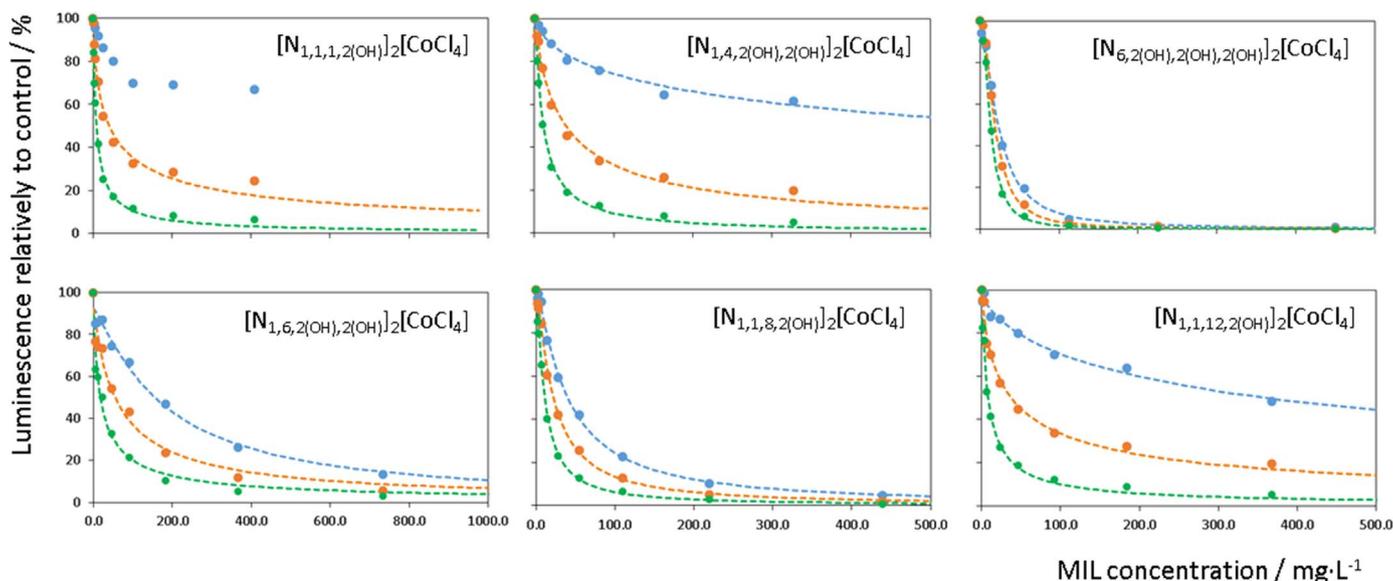

Fig. 3. Concentration response plot for $[CoCl_4]$-based MILs after (🔵) 5, (🟠) 15 and (🟢) 30 min of exposure to the luminescent marine bacteria *V. fischeri*. The standard error associated to these results is lower than 10%.
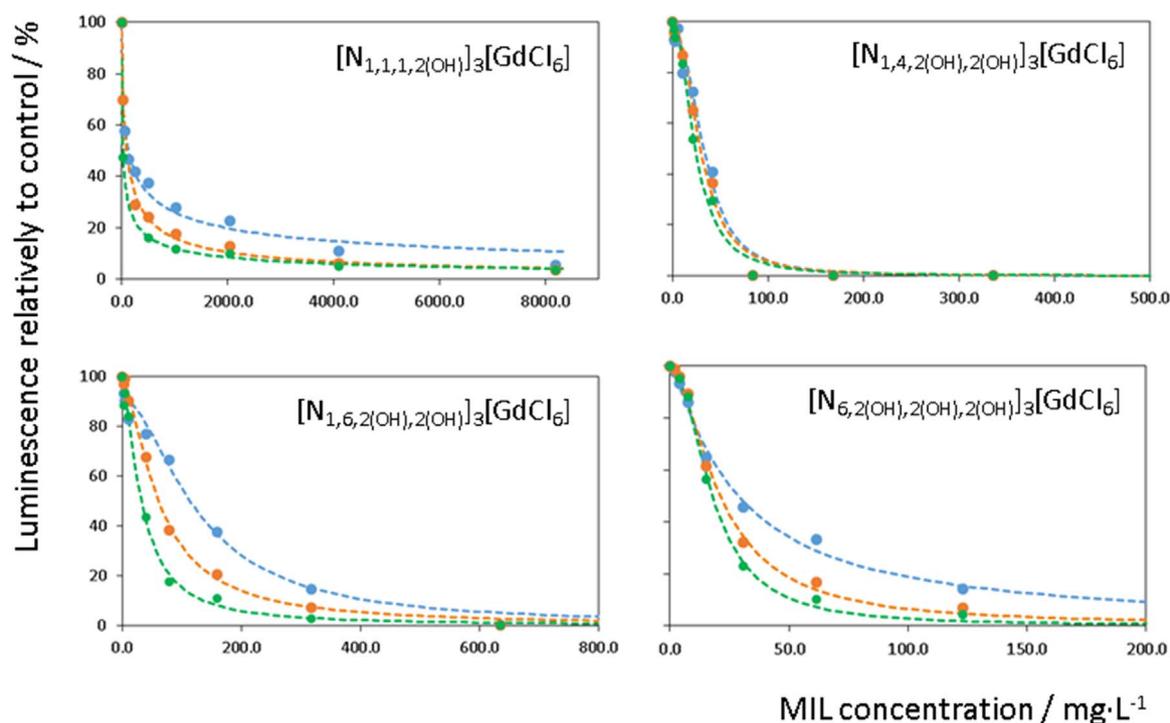
Fig. 4. Concentration response plot for [GdCl$_6$]-based MILs after (🔵) 5, (🟠) 15 and (🟢) 30 min of exposure to the luminescent marine bacteria *V. fischeri*. The standard error associated to these results is lower than 10%.

Table 1
EC$_{50}$ values (mg. L^{-1}) for MILs under study after 30 min of exposure to the luminescent marine bacteria *V. fischeri*, with the respective 95% confidence limits (in brackets). The order of ecotoxicity for the different MILs is also provided.

	EC$_{50}$ / mg L^{-1} at 30 min (lower limit; upper limit)					Order of ecotoxicity According to EC$_{50}$ (μM)
	[FeCl$_4$]$^-$	[MnCl$_4$]$^{2-}$	[CoCl$_4$]$^{2-}$	[GdCl$_6$]$^{3-}$	Br[a]	
[N$_{1,1,1,2(OH)}$]$^+$	14.17 (12.95–15.39)	48.38 (29.06–67.71)	8.90 (7.04–10.76)	26.12 (21.12–31.12)	–	[MnCl$_4$]$^{2-}$ < [FeCl$_4$]$^-$ < [GdCl$_6$]$^{3-}$ < [CoCl$_4$]$^{2-}$
[N$_{1,1,2,2(OH)}$]$^+$	15.49 (13.72–17.25)	n.d.	n.d.	n.d.	25619.07 (17592.81–33645.32)	–
[N$_{1,1,6,2(OH)}$]$^+$	17.19 (13.54–20.85)	37.51 (23.88–51.14)	n.d.	n.d.	746.30 (699.73–792.86)	[MnCl$_4$]$^{2-}$ < [FeCl$_4$]$^-$
[N$_{1,1,8,2(OH)}$]$^+$	17.49 (15.10–19.88)	27.96 (21.10–34.82)	10.75 (8.81–12.69)	n.d.	162.96 (158.59–167.32)	[MnCl$_4$]$^{2-}$ ≈ [FeCl$_4$]$^-$ < [CoCl$_4$]$^{2-}$
[N$_{1,1,12,2(OH)}$]$^+$	1.04 (0.89–1.18)	0.76 (0.53–0.98)	7.84 (5.84–9.84)	n.d.	0.8 (0.79–0.82)	[CoCl$_4$]$^{2-}$ < [FeCl$_4$]$^-$ < [MnCl$_4$]$^{2-}$
[N$_{1,4,2(OH),2(OH)}$]$^+$	n.d.	32.26 (26.56–37.96)	10.41 (8.60–12.22)	24.10 (19.62–28.58)	–	[MnCl$_4$]$^{2-}$ < [GdCl$_6$]$^{3-}$ < [CoCl$_4$]$^{2-}$
[N$_{1,6,2(OH),2(OH)}$]$^+$	16.81 (14.57–19.05)	69.54 (35.95–103.13)	18.08 (10.75–25.42)	34.17 (26.16–42.19)	276.96 (220.22–333.70)	[MnCl$_4$]$^{2-}$ < [FeCl$_4$]$^-$ ≈ [GdCl$_6$]$^{3-}$ ≈ [CoCl$_4$]$^{2-}$
[N$_{6,2(OH),2(OH),2(OH)}$]$^+$	5.99 (5.05–6.92)	10.19 (7.28–13.10)	13.11 (11.81–14.40)	17.52 (15.59–19.44)	19.74 (18.20–21.27)	[CoCl$_4$]$^{2-}$ < [GdCl$_6$]$^{3-}$ ≈ [MnCl$_4$]$^{2-}$ ≈ [FeCl$_4$]$^-$

n.d. – not determined;
[a] values from (e Silva et al., 2014).

V. fischeri (e Silva et al., 2014). The results shown in Fig. 5A indicate that, although the toxicity of the MILs is highly dependent on the magnetic anion, for long alkyl chains (n = 12), the cation toxicity becomes dominant.

The impact of the number of hydroxyethyl groups was also evaluated using the series of [N$_{1,1,6,2(OH)}$]$^+$, [N$_{1,6,2(OH),2(OH)}$]$^+$ and [N$_{6,2(OH),2(OH),2(OH)}$]$^+$ cations conjugated with the four magnetic anions under study. The results presented in Fig. 5C suggest that the insertion of hydroxyethyl groups in the cation alkyl chains increases the toxicity towards the bacteria. This trend is in accordance with what was previously observed in literature (e Silva et al., 2014; Rosatella et al., 2016). Actually, the incorporation of hydroxyl groups of oxygenated alkyl chains is being subject of distinct analysis, not only from the point

of view of potential applications but also due to their increased biodegradable nature (Coleman and Gathergood, 2010). The only exception is the transition from [N$_{1,1,6,2(OH)}$]$_2$[MnCl$_4$] to [N$_{1,6,2(OH),2(OH)}$]$_2$[MnCl$_4$]. For these cations, unlike those discussed above for the long alkyl chain, the EC$_{50}$ values show a significant increase in the toxicity of the MIL with metal containing anions, when compared with the corresponding bromide containing ILs (e Silva et al., 2014). Moreover, and contrary to what is observed in the imidazolium family functionalized with polar groups, the incorporation of oxygenated alkyl chains increases the toxicity of the cholinium derivatives (Stolte et al., 2007). This reinforces the idea that the cholinium derivatives have different mechanisms of toxicity from those of imidazolium-based ILs (Ventura et al., 2014). In order to compare the
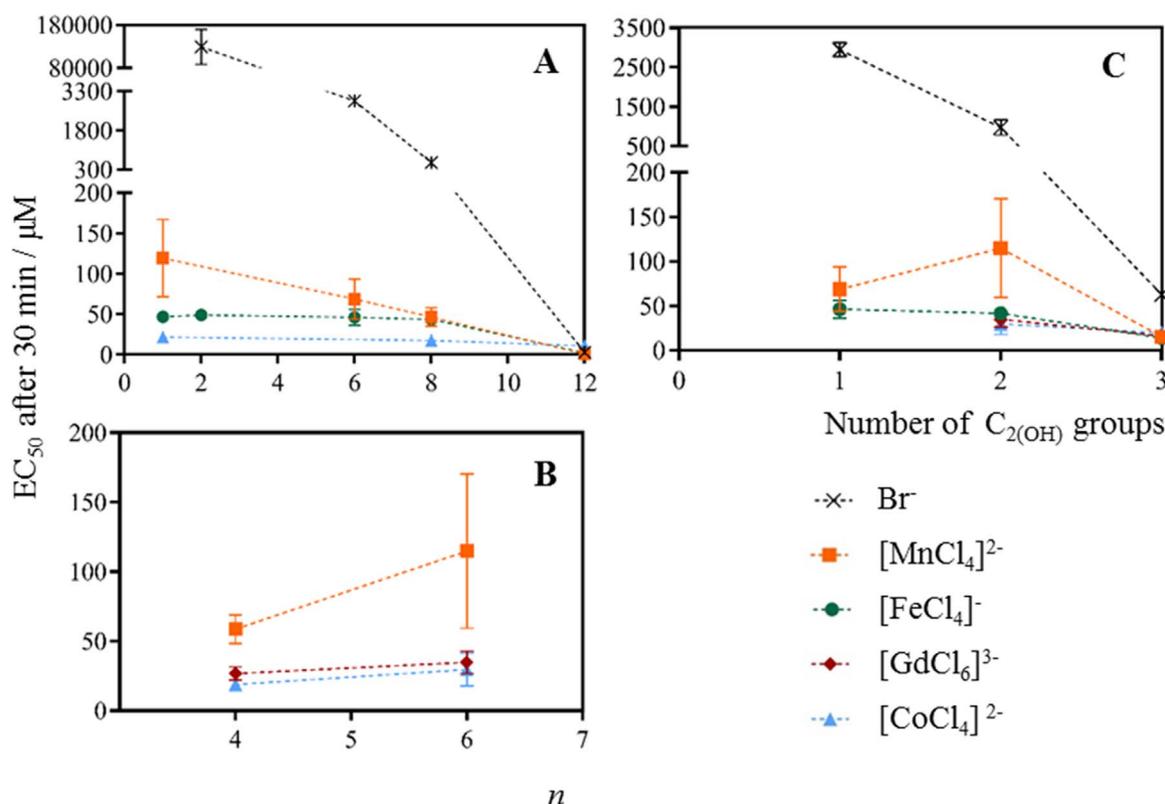
Fig. 5. The effect of the alkyl chain length on the ecotoxicity of the series: (A) $[N_{1,1,n,2(OH)}][M]$ and (B) $[N_{1,n,2(OH),2(OH)}][M]$, where n is the number of carbons and M represents the metal anion. (C) represents the effect of the number of hydroxyethyl groups present in the cations on the ecotoxicity of the MILs. The numbers 1, 2 and 3 represent $[N_{1,1,6,2(OH)}]$, $[N_{1,6,2(OH),2(OH)}]$ and $[N_{6,2(OH),2(OH),2(OH)}]$ cations, respectively. The experimental data presented for the bromide anion (Br) can be checked elsewhere (e Silva et al., 2014).

effect of the magnetic anions, the increasing order of ecotoxicity, decreasing order of EC_{50}, was determined for each cation, as shown in Table 1. From the seven sequences analyzed, the $[MnCl_4]^{2-}$ anion appears consistently as the least toxic (in five of this seven sequences) to *V. fischeri*. However, this same anion, $[MnCl_4]^{2-}$ is the most toxic when conjugated with the $[N_{1,1,12,2(OH)}]^+$ cation, which has itself a pronounced influence on the bacterial toxicity. On the other hand, the $[CoCl_4]^{2-}$ anion seems to present a contradictory behavior. Although the $[CoCl_4]^{2-}$ anion appears as the least toxic when conjugated with $[N_{1,1,12,2(OH)}]^+$ and $[N_{6,2(OH),2(OH),2(OH)}]^+$ cations, it is the anion most frequently reported as the most toxic to the bacteria. Many authors have tried to prove correlations between the physico-chemical properties of different metal ions and their toxicity (e.g., Wolterbeek and Verburg, 2001). In the present work, and not considering the $[GdCl_6]^{3-}$ anion, it seems that an increased toxicity is observed by increasing the atomic number of the metal atoms: Mn (25) < Fe (26) < Co (27). This behavior is in agreement with the results obtained by Li et al. (2013), where a positive correlation between the atomic number of the metal ion and their toxicity against *V. fischeri* was observed. However, this trend was not observed when these magnetic anions were combined with the $[N_{1,1,12,2(OH)}]^+$ and $[N_{6,2(OH),2(OH),2(OH)}]^+$ cations, which can be justified again by the high toxicity associated to these cations, which seems to dominate their toxicity.

The toxicological impact of MILs based in the $[C_8mim]^+$, $[N_{1,1,n,2(OH)}]^+$, $[N_{1,6,2(OH),2(OH)}]^+$ and $[N_{6,2(OH),2(OH),2(OH)}]^+$ cations (n = 1, 2, 3, 4, 6, 8, 12) with the metal anions studied in this work, was evaluated towards the human skin fibroblasts (CRL-1502) and/or human colorectal adenocarcinoma (CaCo-2) cell lines (Frade et al., 2013; Rosatella et al., 2016). Considering the $[C_8mim]^+$ and $[N_{1,1,n,2(OH)}]^+$-based MILs, and contrary to what was observed with the Microtox® assay, these results suggest that $[FeCl_4]^-$ and $[GdCl_6]^{3-}$ were the anions with lower cytotoxicity whereas, $[CoCl_4]^{2-}$ and $[MnCl_4]^{2-}$ were likely the most toxic (Frade et al., 2013). Nevertheless,

with regard to $[N_{1,6,2(OH),2(OH)}]^+$ cation, $[MnCl_4]^{2-}$ seems to be the less cytotoxic (Rosatella et al., 2016). This comparison supports the idea that the toxicological effect of the ILs depends not only of their structure but also on the biological system under study. Thus, in order to evaluate the aquatic toxicity more comprehensively, it is crucial to extend this study to other aquatic organisms of different trophic levels.

4. Conclusion

This work presents the ecotoxicological impact of a series of MILs based on cholinium derivative cations combined with $[FeCl_4]^-$, $[MnCl_4]^{2-}$, $[CoCl_4]^{2-}$ and $[GdCl_6]^{3-}$ anions, towards the marine bacteria *V. fischeri*. Although their interesting broad of applications, all MILs tested are considered moderately toxic or even toxic to the bacteria. The results indicate that their toxicity is highly dependent on the structural modifications of the cation, namely the alkyl side chain length and the number of hydroxyethyl groups, as well as the metal atom of the anion. Long alkyl chains, the increase in the number of hydroxyethyl groups and the increase in the atomic number of the metal atom are structural features capable of significantly increasing the toxicity of the MILs based on cholinium or cholinium derivatives. Moreover, and contrary to what was reported in previous studies with cell lines, the $[MnCl_4]^{2-}$ is the anion presenting low toxicity towards the bacteria, clearly showing that the toxicological effect of the MILs depends not only of their structure but also of the biological system under study. In this sense, it is crucial to expand this study to other aquatic organisms and different trophic levels to improve the knowledge for the prospective design of safer materials.

Acknowledgements

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.05.034.

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