applied sciences

MDPI

Article

Heavy Metal Extraction under Environmentally Relevant Conditions Using 3-Hydroxy-2-Naphthoate-Based Ionic Liquids: Extraction Capabilities vs. Acute Algal Toxicity

Philip Pirkwieser [1,2], José A. López-López [2], Michael Schagerl [3], Wolfgang Kandioller [1], Bernhard K. Keppler [1], Carlos Moreno [2] and Franz Jirsa [1,4,*]

1 Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Strasse 42, 1090 Vienna, Austria; philip.pirkwieser@univie.ac.at (P.P.); wolfgang.kandioller@univie.ac.at (W.K.); bernhard.keppler@univie.ac.at (B.K.K.)

2 Department of Analytical Chemistry, Faculty of Marine and Environmental Sciences, Instituto de Investigación Marina (INMAR), University of Cádiz, 11510 Puerto Real, Spain; joseantonio.lopezlopez@uca.es (J.A.L.-L.); carlos.moreno@uca.es (C.M.)

3 Department of Limnology and Bio-Oceanography, Faculty of Life Sciences, Althanstraße 14, 1090 Vienna, Austria; michael.schagerl@univie.ac.at

4 Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 Johannesburg, South Africa

* Correspondence: franz.jirsa@univie.ac.at

Received: 15 April 2020; Accepted: 28 April 2020; Published: 1 May 2020

Abstract: We investigated the applicability of three task-specific ionic liquids (ILs) as heavy metal extracting agents by contrasting extraction capabilities with algal toxicity. The compounds tested were trihexyltetradecylphosphonium-, methyltrioctylphosphonium- and methyltrioctylammonium 3-hydroxy-2-naphthoates. Experiments were performed to assess if these ILs can provide environmentally safe residual concentrations of the target metals after extraction. Both pure water and natural mineral water samples were spiked with 20 µg L^{-1} of Cu, Ag, Cd, Hg and Pb, respectively. Quantitative extraction (> 99%) of Hg and Ag was achieved. Cu and Hg were below the respective no-observed-effect-concentrations (NOECs) after extraction and Ag below 0.03 µg L^{-1}. Acute toxicity assays were conducted using two freshwater green algae *Raphidocelis subcapitata* and *Tetradesmus obliquus*. Growth inhibition and maximum photochemical quantum yield of photosystem II after 72 h were assessed. ILs were less toxic than similar compounds, but still must be classified as acute toxicants for algae. An inhibiting effect on both growth and chlorophyll fluorescence was observed. The leaching of the ILs into the samples remains a limitation regarding their environmental-friendly applicability. Nonetheless, the extremely efficient removal of Cu, Ag and Hg under environmentally relevant conditions calls for further research, which should focus on the immobilization of the ILs.

Keywords: task-specific ionic liquids; heavy metal extraction; algal toxicity; growth inhibition; chlorophyll fluorescence

1. Introduction

Ionic liquids (ILs) are generally defined as low-melting organic salts, often described as liquid below 100 °C, and represent a relatively new class of chemical compounds [1]. They exhibit a variety of useful physico-chemical properties, including very low volatility, high thermal, chemical and electrochemical stability, a wide liquid range and varying lipophilicity. Some of these characteristics are sought-after, environmentally favorable attributes, which has led to frequent proposal for ILs as more

sustainable alternatives in numerous applications [2]. Successful uses of ILs were reported in the fields of solvent extraction and separation [3,4], organic synthesis [5], electrochemistry [6], among many others. Their versatile properties enable numerous applications ranging from carbon dioxide ($CO_{2)}$ capture [7] to their use as a drug carrier in the pharmaceutical industry [8]. An increasing number of studies has also exploited these properties to extract heavy metals, in the desire to improve the applicability and sustainability of traditional methods [9]. In this regard, the synthesis of task-specific ionic liquids (TSILs) has received growing interest because they can be "tailored" to meet the intended properties: Introducing metal-chelating functional groups to either the respective cation or anion can fine-tune the affinity of the ILs towards metal ions, improving the efficacies in the extraction processes [10]. Incorporating anions with functional groups has proved to be successful in extracting various heavy metals or trace elements. For instance, ILs with a Sulphur functionality, e.g., thiosalicylate, thioglycolate or thiocyanate groups, showed a high affinity towards copper, silver, cadmium, platinum or mercury [11–13].

Meeting the claim of ILs as "green" solvents, starting with their production, involved proposing enhanced synthesis routes achieving higher yields and fewer byproducts while avoiding environmentally harmful chemicals [14,15]. Regarding green applicability, however, the liquid–liquid extraction of metals from aqueous phases revealed a major drawback: partial dissolution of the ILs during extraction, so-called leaching [9]. Moreover, the prevalent extraction mechanism can also decompose the IL: excellent extraction capabilities can be accomplished through a cation-exchange mechanism, which considerably limits the practicability of ILs in liquid-liquid extraction [16]. Utilizing more hydrophobic ILs, e.g., by increasing the length of alkyl chains on the cation, combined with a strongly coordinating, functional anion, enables a shift from ion exchange to neutral extraction mechanisms, thus improving the leaching behavior [17]. However, even using highly hydrophobic ILs cannot guarantee the complete inhibition of ion exchange [18]. The antimicrobial activity of quaternary ammonium compounds has long been known, and imidazolium-based ILs, for example, were recently tested for their antibiofilm activity [19]. Accordingly, this inevitable (even if only partial) loss of the ILs during extraction requires a stronger focus on their hazard potential [20]. The regularly attributed green status of ILs is mainly derived from their non-volatile nature, ignoring their potential bioactivity [21], which represents an often-neglected parameter in the optimization of TSILs [22]. For example, the hydrolysis of fluorinated anions and the subsequent toxicity makes ILs based on fluorinated anions inadequate for use in extraction processes [21,23]. Recent work has therefore highlighted both the ecotoxicity and degradability of ILs [21,24]. Kumar et al. [25] investigated the in vitro cytotoxicity of various TSILs using human breast cancer cells. The authors showed a positive correlation between alkyl chain length and cytotoxicity. Determining the ecotoxicity through algal growth assays proved to be another useful tool, because this approach is easy to perform, and algae are important primary producers. For instance, the toxicity of pyridinium and imidazolium ILs for the alga *Selenastrum capricornutum* was reported to be between two and four orders of magnitude higher than that of traditional organic solvents [26]. Likewise, phosphonium- and ammonium-based TSILs used in heavy metal extraction were acutely toxic to freshwater algae [13]. While toxicity depends on several factors, e.g., the alkyl chain length or the nature of the functional group [27], the results vary tremendously depending on the applied biological test [22]. These findings underpin that testing the ecotoxic potential of TSILs proposed as extracting agents is a prerequisite before use: improved extraction capabilities should not be accompanied by increased toxicity. Furthermore, these assays should be straight-forward, reproducible and comparable to each other.

The recently characterized TSILs trihexyltetradecylphosphonium-, methyltrioctylphosphonium- and methyltrioctylammonium 3-hydroxy-2-naphthoates displayed great potential as heavy metal extractants; the best results were achieved for Cu, Ag, Cd and Pb [28]. The performed extraction experiments with model solutions were in the mg L^{-1} concentration range, which is commonly performed. Nonetheless, follow-up investigations using trace level concentrations are rarely reported. Fischer et al. [11] found a positive correlation between extraction efficacies and initial concentrations

for several cases, emphasizing that high extraction efficacies in the mg L^{-1} concentration range do not necessarily apply in lower concentration ranges. This highlights the importance of using trace level concentrations in extraction experiments, which are reported here for the first time for the ILs under investigation. The experiments were designed to show if environmentally safe residual concentrations after extraction could be achieved, even when initial metal concentrations were in the µg L^{-1} range. For this study, we also included investigations on mercury, for which experiments have not been conducted before. To further assess their applicability with respect to toxicological concerns, the toxicological potential of the Ils towards the freshwater green algae *Raphidocelis subcapitata* and *Tetradesmus obliquus* was investigated in acute toxicity assays. These tests included growth inhibition assays and were enhanced by monitoring the photosynthetic performance via chlorophyll fluorescence. We propose this non-invasive and fast method as a first ecotoxicity screening of novel TSILs used as extracting agents in aqueous phases. The combined results from extraction experiments and acute toxicity assays should help to shed light on the practicability and feasibility of using highly hydrophobic ammonium- and phosphonium-based TSILs as metal extracting agents.

2. Materials and Methods

2.1. Materials

The ionic liquids trihexyltetradecylphosphonium 3-hydroxy-2-naphthoate ([P_{66614}][HNA]), methyltrioctylphosphonium 3-hydroxy-2-naphthoate ([P_{1888}][HNA]) and methyl-trioctylammonium 3-hydroxy-2-naphthoate ([N_{1888}][HNA]) were synthesized as described elsewhere [29]. For the preparation of feed solutions as well as instrument calibration, 1000 mg L^{-1} atomic absorption spectrometry elemental standards of Cu, Cd, Pb (Honeywell Fluka, Charlotte, NC, USA) and Ag (VWR, Radnor, PA, USA) in 2 wt% HNO_3 and Hg in 12 wt% HNO_3 (Fluka) were used. HNO_3 (trace select, ≥ 69%) purchased from Fluka and NaOH (50% in water) from Sigma-Aldrich (St. Louis, MO, USA) were used to adjust feed solution pH. Ultra-pure water (resistivity < 18.2 MΩ cm) was obtained from a Millipore Milli-Q Advantage A10 apparatus (Merck Millipore, Burligton, MA, USA).

Batch cultures of the freshwater green algae species *Tetradesmus obliquus* (strain SAG 276-1) and *Raphidocelis subcapitata* (strain ASW05231) were obtained from the Culture Collection of Algae at the University of Göttingen (Göttingen, Germany) and from the Algensammlung Wien at the University of Vienna (Vienna, Austria), respectively. Studies were performed using Bold's Basal Medium with vitamins (BBM + V) [29].

2.2. Apparatus

Extraction samples were put on a Vibramax 100 shaker (Heidolph, Schwabach, Germany) and centrifuged using an EBA 20 centrifuge manufactured by Hettich (Tuttlingen, Germany). pH values were measured with a Lab 850 pH Meter (SI Analytics, Mainz, Germany). The metals Cu, Ag, Cd and Pb were quantified by graphite furnace atomic absorption spectrometry using a PinAAcle 900 z spectrometer by Perkin Elmer (Waltham, MA, USA). In the case of Hg, cold vapor atomic absorption spectrometry was utilized, using a FIMS 400 system by Perkin Elmer (Waltham, MA, USA). Dissolved organic carbon (DOC) was measured with the TOC Analyzer TOC-V CHP (Shimadzu, Kyoto, Japan). The major components of mineral water were determined using flame atomic absorption spectrometer AAnalyst 200 (Perkin Elmer, Waltham, MA, USA), photometer Spectroquant NOVA 60a (Merck Millipore, Burlington, MA, USA) and conductivity meter FiveGo F3 (Mettler-Toledo, Columbus, OH, USA).

For growth inhibition assays, cell numbers were estimated using a Neubauer improved cell counting chamber with 0.1 mm depth (Marienfeld, Lauda-Königshofen, Germany) under a compound microscope Neovar 2 (Reichert-Jung, Vienna, Austria). Chlorophyll fluorescence was measured with the pulse-amplified modulated ("PAM") fluorometer PAM-2500/USD (Walz, Effeltrich, Germany).

2.3. Extraction Experiments

In order to evaluate the potential of the synthesized ILs for metal extraction, 100 mg of the respective IL were weighed into glass vials and 5 mL of the respective feed solution were added. The two ILs that were solid at room temperature, $[P_{1888}][HNA]$ and $[N_{1888}][HNA$, were melted, weighed in molten state, and the feed solution was added after solidification. The ILs remained solid during extraction, resulting in a solid–liquid extraction in these cases. Firstly, a time-dependent extraction using a feed solution with 1 mg L^{-1} mercury adjusted to an initial pH of 3.5 was performed. For this, samples were kept at room temperature and shaken with 300 rpm for 1, 2, 3, 4, 5 and 6 h, respectively. Afterwards, the aqueous phase was recovered by pipetting and centrifuged at 5000 rpm before being measured for the respective metal and DOC content. Experiments were conducted in triplicates. To consider a loss of metal due to possible oxidation, precipitation and adhesion effects in the glass vials, reference samples containing the respective metal concentrations without IL were treated equally to the samples with ILs; the metal concentration was determined before and after the respective extraction time. Secondly, extraction experiments were carried out for an extraction time of 2 h with spiked pure water feed solutions adjusted to pH 3.5 and pH 8.0, as well as with bottled natural mineral water whose major composition was characterized before use (Table 1). These feed solutions were spiked with 20 µg L^{-1} of the respective metal.

Table 1. Major parameters and components of the mineral water feed solution; El.Cond.: Electrical Conductivity, all ions in (mg L^{-1}).

pH	El. Cond.	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}
7.22	846 (µS cm^{-1})	14.2	1.8	39.5	95	23	221

The extraction efficacy was calculated as the percentage of metal removed from the feed solution after the extraction experiment compared to reference samples, using Equation (1)

$$\textbf{Extraction efficacy } (\%) = \frac{\mathbf{C_{Ref} - C_t}}{\mathbf{C_{Ref}}} * \mathbf{100} \quad (1)$$

C_{Ref} represents the metal concentration in the reference samples after the respective extraction time and C_t the metal concentration in the sample after extraction.

To evaluate the degree of leaching, the dissolved organic carbon concentration in samples after extraction was measured and leaching expressed in mg L^{-1} as well as relative loss of IL (Equations (2) and (3))

$$\textbf{Leaching } \left(\mathbf{mg\ L^{-1}}\right) = \frac{\mathbf{DOC}}{\mathbf{C_{IL}}} \quad (2)$$

$$\textbf{Leaching } (\%) = \frac{\mathbf{DOC} * \mathbf{V_S}}{\mathbf{m_{IL}}} * \mathbf{100} * \mathbf{C_{IL}} \quad (3)$$

DOC (mg L^{-1}) was the measured concentration of dissolved organic carbon in the sample after extraction, vs. (L) the feed solution volume and m_{IL} (mg) the mass of IL used. C_{IL} represents the carbon content of the respective IL and was used to calculate the leaching of the entire liquid based on the carbon leaching, whereby the cation and anion of the IL were assumed to leach equally. The calculated value therefore represents the percent loss of IL during extraction and serves as an estimation of the reusability in liquid–liquid/solid–liquid extraction setups.

2.4. Growth Inhibition Assays

The first step involved preparing an aqueous phase saturated with the respective IL. Here, 100 mL of pure water were added to 1 g of the respective IL and shaken overnight. Afterwards, the dissolved organic carbon (DOC) content in the aqueous phase was measured in order to calculate

the IL concentration for the subsequent acute toxicity assays. Algal growth was then monitored based on ISO Norm 8692, with cultures of the freshwater green algae species *Tetradesmus obliquus* and *Raphidocelis subcapitata*. All tests were performed as batch cultures in sterilized 200 mL Schott flasks in 0.45 µm filtered (cellulose acetate, Sartorius, Göttingen, Germany) Bold's Basal Medium with vitamins (BBM + V) [13].. The algae stock solution was grown under photoautotrophic conditions in a water bath at 20 °C (± 0.5 °C) and stirred at 300 rpm, using plant growth fluorescence lamps (mean PAR intensities of 165 µmol m^{-2} s^{-1}, measured at the flask surface), until a sufficient cell density was reached to conduct growth inhibition experiments. Algal density was then diluted to 10^5 cells mL^{-1} using an inoculum from the stock algae solution and subsequently exposed to different concentrations of the test substances for 72 h. All samples were prepared as triplicate and were stirred at 300 rpm throughout the test period. After the test time, cell densities were estimated and the growth rate µ was calculated as follows (Equation (4))

$$\mu = \frac{\mathbf{\ln N_{72} * \ln N_0}}{\mathbf{t}} \quad (4)$$

where N_0 is the initial algal concentration, N_{72} the final algal concentration after 72 h and t the exposition time (72 h). The inhibition (I) in % was then calculated for the respective toxicant concentrations using Equation (5)

$$\mathbf{I} = \frac{\mu_c - \mu_i}{\mu_c} \quad (5)$$

where I is the growth inhibition after 72 h, μ_c the growth rate in control and μ_i the growth rate of IL-exposed cells after 72 h. Ultimately, fitted dose–response curves were obtained by plotting the inhibition and logarithm of the IL concentration using Origin®2015 (Vers. 9.2, OriginLab, Northampton, MA, USA).

2.5. Chlorophyll Fluorescence

Maximum photochemical quantum yield of PS II (φ_M = Fv/Fm) was measured by means of pulse-amplified modulated fluorescence. First, samples were pre-darkened for 10 min. Then, minimum chlorophyll fluorescence (Fo) was determined, followed by maximal fluorescence (Fm) during a saturation pulse. Fv/Fm = (Fm-Fo)/Fm is treated as a proxy parameter for the overall photosynthetic performance of plants and for healthy plants around 0.75–0.80; lowered values indicate stress [30]. The results for chlorophyll fluorescence parameters were evaluated as described elsewhere [31,32], the relevant parameters are given in Equation (6)

$$\mathbf{\Phi_M} = \frac{\mathbf{F_m - F_0}}{\mathbf{F_m}} = \frac{\mathbf{F_v}}{\mathbf{F_m}} \quad (6)$$

By way of illustration, these data are presented in percent with reference to φ_M values obtained for reference samples not exposed to the ILs (Equation (7))

$$\mathbf{rel.}\ \mathbf{\Phi_M}(\%) = \left(\frac{\mathbf{\Phi_{M(sample)}}}{\mathbf{\Phi_{M(ref)}}}\right) * \mathbf{100} \quad (7)$$

For comparison, EC_{50} (refers to the concentration of a toxicant that causes 50% of an observed effect, e.g., growth inhibition or a maximum photochemical quantum yield of PS II after the specified time of the test compared to samples not exposed to the toxicant (reference sample)) were calculated equal to the growth inhibition results, as proposed by, e.g., [33].

3. Results and Discussion

3.1. Extraction Experiments

The three ILs utilized in this study have been previously tested for their capability to extract the heavy metals Cu, Ag, Cd and Pb from pure water as well as from several synthetic and natural waters, including drinking water and seawater. High extraction efficacies have been described at mg L^{-1} levels in liquid–liquid as well as in a solvent bar micro-extraction setup [28,34]. The extraction of Hg had not been tested before.

3.1.1. Extraction of Hg

Before conducting extraction experiments at trace level concentrations, we investigated if the three ILs are adequate extracting agents for Hg. The results for this time-dependent extraction are summarized in Figure 1. All three ILs displayed high extraction efficacies, with $[P_{66614}]$[HNA] achieving the highest value of 95.2% ± 1.7% after 1 h.

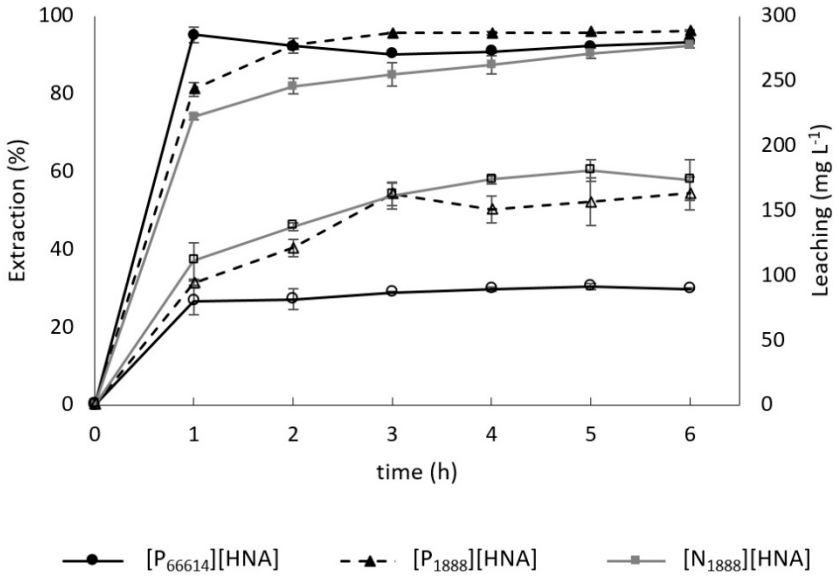

Figure 1. Time-dependent Hg extraction (filled symbols) from a pure water feed solution at pH 3.5 containing 1 mg L^{-1} Hg and leaching of the ILs into the aqueous phase (open symbols); n = 3, error bars = ± standard deviation (SD).

The results are in good agreement with observations by Germani et al., where increasing alkyl chain lengths, and therefore the hydrophobicity, correlated negatively with the time needed for quantitative extraction of Hg [35]. Leaching values also are in line with previous data, with highly hydrophobic $[P_{66614}]$[HNA] achieving the lowest leaching of 80.0 ± 10.4 mg C L^{-1} (0.40% ± 0.05%), while similar but slightly higher values were obtained for $[P_{1888}]$[HNA] and $[N_{1888}]$[HNA] [28]. These results promise high reusability of the ILs because the relative dissolution ranged from 0.4% to 0.9% and separation from the aqueous phase after extraction was simple. This warrants further experiments regarding the back-extraction of the target metals and subsequent cycles of extraction and back extraction, which was successfully achieved with similar compounds by Platzer et al. [15] using, e.g., simple washing steps with 0.5 M HNO_3.

3.1.2. Extraction of Cu, Ag, Cd, Hg and Pb from 20 µg L^{-1} Feed Solutions

The results on the extraction capability are summarized in Table 2, given as residual concentration remaining in feed solutions after extraction and the respective relative efficacy in brackets. The highest

extraction efficacies were achieved for Hg and Ag using [P_{66614}][HNA], which is additionally depicted in Figure 2.

Table 2. Residual concentrations of metals after extraction experiments from pure water at pH 3.5, pure water at pH 8.0 and spiked natural mineral water for an extraction time of 2 h; extraction efficacies in percent are given in brackets, n = 3. n/a = not applicable: no metal in solution in reference samples after 2 h.

		Residual concentration mean ± SD (µg L^{-1})																					
Sample	**IL**	**Cu**				**Ag**				**Cd**				**Hg**				**Pb**			
Pure water pH 3.5	[P_{66614}][HNA]	16.2	±	0.5	(21.3)	< 0.03			(> 99)	7.5	±	0.3	(64.4)	< 0.1			(> 99)	17.7±0.1			(< 5)
	[P_{1888}][HNA]	7.8	±	2.3	(62.4)	13.2	±	0.1	(14.3)	18.5	±	1.0	(13.3)	1.7	±	0.2	(91.3)	4.3	±	0.1	(75.6)
	[N_{1888}][HNA]	2.2	±	0.8	(89.1)	3.6	±	0.2	(76.1)	14.8	±	1.3	(30.4)	2.6	±	0.4	(86.7)	1.2	±	0.6	(93.0)
Pure water pH 8.0	[P_{66614}][HNA]	10.5±1.8			(< 5)	< 0.03			(> 99)	6.5	±	0.9	(37.3)	< 0.1			(> 99)	n/a			
	[P_{1888}][HNA]	6.6	±	0.8	(36.1)	7.0	±	0.9	(33.1)	9.3	±	0.2	(10.5)	2.4	±	0.2	(87.8)	n/a			
	[N_{1888}][HNA]	4.2	±	0.7	(59.7)	6.8	±	0.5	(35.0)	11.7±0.8			(< 5)	2.9	±	1.2	(85.4)	n/a			
Mineral water	[P_{66614}][HNA]	1.3	±	0.4	(90.4)	< 0.03			(> 99)	12.7	±	0.1	(35.4)	< 0.1			(> 99)	6.0	±	1.2	(28.2)
	[P_{1888}][HNA]	1.1	±	0.2	(90.8)	3.5	±	0.3	(75.3)	16.0	±	0.9	(18.8)	6.4	±	0.1	(68.0)	4.5	±	0.6	(45.6)
	[N_{1888}][HNA]	0.8	±	0.3	(93.3)	2.3	±	0.7	(83.6)	13.5	±	0.7	(31.3)	6.7	±	0.2	(66.3)	5.2	±	1.2	(38.3)

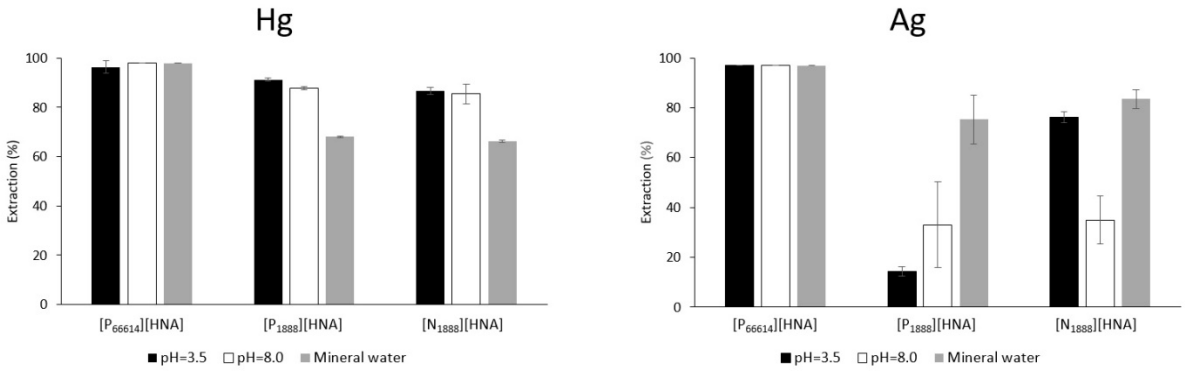

Figure 2. Extraction of 20 µg L^{-1} Hg and Ag, respectively, from pure water feed solutions at pH 3.5 and 8.0 as well as a spiked mineral water sample. Extraction time = 2 h, n= 3, error bars = ± SD.

Remarkably, Hg could be extracted to final concentrations under the limit of detection of 0.1 µg L^{-1} from all three feed solutions using [P_{66614}][HNA]. Changing the feed solution concentration from 1 to 20 µg L^{-1} decreased the metal content after extraction from 50 to less than 0.1 µg L^{-1}, displaying a successful removal even of trace levels of Hg. In both cases, the residual concentration would conform to the Water Framework Directive 2000/60/EC of the European Union concerning limit values and quality objectives for industrial mercury discharges, with a threshold value of 50 µg L^{-1} [36].

Furthermore, concentrations after the trace level extraction experiment are below the Austrian drinking water act threshold of 1.0 µg L^{-1} [37] and potentially comply with the threshold value of 0.07 µg L^{-1} defined in the directive on environmental quality standards for surface waters in the European Union [38]. Regarding toxicity to freshwater algae, the achievable extraction also conforms with predicted no observed effect concentrations (NOEC) for Hg towards several freshwater algae, including *R. subcapitata*, of 0.6–3.2 µg L^{-1}, reported by Rodrigues et al. (2013) [39].

[P_{66614}][HNA] was equally suitable for extracting Ag, achieving > 99% extraction from the three feed solutions. The remaining concentrations below the limit of detection of 0.03 µg L^{-1} were significantly lower than the EC_{50} values found in the literature: the lowest EC_{50} for aquatic biota have been reported for fish, with LC_{50} (refers to the concentration of a toxicant in the respective medium, that causes the death of 50% of a tested population after a specified time) between 2 and 30 µg L^{-1} [40]; EC_{50} for certain freshwater algae are as low as 10 µg L^{-1} [41], whereas 125 µg L^{-1} were reported for *R. subcapitata* [42].

Cu was extracted > 90% from the mineral water feed solution for all three investigated ILs. The positive effect that we previously reported [28] of a mineral water composition on Cu extraction at mg L^{-1} levels remained valid in the present extraction setup. The low residual concentration after

extraction, approx. 1 µg L^{-1}, is below the lowest reported NOEC of 4.2 µg L^{-1} for *R. subcapitata* in natural waters [43], underlining the high capability of the three ILs to extract Cu from freshwater matrices. Regarding Pb, $[N_{1888}]$[HNA] achieved an extraction of 93.0% ± 0.6%, but only from pure water at pH 3.5. For mineral water, the extraction efficacies were comparably low for all three ILs, with values ranging from 28% to 45%, equivalent to residual concentrations of 4.5–6.0 µg L^{-1}. Cd extraction likewise was decreased compared to studies using 1 mg L^{-1} feed solutions [28], revealing comparably high residual concentrations between 6.5 and 18.5 µg L^{-1}. The highest efficacy was achieved from pure water at pH 3.5 using $[P_{66614}]$[HNA]: 64.4% ± 2.1%. Both Pb and Cd displayed a dependence on initial metal concentration, as described by Fischer et al. [11], and furthermore suggest that the extraction of trace level concentrations is more susceptible to sample matrix effects.

In sum, beyond the high extraction efficacies from mg L^{-1} feed solutions reported in previous studies, the investigated ILs also displayed a high capability to extract trace level concentrations of the target metals. The residual concentrations achieved for Cu, Ag and Hg were well below known concentrations of concern regarding algae and fish.

3.2. Leaching

Results for IL leaching into the aqueous phase during extraction are summarized in Table 3.

Table 3. Leaching of the respective ionic liquid into the aqueous feed solution during extraction for an extraction time of 2 h; percentage of dissolution given in brackets, n = 3.

	Leaching ± SD (mg L^{-1})					
Sample	**$[P_{66614}]$[HNA]**		**$[P_{1888}]$[HNA]**		**$[N_{1888}]$[HNA]**	
Cu						
Pure water pH 3.5	76.7 ± 1.1	(0.38)	24.7 ± 2.0	(0.12)	139.3 ± 23.8	(0.70)
Pure water pH 8.0	75.9 ± 0.9	(0.38)	25.7 ± 2.3	(0.13)	136.8 ± 30.7	(0.68)
Mineral water	62.9 ± 1.1	(0.31)	27.6 ± 3.8	(0.14)	78.6 ± 6.0	(0.39)
Ag						
Pure water pH 3.5	75.0 ± 2.4	(0.38)	25.3 ± 0.4	(0.13)	168.1 ± 5.7	(0.83)
Pure water pH 8.0	80.3 ± 0.7	(0.40)	23.2 ± 0.9	(0.12)	132.4 ± 9.8	(0.66)
Mineral water	74.4 ± 1.6	(0.38)	24.8 ± 7.9	(0.12)	99.8 ± 9.3	(0.50)
Cd						
Pure water pH 3.5	83.7 ± 2.5	(0.42)	25.6 ± 1.5	(0.13)	97.4 ± 6.1	(0.48)
Pure water pH 8.0	78.6 ± 2.5	(0.39)	25.4 ± 1.2	(0.24)	80.4 ± 10.4	(0.39)
Mineral water	84.3 ± 0.5	(0.42)	43.5 ± 3.2	(0.33)	81.8 ± 6.0	(0.35)
Hg						
Pure water pH 3.5	80.2 ± 1.6	(0.40)	25.6 ± 2.0	(0.13)	137.1 ± 15.0	(0.68)
Pure water pH 8.0	78.4 ± 0.6	(0.39)	24.8 ± 0.4	(0.12)	87.5 ± 2.3	(0.44)
Mineral water	65.4 ± 0.8	(0.33)	27.6 ± 2.4	(0.14)	80.6 ± 6.0	(0.40)
Pb						
Pure water pH 3.5	73.2 ± 8.0	(0.36)	25.4 ± 2.4	(0.13)	76.7 ± 2.7	(0.38)
Pure water pH 8.0	74.6 ± 1.7	(0.37)	28.5 ± 1.5	(0.14)	107.1 ± 12.5	(0.53)
Mineral water	63.6 ± 1.2	(0.32)	27.3 ± 0.2	(0.14)	110.8 ± 4.8	(0.55)

In general, we were able to reproduce the low leaching values reported in previous extraction experiments [28]. Values ranged between a partial dissolution of 0.1%–0.8%, implying a high potential for reusability of the three ILs in liquid–liquid extraction setups. In contrast to the multi-elemental setup of past works [28,34], the single metal experiments in the present study enabled an examination of metal-dependent changes in leaching. This provides insights into the prevalent extraction mechanisms. Overall, differences in leaching due to varying sample matrices or the respective target metal, however, were small. The most pronounced effects were recorded for $[N_{1888}]$[HNA], which we previously reported to be more susceptible to sample composition [28]. This difference could reflect a higher probability of a cation exchange extraction mechanism for $[N_{1888}]$[HNA] in the pure water feed

solution at pH 3.5, enabled through the weak ionic strength and acidic conditions in the sample [17,44]. This effect is best visible for the extraction of Ag, where leaching decreased from 0.83% to 0.50% for the mineral water solution. $[P_{66614}][HNA]$ and $[P_{1888}][HNA]$ displayed almost equal leaching data for each metal and feed solution, respectively, suggesting that the mode of extraction remained constant under all conditions. Based on the consistent data and the highly hydrophobic character of the two ILs, we assume that all target metals were mainly extracted via a neutral extraction mechanism. This is further supported by constant leaching, regardless of whether or not the respective target metal could be extracted.

3.3. Acute Toxicity Assays

Results for the concentration-dependent growth inhibition and maximum photochemical quantum yield of PS II of *R. subcapitata* are depicted in Figure 3, for *T. obliquus* they are given in Figure S1. The EC_{50} (72 h) values calculated from the data of both parameters are summarized in Table 4. Most importantly, our data do not support the general assumption that the effect of anions on acute toxicity is small compared to alkyl chain lengths of the cation [27,45]. This means that an overall consensus on this matter remains elusive. Compared to previous studies that utilized ILs with the same cations, we achieved lower toxicity using $[HNA]^-$ as anion in our work [13]. At the same time, the effects on the toxicity of different alkyl chains or the central atom in the cation were comparably small. The detailed results are discussed in the following paragraphs.

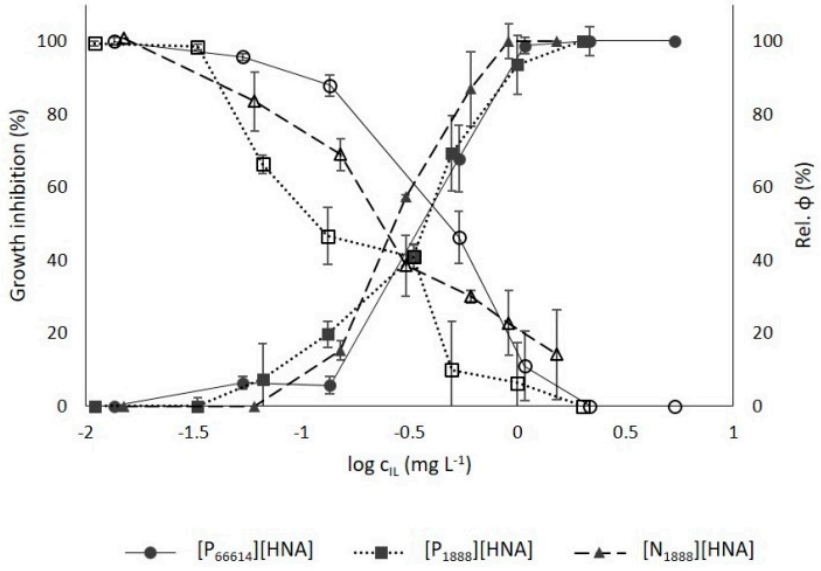

Figure 3. Effect of the three ILs on algal growth (filled symbols) and chlorophyll fluorescence (Rel. φ, open symbols) in 72 h acute toxicity assays with *Raphidocelis subcapitata*, n = 3, error bars = ± SD.

Table 4. Influence of the ILs on growth and chlorophyll fluorescence after 72 h (EC_{50} values ± SD) in acute toxicity assays.

Ionic Liquid	M_W [g mol^{-1}]	Growth inhibition		Max. PSII quantum yield	
		T. obliquus EC_{50}± SD	*R. subcapitata* EC_{50}± SD	*T. obliquus* EC_{50}± SD	*R. subcapitata* EC_{50}± SD
$[P_{66614}][HNA]$	671.03	1.76 ± 0.17	0.47 ± 0.01	1.81 ± 0.03	0.52 ± 0.05
$[P_{1888}][HNA]$	572.86	2.61 ± 0.06	0.39 ± 0.05	1.86 ± 0.06	0.13 ± 0.03
$[N_{1888}][HNA]$	555.88	2.68 ± 0.44	0.28 ± 0.01	2.04 ± 0.22	0.24 ± 0.06
$[N_{1888}][C_6SAc]$ *	543.98	0.93 ± 0.16	0.05 ± 0.01	n/a	n/a
$[P_{1888}][C_6SAc]$ *	560.95	8.96 ± 0.43	0.04 ± 0.01	n/a	n/a
$[N_{1888}][Cl]$ *	404.16	0.30 ± 0.03	0.07 ± 0.01	n/a	n/a
$[P_{66614}][Cl]$ *	519.31	0.39 ± 0.02	0.10 ± 0.01	n/a	n/a

* Platzer et al. [13]; n/a = not available

3.3.1. Growth Inhibition

EC_{50} (72 h) values were significantly lower for *R. subcapitata* than for *T. obliquus*: the values range from 0.28 to 0.47 mg L^{-1} for the former and 1.76–2.68 mg L^{-1} for the latter (Table 4). This agrees with an observation by Platzer et al. [13], that thioglycolate-based ILs $[N_{1888}][C_6SAc]$ and $[P_{1888}][C_6SAc]$ as well as commercially available ILs $[N_{1888}][Cl]$ and $[P_{66614}][Cl]$ exhibited higher values for *T. obliquus*. This was attributed to the inert character of the biological polymer sporopollenin, covering the cell walls of *T. obliquus*, thus reducing diffusion into the cells.

In further comparing our results to [13], our EC_{50} values were significantly higher for *R. subcapitata* and, additionally, the chosen anion had a clear effect on acute toxicity. Moreover, a comparison of three different anions is possible for cation $[N_{1888}]$, with acute toxicity decreasing in the order $[C_6SAc]^-$ > $[Cl]^-$ > $[HNA]^-$. For the latter, a value of 0.28 ± 0.01 mg L^{-1} signifies a decreased toxicity by a factor of 4 compared with $[Cl]^-$ and by a factor of 5.6 for $[C_6SAc]$. The same holds true for $[P_{66614}][Cl]$ and $[P_{66614}][HNA]$ and is highly pronounced for $[P_{1888}][C_6SAc]$ and $[P_{1888}][HNA]$, with EC_{50} values of 0.04 ± 0.01 and 0.39 ± 0.05 mg L^{-1}, respectively. The chloride anion was proposed as a reference point in determining anion toxicity because no effect of chloride on the overall toxicity of the compound is assumed [45]. Accordingly, $[HNA]^-$ has a positive effect on IL acute toxicity. The exact interaction with cells is unclear, but recent work revealed a positive influence of 3-hydroxy-2-naphthoate on cell activity [46], which indicates a generally lower toxicity of $[HNA]^-$ compared to $[C_6SAc]^-$.

Whereas no effect of alkyl chain length could be detected for growth of *R. subcapitata*, $[P_{66614}][HNA]$ exhibited a slightly higher toxicity compared to the less hydrophobic cations $[P_{1888}]^+$ and $[N_{1888}]^+$ for *T. obliquus*. This agrees with the literature on the alkyl-length-dependent toxicity of imidazolium-based ILs on *T. obliquus* [47]. EC_{50} values (72 h) for ILs with alkyl chains ≥ C_{10} and chloride anion were below 0.06 mg L^{-1} [48] and below 0.11 mg L^{-1} in the case of bromide [47], which indicates a higher acute toxicity compared to the ILs we tested. Likewise, [49] reported an EC_{50} value (72 h) of 0.06 mg L^{-1} for 1-decylpyridinium bromide.

Comparing cations with the same alkyl chains revealed no influence of the central atom on toxicity, yielding similar results for $[P_{1888}][HNA]$ and $[N_{1888}][HNA]$ towards both *T. obliquus* and *R. subcapitata*. As is frequently suggested, predicting the toxicity of ILs based on chemical structures is complicated by the distinct reactions of different test organisms. Moreover, Stolte et al. [45] described overadditive effects on toxicity by specific cation–anion combinations.

Although the three ILs we tested here displayed a lower acute toxicity than similar compounds in previous studies, it is important to emphasize that they nevertheless are classified as "acute toxic 1" (EC_{50} ≤ 1 mg L^{-1}) towards *R. subcapitata* and as "acute toxic 2" (1 mg L^{-1} ≤ EC_{50} ≤ 10 mg L^{-1}) towards *T. obliquus* for an exposure time of 72 h in the *Globally Harmonized System of the Classification and Labelling of Chemicals* (GHS) [50]. As such, their potential application must be further reviewed in greater detail, e.g., by assessing their toxicity on additional organisms or conducting studies on biodegradability.

3.3.2. Chlorophyll Fluorescence

PAM fluorometry has been established as a simple and rapid screening method to determine a compound's toxicity on photoautotrophs [51]. We therefore used this method to obtain additional information complementing the results of the growth inhibition assays. Figure 3 depicts the results of the two tests, displaying the concentration-dependent growth inhibition as well as the maximum photochemical quantum yield of photosystem II. Table 4 provides the EC_{50} values calculated from both assays.

These results demonstrate a clear negative correlation between growth inhibition and photosynthetic performance. For instance, NOECs for both parameters towards *T. obliquus* are estimated to be 0.33 mg L^{-1} for $[P_{1888}][HNA]$ and 0.52 mg L^{-1} for $[P_{66614}][HNA]$. Intermediate concentrations likewise displayed similarly pronounced impacts on both parameters. This signifies that the tested ILs simultaneously affect PSII and inhibit algal growth to equal extent, which is also reflected in the comparable EC_{50} values of both tests (Table 4): EC_{50} values of e.g., $[P_{66614}][HNA]$ were equal among the same algae species, with values of 1.76 ± 0.17 mg L^{-1} for growth inhibition vs. 1.81 ± 0.03 mg L^{-1} for Fv/Fm of *T. obliquus*. The same pattern between the two parameters for *T. obliquus* was reported by [49] at approximately 60 µg L^{-1} for IL 1-decylpyridinium bromide. In contrast, no correlation between growth inhibition and Fv/Fm has been found for explicitly non-photosynthetic inhibitors. In this respect, Fai et al. [52] reported on the behavior of herbicide Alachlor: it did not affect the chlorophyll fluorescence of *R. subcapitata*, even though it displayed the highest growth inhibition of the tested compounds.

EC_{50} values for $[N_{1888}][HNA]$ were equal regarding *R. subcapitata* and similar for *T. obliquus*, whereas a significantly stronger impact of $[P_{1888}][HNA]$ on chlorophyll fluorescence was observed at lower concentrations than its effect on growth inhibition. Nevertheless, the overall strong relation between both parameters qualifies the fast and non-invasive PAM fluorescence measurement as a suitable first screening of IL toxicity on photoautotrophs.

3.3.3. Ionic Liquid vs. Heavy Metal Toxicity

Concentrations of dissolved IL after extraction significantly exceeded the calculated EC_{50} values in all samples. Even in the most favorable cases, leaching still equaled 18 mg IL L^{-1} (Table 3). To better understand these results in light of possible applications, the retention in treatment plants and biodegradability of these ILs needs to be assessed. Amongst others, several readily biodegradable (> 60% in the 28-d bottle test) naphthenic acid-based ILs were synthesized [53]. Moreover, guidelines on designing biodegradable ILs recommend long, hydrophobic alkyl chains on the cation and anions derived from organic salts [24]. This suggests that the ILs studied in this work have potential in this regard. This should be the subject of further investigation. The potential biodegradability described for ILs stands in contrast to the environmental properties of heavy metals, which are persistent and non-degradable [54]. Algal toxicity for heavy metals already has been reported for different algal strains and under various testing conditions. For the two target algae of interest, diverging results have been published: For instance, EC_{50} values regarding the growth inhibition of *R. subcapitata* range from 13 to 110 µg L^{-1} for Cd, 10–280 µg L^{-1} for Cu or 27–330 µg L^{-1} for Hg [55–58]. Concerning *T. obliquus*, the reported values lie in the same order of magnitude, with, e.g., 58 µg L^{-1} for Cd [59]. Likewise, a significantly higher impact on overall photosynthetic performance was reported for the target metals compared to the tested ILs. Thus, Juneau et al. determined EC_{50} (96 h) values of 50 µg L^{-1} for Cu [60] and 37 µg L^{-1} for Hg [33] towards *R. subcapitata*. Although the different data are not fully comparable due to varying setups, available data suggest that heavy metal toxicity greatly exceeds that of the tested ILs.

Our results clearly indicate that environmentally favorable conditions in the sample solution after extraction cannot be easily achieved even when using highly hydrophobic ILs. Certainly, the leaching behavior of ILs can be improved in several ways: we demonstrated that immobilizing ILs on solid

supports can substantially decrease leaching by up to 88% [34,61], leaving residual IL concentrations in the feed solution at concentrations approximate to the EC_{50} values.

4. Conclusions

We examined the suitability of three recently developed ionic liquids, namely trihexyltetradecylphosphonium-, methyltrioctylphosphonium- and methyltrioctylammonium 3-hydroxy-2-naphthoate, as heavy-metal-extracting agents by contrasting their extraction capabilities with acute algal toxicity. To elucidate their extraction behavior under environmentally relevant conditions, we spiked water and natural mineral water samples with 20 µg L^{-1} of the heavy metals Cu, Ag, Cd, Hg and Pb and extracted them using a liquid–liquid extraction setup. We were able to reproduce the high extraction capabilities we previously reported towards mg L^{-1} concentrations. Moreover, the ILs displayed great potential in achieving residual metal concentrations well below hazardous concentrations for algae and fish. The best results were obtained for the extraction of Ag and Hg from a spiked mineral water feed solution using trihexyltetradecylphosphonium 3-hydroxy-2-naphthoate. This yielded final metal concentrations below 0.03 and 0.1 µg L^{-1}, respectively.

Acute toxicity assays revealed comparable EC_{50} (72 h) values of the ILs on both growth and photosynthetic performance for two freshwater green algae *Raphidocelis subcapitata* and *Tetradesmus obliquus*. The functional anion 3-hydroxy-2-naphthoate was less toxic than similar compounds; nonetheless, all three compounds must be considered as acute toxicants for algae according to the *Globally Harmonized System of the Classification and Labelling of Chemicals*. The leaching of the ionic liquids into the samples was low but remains a limitation regarding technical applications due to their ecotoxicity. Further research should therefore focus on IL ecotoxicity and biodegradability on one hand and on the development of environmentally friendly extraction setups on the other.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/9/3157/s1, Figure S1: Effect of the three ILs on algal growth (filled symbols) and chlorophyll fluorescence (Rel. φ, open symbols) in 72 h acute toxicity assays with *T. obliquus*, n = 3, error bars = ± SD.

Author Contributions: Conceptualization, J.A.L.-L. and F.J.; Data curation, M.S. and W.K.; Formal analysis, P.P. and M.S.; Investigation, P.P.; Resources, B.K.K. and C.M.; Supervision, F.J.; Validation, M.S., B.K.K. and C.M.; Writing-original draft, P.P.; Writing-review & editing, J.A.L.-L., W.K. and F.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to thank Michael Stachowitsch for proofreading the English of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Welton, T. Ionic liquids: A brief history. *Biophys. Rev.* **2018**, *10*, 691–706. [CrossRef] [PubMed]
2. Lei, Z.; Chen, B.; Koo, Y.-M.; Macfarlane, D.R. Introduction: Ionic Liquids. *Chem. Rev.* **2017**, *117*, 6633–6635. [CrossRef] [PubMed]
3. Zhao, H.; Xia, S.; Ma, P. Use of ionic liquids as 'green' solvents for extractions. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1089–1096. [CrossRef]
4. Berthod, A.; Ruiz-Angel, M.-J.; Carda-Broch, S. Recent advances on ionic liquid uses in separation techniques. *J. Chromatogr. A* **2018**, *1559*, 2–16. [CrossRef]
5. Feldmann, C.; Ruck, M. Ionic Liquids—Designer Solvents for the Synthesis of New Compounds and Functional Materials. *Z. Anorg. Allg. Chem.* **2017**, *643*, 2. [CrossRef]
6. Yang, Q.; Zhang, Z.; Sun, X.-G.; Hu, Y.-S.; Xing, H.; Dai, S. Ionic liquids and derived materials for lithium and sodium batteries. *Chem. Soc. Rev.* **2018**, *47*, 2020–2064. [CrossRef]
7. Zeng, S.; Zhang, X.; Bai, L.; Zhang, X.; Wang, H.; Wang, J.; Bao, D.; Li, M.; Liu, X.; Zhang, S.-J. Ionic-Liquid-Based CO2 Capture Systems: Structure, Interaction and Process. *Chem. Rev.* **2017**, *117*, 9625–9673. [CrossRef]

8. Shamshima, J.L.; Berton, P.; Wang, H.; Zhou, X.; Gurau, G.; Rodgers, R.D. Ionic Liquids in Pharmaceutical Industry. In *Green Techniques for Organic Synthesis and Medicinal Chemistry*; Zhang, W., Cue, B.W., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2018; pp. 539–577.
9. Stojanovic, A.; Keppler, B.K. Ionic Liquids as Extracting Agents for Heavy Metals. *Sep. Sci. Technol.* **2012**, *47*, 189–203. [CrossRef]
10. Visser, A.E.; Swatloski, R.P.; Reichert, W.M.; Mayton, R.; Sheff, S.; Wierzbicki, A.; Davis, J., Jr.; Rogers, R.D. Task-Specific Ionic Liquids for the Extraction of Metal Ions from Aqueous Solutions. *Chem. Commun.* **2001**, *1*, 135–136. [CrossRef]
11. Fischer, L.; Falta, T.; Koellensperger, G.; Stojanovic, A.; Kogelnig, D.; Galanski, M.; Krachler, R.; Keppler, B.K.; Hann, S. Ionic liquids for extraction of metals and metal containing compounds from communal and industrial waste water. *Water Res.* **2011**, *45*, 4601–4614. [CrossRef]
12. Leyma, R.; Platzer, S.; Jirsa, F.; Kandioller, W.; Krachler, R.; Keppler, B.K. Novel thiosalicylate-based ionic liquids for heavy metal extractions. *J. Hazard. Mater.* **2016**, *314*, 164–171. [CrossRef] [PubMed]
13. Platzer, S.; Leyma, R.; Wolske, S.; Kandioller, W.; Heid, E.; Schröder, C.; Schagerl, M.; Krachler, R.; Jirsa, F.; Keppler, B.K. Thioglycolate-based task-specific ionic liquids: Metal extraction abilities vs. acute algal toxicity. *J. Hazard. Mater.* **2017**, *340*, 113–119. [CrossRef] [PubMed]
14. Kogelnig, D.; Stojanovic, A.; Galanski, M.; Groessl, M.; Jirsa, F.; Krachler, R.; Keppler, B.K.; Krachler, M. Greener synthesis of new ammonium ionic liquids and their potential as extracting agents. *Tetrahedron Lett.* **2008**, *49*, 2782–2785. [CrossRef]
15. Platzer, S.; Kar, M.; Leyma, R.; Chib, S.; Roller, A.; Jirsa, F.; Krachler, R.; Macfarlane, D.R.; Kandioller, W.; Keppler, B.K. Task-specific thioglycolate ionic liquids for heavy metal extraction: Synthesis, extraction efficacies and recycling properties. *J. Hazard. Mater.* **2017**, *324*, 241–249. [CrossRef]
16. Dietz, M.L.; A Dzielawa, J. Ion-exchange as a mode of cation transfer into room-temperature ionic liquids containing crown ethers: Implications for the 'greenness' of ionic liquids as diluents in liquid-liquid extraction. *Chem. Commun.* **2001**, *20*, 2124–2125. [CrossRef]
17. Messadi, A.; Mohamadou, A.; Boudesocque, S.; Dupont, L.; Guillon, E. Task-specific ionic liquid with coordinating anion for heavy metal ion extraction: Cation exchange versus ion-pair extraction. *Sep. Purif. Technol.* **2013**, *107*, 172–178. [CrossRef]
18. Dietz, M.L.; Stepinski, D.C. A ternary mechanism for the facilitated transfer of metal ions into room-temperature ionic liquids (RTILs): implications for the "greenness" of RTILs as extraction solvents. *Green Chem.* **2005**, *7*, 747. [CrossRef]
19. Reddy, G.K.K.; Nancharaiah, Y.; Venugopalan, V. Long alkyl-chain imidazolium ionic liquids: Antibiofilm activity against phototrophic biofilms. *Colloids Surfaces B Biointerfaces* **2017**, *155*, 487–496. [CrossRef]
20. Scammells, P.J.; Scott, J.L.; Singer, R.D. Ionic Liquids: The Neglected Issues. *Aust. J. Chem.* **2005**, *58*, 155–169. [CrossRef]
21. Pham, T.P.T.; Cho, C.-W.; Yun, Y.-S. Environmental fate and toxicity of ionic liquids: A review. *Water Res.* **2010**, *44*, 352–372. [CrossRef]
22. Egorova, K.S.; Ananikov, V.P. Toxicity of Ionic Liquids: Eco(Cyto)Activity as Complicated, but Unavoidable Parameter for Task-Specific Optimization. *ChemSusChem* **2014**, *7*, 336–360. [CrossRef] [PubMed]
23. Wang, B.; Qin, L.; Mu, T.; Xue, Z.; Gao, G. Are Ionic Liquids Chemically Stable? *Chem. Rev.* **2017**, *117*, 7113–7131. [CrossRef] [PubMed]
24. Bubalo, M.C.; Radošević, K.; Redovniković, I.R.; Halambek, J.; Srček, V.G. A brief overview of the potential environmental hazards of ionic liquids. *Ecotoxicol. Environ. Saf.* **2014**, *99*, 1–12. [CrossRef] [PubMed]
25. Kumar, R.A.; Papaiconomou, N.; Lee, J.-M.; Salminen, J.; Clark, D.S.; Prausnitz, J.M. In vitrocytotoxicities of ionic liquids: Effect of cation rings, functional groups, and anions. *Environ. Toxicol.* **2009**, *24*, 388–395. [CrossRef] [PubMed]
26. Cho, C.-W.; Jeon, Y.-C.; Pham, T.P.T.; Vijayaraghavan, K.; Yun, Y.-S. The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum*. *Ecotoxicol. Environ. Saf.* **2008**, *71*, 166–171. [CrossRef]
27. Matzke, M.; Stolte, S.; Thiele, K.; Juffernholz, T.; Arning, J.; Ranke, J.; Welz-Biermann, U.; Jastorff, B. The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery. *Green Chem.* **2007**, *9*, 1198. [CrossRef]

28. Pirkwieser, P.; López-López, J.A.; Kandioller, W.; Keppler, B.K.; Moreno, C.; Jirsa, F. Novel 3-Hydroxy-2-Naphthoate-Based Task-Specific Ionic Liquids for an Efficient Extraction of Heavy Metals. *Front. Chem.* **2018**, *6*, 172. [CrossRef]
29. Stein, J.R. *Handbook of Phycological Methods: Culture Methods and Growth Measurements*; Cambridge University Press: Cambridge, UK, 1980.
30. Maxwell, K.; Johnson, G.N. Chlorophyll Fluorescence-a Practical Guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef]
31. Kitajima, M.; Butler, W. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochim. Biophys. Acta (BBA) Bioenergy* **1975**, *376*, 105–115. [CrossRef]
32. Lichtenthaler, H.K.; Buschmann, C.; Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica* **2005**, *43*, 379–393. [CrossRef]
33. Juneau, P.; Dewez, D.; Matsui, S.; Kim, S.-G.; Popovic, R. Evaluation of different algal species sensitivity to mercury and metolachlor by PAM-fluorometry. *Chemosphere* **2001**, *45*, 589–598. [CrossRef]
34. Pirkwieser, P.; López-López, J.A.; Kandioller, W.; Keppler, B.K.; Moreno, C.; Jirsa, F. Solvent Bar Micro-Extraction of Heavy Metals from Natural Water Samples Using 3-Hydroxy-2-Naphthoate-Based Ionic Liquids. *Molecules* **2018**, *23*, 3011. [CrossRef] [PubMed]
35. Germani, R.; Mancini, M.V.; Savelli, G.; Spreti, N. Mercury extraction by ionic liquids: temperature and alkyl chain length effect. *Tetrahedron Lett.* **2007**, *48*, 1767–1769. [CrossRef]
36. EU. *Directive 2000/60/Ec of the European Parliament and the Council Establishing a Framework for the Community in the Field of Water Policy*; European Union: Brussels, Belgium, 2000.
37. Republic of Austria. Verordnung des Bundesministers Für Soziale Sicherheit Und Generationen Über Die Qualität Von Wasser Für Den Menschlichen Gebrauch (Trinkwasserverordnung—TWV). In *BGBl. II Nr. 304/2001*; Bundeskanzleramt Österreich: Vienna, Austria, 2001.
38. EU. *Directive 2008/105/Ec of the European Parliament and of the Council of 16 December 2008 on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/Eec, 83/513/Eec, 84/156/Eec, 84/491/Eec, 86/280/Eec and Amending Directive 2000/60/Ec of the European Parliament and of the Council*; European Parliament: European Union: Brussels, Belgium, 2008.
39. Rodrigues, A.; Jesus, F.; Fernandes, M.; Morgado, F.; Soares, A.M.; Abreu, S. Mercury Toxicity to Freshwater Organisms: Extrapolation Using Species Sensitivity Distribution. *Bull. Environ. Contam. Toxicol.* **2013**, *91*, 191–196. [CrossRef]
40. Wood, C.M. Silver. In *Homeostasis and Toxicology of Non-Essential Metals*; Elsevier: Amsterdam, The Netherlands, 2012.
41. Ratte, H.T. Bioaccumulation and Toxicity of Silver Compounds: A Review. *Environ. Toxicol. Chem.* **1999**, *18*, 89–108. [CrossRef]
42. Schmittschmitt, J.P.; Shaw, J.R.; Birge, W.J. Effects of Silver on Green Algae and Prospects for Trophic Transfer. In Proceedings of the 4th International Conference Proceedings: Transport, Fate and Effects of Silver in the Environment, Madison, WI, USA, 25–28 August 1996.
43. Heijerick, D.G.; Bossuyt, B.T.; De Schamphelaere, K.A.; Indeherberg, M.; Mingazzini, M.; Janssen, C.R. Effect of Varying Physicochemistry of European Surface Waters on the Copper Toxicity to the Green Alga *Pseudokirchneriella subcapitata*. *Ecotoxicology* **2005**, *14*, 661–670.
44. Janssen, C.; Macías-Ruvalcaba, N.A.; Aguilar-Martínez, M.; Kobrak, M. Metal extraction to ionic liquids: the relationship between structure, mechanism and application. *Int. Rev. Phys. Chem.* **2015**, *34*, 591–622. [CrossRef]
45. Stolte, S.; Arning, J.; Bottin-Weber, U.; Matzke, M.; Stock, F.; Thiele, K.; Uerdingen, M.; Welz-Biermann, U.; Jastorff, B.; Ranke, J. Anion effects on the cytotoxicity of ionic liquids. *Green Chem.* **2006**, *8*, 621. [CrossRef]
46. Park, S.-M.; Choi, J.; Nam, T.-G.; Ku, J.-M.; Jeong, K. Anti-diabetic effect of 3-hydroxy-2-naphthoic acid, an endoplasmic reticulum stress-reducing chemical chaperone. *Eur. J. Pharmacol.* **2016**, *779*, 157–167. [CrossRef]
47. Ma, J.-M.; Cai, L.-L.; Zhang, B.-J.; Hu, L.-W.; Li, X.-Y.; Wang, J. Acute toxicity and effects of 1-alkyl-3-methylimidazolium bromide ionic liquids on green algae. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 1465–1469. [CrossRef]

48. Liu, H.; Zhang, X.; Chen, C.; Du, S.; Dong, Y. Effects of imidazolium chloride ionic liquids and their toxicity to Scenedesmus obliquus. *Ecotoxicol. Environ. Saf.* **2015**, *122*, 83–90. [CrossRef] [PubMed]
49. Liu, D.; Liu, H.; Wang, S.; Chen, J.; Xia, Y. The toxicity of ionic liquid 1-decylpyridinium bromide to the algae *Scenedesmus obliquus*: Growth inhibition, phototoxicity, and oxidative stress. *Sci. Total Environ.* **2018**, *622–623*, 1572–1580. [CrossRef] [PubMed]
50. UN. *Globally Harmonized System of Classification and Labelling of Chemicals (Ghs)*; United Nations: New York, NY, USA; Geneva, Switzerland, 2011.
51. Kumar, K.S.K.; Dahms, H.-U.; Lee, J.-S.; Kim, H.C.; Lee, W.C.; Shin, K.-H. Algal photosynthetic responses to toxic metals and herbicides assessed by chlorophyll a fluorescence. *Ecotoxicol. Environ. Saf.* **2014**, *104*, 51–71. [CrossRef] [PubMed]
52. Fai, P.B.; Grant, A.; Reid, B. Chlorophyll a fluorescence as a biomarker for rapid toxicity assessment. *Environ. Toxicol. Chem.* **2007**, *26*, 1520–1531. [CrossRef] [PubMed]
53. Yu, Y.; Lu, X.; Zhou, Q.; Dong, K.; Yao, H.; Zhang, S.-J. Biodegradable Naphthenic Acid Ionic Liquids: Synthesis, Characterization, and Quantitative Structure-Biodegradation Relationship. *Chem. A Eur. J.* **2008**, *14*, 11174–11182. [CrossRef]
54. Pacyna, E.G.; Pacyna, J.; Fudała, J.; Strzelecka-Jastrząb, E.; Hlawiczka, S.; Panasiuk, D.; Nitter, S.; Pregger, T.; Pfeiffer, H.; Friedrich, R. Current and future emissions of selected heavy metals to the atmosphere from anthropogenic sources in Europe. *Atmospheric Environ.* **2007**, *41*, 8557–8566. [CrossRef]
55. Vasseur, P.; Pandard, P.; Burnel, D. Influence of Some Experimental Factors on Metal Toxicity to *Selenastrum capricornutum*. *Toxic. Assess.* **1988**, *3*, 331–343. [CrossRef]
56. Chen, C.Y.; Lin, K.C. Optimization and Performance Evaluation of the Continuous Algal Toxicity Test. *Environ. Toxicol. Chem.* **1997**, *16*, 1337–1344. [CrossRef]
57. Lin, J.-H.; Kao, W.-C.; Tsai, K.-P.; Chen, C.-Y. A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants. *Water Res.* **2005**, *39*, 1869–1877. [CrossRef]
58. Horvatic, J.; Persic, V.; Pavlic, Z.; Stjepanovic, B.; Has-Schön, E. Toxicity of Metals on the Growth of *Raphidocelis Subcapitata* and *Chlorella Kessleri* Using Microplate Assays. *Fresenius Environ. Bull.* **2007**, *16*, 826.
59. Monteiro, C.; Caldas-Fonseca, S.; Castro, P.; Malcata, F.X. Toxicity of cadmium and zinc on two microalgae, *Scenedesmus obliquus* and *Desmodesmus pleiomorphus*, from Northern Portugal. *Environ. Biol. Fishes* **2010**, *23*, 97–103. [CrossRef]
60. Juneau, A.E.B.P.; Juneau, P.; El Berdey, A.; Popović, R. PAM Fluorometry in the Determination of the Sensitivity of *Chlorella vulgaris*, *Selenastrum capricornutum*, and *Chlamydomonas reinhardtii* to Copper. *Arch. Environ. Contam. Toxicol.* **2002**, *42*, 155–164. [CrossRef] [PubMed]
61. López-López, J.A.; Pirkwieser, P.; Leyma, R.; Kandioller, W.; Krachler, R.; Keppler, B.K.; Jirsa, F.; Moreno, C. Solvent bar micro-extraction for greener application of task specific ionic liquids in multi-elemental extraction. *J. Clean. Prod.* **2018**, *201*, 22–27. [CrossRef]