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Study of the toxicity of five quaternary ammonium ionic liquids to aquatic organisms

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ABSTRACT

The study aimed to test the toxicity of five quaternary ammonium ionic liquids with varying numbers (from one to three) of didecyldimethylammonium cations and single nitrite, nitrate, or citrate anions toward *Vibrio fischeri* (5- and 15-min acute luminescence inhibition), *Daphnia magna* (24- and 48-h acute immobilization test), *Artemia salina* (24-h acute immobilization test), *Pseudokirchneriella subcapitata* (72-h chronic growth inhibition test), and *Lemna minor* (7-d chronic growth inhibition test). Subsequently, the activities of catalase, superoxide dismutase, and glutathione S-transferase were measured in *D. magna* and *L. minor* after treatment with 25% and 50% effective concentrations of the most toxic compound. The results clearly indicate that the toxicity depends on the number of cations in the molecule: the more cations there are, the higher the toxicity. The toxic effects at 50% calculated in this study ranged between 0.01 and 100 μM depending on the test organism. Of all the test species, *D. magna* was the most and *A. salina* the least sensitive to the tested compounds. The most toxic ionic liquid inhibited catalase and increased superoxide dismutase activity in both organisms. Oxidative stress is either directly or indirectly involved in the toxic mechanism of the tested ionic liquid action.

Keywords: Aquatic toxicity; Ionic liquids; Quaternary ammonium compounds

1. Introduction

Quaternary ammonium ionic liquids (QAILs) belong to the class of quaternary ammonium compounds (QACs) used on a large scale in industry, households, and agriculture. They are applied mainly as surface-active agents, emulsifiers, antistatics, corrosion inhibitors, phase-transfer catalysts, and cosmetic ingredients [1]. Due to their strong antimicrobial activity, they constitute a very popular class of disinfectants. QAILs are also tested as fungicides in wood protection [2] and antifeedants in crop protection [3]. QACs are released into the environment via effluents from industries, laundries, households, and hospitals, or by inadvertent spills [4]. The sources of contamination can also be the leachates from the application site (wooden surfaces, and crops) or landfills. QACs have already been detected in different environmental samples, including surface water, wastewater, marine water, urban storm water, and river sediments [5–10].

There is a strong need to explore the field of the toxicity of QAILs and its mechanisms, because this family of compounds is growing rapidly as producers seek new and effective formulations. However, the environmental impact of QAILs is still insufficiently described. Although there is a vast amount of literature available on the toxicity of ionic liquids (ILs), studies on QAILs are not reported very often. The focus is being placed mainly on imidazolium ionic liquids (IMILs), making this group of ILs the most popular subject of study. Many ILs have already been proven to be toxic to different organisms, including bacteria and fungi [11], luminescent marine bacteria [12], algae [13], crustaceans [14],

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plants [15], fish [16], as well as rats and mice [17,18]. In many cases, their possible occurrence in the environment has been deemed threatening to ecosystems. Nevertheless, in the majority of reports, the toxicity mechanism is not studied.

All ILs are composed of cations and anions. The cation structure is mainly responsible for their toxicity, which depends on the number, type, and length of substituents attached to the head group. Generally, the longer the side chain, the more toxic the compound [19]. ILs with long alkyl chains in the cation group are believed to disturb biological membranes and that is how their toxicity is explained in most cases [20]. However, many recent reports state that ILs can also cause oxidative stress in different organisms [21–24]. Oxidative stress is the imbalance between the production of reactive oxygen species and the natural protection from their harmful actions [25]. Oxidative stress can lead to disfunctioning of many organelles, natural metabolism disturbance, DNA damage, and cell death [26]. In the presence of oxidative stress, the antioxidant system is triggered, and the alterations in its components' natural activity can constitute valuable biomarkers of oxidative stress presence [27].

The aim of this study was to test the toxicity of five QAILs to four aquatic species representing different trophic levels: *Vibrio fischeri* (decomposers), *Daphnia magna* (primary consumers), *Artemia salina* (primary consumers), *and Lemna minor* (producers), as well as the toxicity of three QAILs to *Pseudokirchneriella subcapitata* (producers). Subsequently, the activities of catalase (CAT), superoxide dismutase (SOD), and S-glutathione transferase (GST) were measured in *D. magna* and *L. minor* after exposure to 25% and 50% effective concentrations of the most toxic QAIL.

2. Materials and methods

2.1. Test compounds and chemicals

Five QAILs with didecyldimethylammonium (DDA) cations were tested, as presented in Table 1. The compounds were synthesized by a team led by Juliusz Pernak from Poznań University of Technology (Poland). Stock solutions of ILs for tests were prepared in deionized Millipore water. All chemicals and reagents used were of analytical grade.

Table 1
The structures of tested QAILs

Molecule name	Formula	Molecular weight, g/mol
Didecyldimethylammonium nitrite	[DDA] [NO_2]	372.6
Didecyldimethylammonium nitrate	[DDA] [NO_3]	388.6
Didecyldimethylammonium dihydrocitrate	[DDA] [H_2Cytr]	517.7
Bis-(didecyldimethylammonium) hydrocitrate	[$DDA]_2$ [HCytr]	843.4
Tris-(didecyldimethylammonium) citrate	[$DDA]_3$ [Cytr]	1,169.0

QAILs, quaternary ammonium ionic liquids.

2.2. Toxicity tests

2.2.1. Acute toxicity toward V. fischeri

A test for acute toxicity toward luminescent marine bacteria *V. fischeri* was performed for 5 and 15 min using standard Microtox test system (MicroBioTests Inc., Belgium). The bacteria were activated by reconstitution solution (0.01% of NaCl) and exposed to QAIL solutions (2% of NaCl). The luminescence inhibition after treatment with QAILs was measured with Microtox 500 analyzer and MicrotoxOmni software (Modern Water, UK). The calculated effects were expressed as $IC_{50/5\ min}$ and $IC_{50/15\ min}$.

2.2.2. Acute toxicity toward D. magna

D. magna ephippia were purchased from MicroBioTests Inc. (Belgium). They were kept in aerated tap water at 22 ± 1°C, over a photoperiod of 16 h: 8 h light/darkness until they hatched and further for several parthenogenetic generations prior to toxicity tests. They were fed three times a week with *Spirulina* (Cyanotech Corporation, USA). The acute toxicity was tested according to OECD 202 Guideline [28]. Neonates not older than 24 h were used for testing. The tests were performed in plastic 30-well plates. The toxicant dilutions were prepared in aerated tap water. Five toxicant dilutions and one control, which was aerated tap water, were used in each experiment in four technical replicates. Five neonates were placed in each test well, which contained 10 mL of test solution, and incubated under culture cultivation conditions. Subsequently, the number of dead (motionless) and living individuals was noted after 24 and 48 h, respectively. The calculated effects were expressed as $LC_{50/24\ h}$ and $LC_{50/48\ h}$.

2.2.3. Acute toxicity toward A. salina

A. salina ephippia were purchased from Sera (Germany). They were kept in aerated tap water with 0.9% of NaCl at 22 ± 1°C and over a photoperiod of 16 h: 8 h light/darkness until they hatched. The acute toxicity was tested according to ASTM E1440—91 [29]. Neonates not older than 24 h were used for testing. The tests were performed in plastic 24-well plates. The toxicant dilutions were prepared in aerated tap water with 0.9% of NaCl. Five toxicant dilutions and one control, which was aerated tap water with 0.9% of NaCl, were used in each experiment in three technical replicates. Ten neonates were placed in each test well, which contained 2 mL of test solution, and incubated at 22 ± 1°C and over a photoperiod of 16 h: 8 h light/darkness. Subsequently, the number of dead (motionless) and living individuals was noted after 24 h. The calculated effects were expressed as $LC_{50/24\ h}$.

2.2.4. Chronic toxicity toward P. subcapitata

The toxicity of [DDA] [H_2Cytr], $[DDA]_2$ [HCytr], and $[DDA]_3$ [Cytr] was tested toward algae. *P. subcapitata* algae were purchased in an immobilized form from MicroBioTests Inc. (Belgium). They were reconstituted and cultivated in sterile conditions in an AAP growth medium in 100 mL glass flasks in 24°C and over a photoperiod of 16 h: 8 h light/darkness. The chronic toxicity was tested according to OECD 201 Guideline [30]. Exponentially growing algae

were exposed to 50 mL of five or six concentrations of QAILs in an AAP growth medium in 100 mL glass flasks in three technical replicates obtaining 10^4 cells/mL. The algae in the control samples were exposed to a 50 mL of AAP growth medium. They were incubated for 72 h in culture cultivation conditions. At the beginning and in the end of the test period, the optical density of each test replicate was measured using Jenway 6300 spectrophotometer and 10 cm plastic cuvettes at λ = 670 nm. The calculated effects were expressed as $IC_{50/72\,h}$.

2.2.5. Chronic toxicity toward L. minor

L. minor were originally collected from a small eutrophic pond in Tarnowskie Góry (Silesian Voivodeship, Poland). They were cultured in a 50 L glass aquarium in a 20× AAP growth medium with aerated tap water as a diluent at 22 ± 1°C, over a photoperiod of 16 h: 8 h light/darkness since collection. The chronic toxicity toward *L. minor* was tested according to OECD 221 Guideline [31]. The toxicant dilutions were prepared in a 20× AAP growth medium. The test was performed in glass petri dishes (8 cm in diameter) in technical triplicates for each dilution. Each dish contained 15 mL of test solutions. The control dishes contained 15 mL of a 20× AAP growth medium. Several healthy plants with 2–4 fronds were transferred onto each dish. Plants in dishes with glass covers were incubated under culture cultivation conditions. Subsequently, the number of fronds in all the dishes was noted.

2.3. Calculations of toxicity effects

Regression curves were fitted to data points using MATLAB 10 software with the application of the following four-parametric Hill equation:

$$y = \min + \frac{\max - \min}{1 + \left(\frac{x}{EC_{50}}\right)^{-HS}} \quad (1)$$

where y represents toxic effect (%), x is the toxicant concentration (μM), min and max are the minimal and maximal data values set to 0% and 100%, respectively, EC_{50} is the concentration causing toxic effect at 50% (μM), and HS is the Hill slope.

2.4. Enzyme activity measurements

D. magna neonates and *L. minor* were exposed to LC_{25} and LC_{50} of $[DDA]_3$ [Cytr] for 24 h and 7 d, respectively, at 22 ± 1°C, over a photoperiod of 16 h: 8 h light/darkness. For a positive control (PC), organisms were exposed to 0.002 M H_2O_2 for 30 min. From each treatment group, 15 green *L. minor* plants with double fronds and 30 living daphnids were chosen for enzyme assays. Organisms from each sample were rinsed with ice-cold phosphate-buffered saline (PBS) and homogenized on ice in PBS with MHX/E device (Xenox, Niersbach, Germany). Each homogenate was centrifuged at 12,000 rcf for 15 min, at 4°C. Supernatants were collected and stored in –80°C for enzyme assays.

The activities of CAT, SOD, and GST were analyzed in samples with Epoch microplate spectrophotometer (BioTek, Winooski, Vermont, USA) according to Góth [32], Misra and Fridovich [33], and Habig et al. [34], respectively. The principle of the CAT activity assay is the binding of the remaining H_2O_2 unconverted by CAT in the solution with ammonium molybdate, which together form a colorful complex, measured at λ = 405 nm. The results of the CAT activity were expressed as (μM H_2O_2/min/$mg_{protein}$). The activity of SOD was evaluated by measuring the inhibition of adrenaline autooxidation. Adrenaline autooxidates to adenochrome in pH 10.2 and the side product is superoxide anion radical catalyzed by SOD. The absorbance was measured for 4 min (in 1-min intervals) at λ = 480 nm. The SOD activity was expressed as (unit of activity [UA]/min/$mg_{protein}$). One UA is the amount of enzyme which inhibits autooxidation at 50%. GST catalyzes the oxidation of reduced glutathione (GSH) in reaction with nucleo- and electrophile compounds. The amount of the complex between GSH and 1-chloro-2,4-dinitrobenzene (CDNB) in the supernatants immediately after adding CDNB was measured for 5 min (in 1-min intervals) at λ = 340 nm. The GST activity was expressed as (μM CDNB/min/$mg_{protein}$).

Each supernatant sample in all three assays was measured in four technical replicates. The experiments were performed on 96-well plates. The protein content was measured using Protein Quantification Kit-Rapid (Fluka) with bovine serum albumin as a standard. Each assay was repeated three times.

2.5. Statistical analyses

The results were analyzed statistically with the use of Statistica 10 software. Shapiro–Wilk test was used to analyze the normality of distribution. The results of enzyme activity measurements were analyzed with one-way analysis of variance (ANOVA) and post hoc Tukey test ($p < 0.05$). Asterisks used in the figure mark those results which are statistically different from controls.

3. Results and discussion

3.1. Toxicity tests

The results of toxicity tests are presented in Table 2. The toxicity of QAILs was classified according to EU criteria (Table 3) [35]: [DDA] [NO_2] and [DDA] [H_2Cytr] were classified as very toxic, whereas [DDA] [NO_3], $[DDA]_2$ [HCytr], and $[DDA]_3$ [Cytr] were classified as extremely toxic to aquatic biocenoses. $[DDA]_3$ [Cytr] compound was the most toxic toward almost all of the tested species. One exception was *P. subcapitata*, in which $[DDA]_2$ [HCytr] was the most toxic comparable to $[DDA]_3$ [Cytr]. The results clearly indicate that the toxicity is highly dependent on the number of DDA cations in a molecule: the more cations there are, the higher the toxicity. The toxicity is less dependent on the anion type. These findings are in agreement with most of the literature [36]. The species that are most sensitive to the tested QAILs were *V. fischeri* (toxic effects were comparable to the ones observed in other tested species despite very short exposure time) and *D. magna*, in which the strongest effects were observable.

Table 2
Toxicity of QAILs to tested species

Compound	Organism	Toxic effect		
		Type	Value	
			μM	mg/L
[DDA] [NO_2]	*Vibrio fischeri*	$IC_{50/5\ min}$	1.35 (1.20–1.51)	0.50
		$IC_{50/15\ min}$	0.75 (0.67–0.83)	0.28
	Daphnia magna	$LC_{50/24\ h}$	2.32 (2.0–2.77)	0.86
		$LC_{50/48\ h}$	0.59 (0.34–0.80)	0.22
	Artemia salina	$LC_{50/24\ h}$	57.98 (50.43–66.77)	21.60
	Lemna minor	$IC_{50/7\ d}$	4.27 (3.74–4.81)	1.59
[DDA] [NO_3]	*V. fischeri*	$IC_{50/5\ min}$	1.33 (1.18–1.52)	0.52
		$IC_{50/15\ min}$	0.72 (0.64–0.79)	0.28
	D. magna	$LC_{50/24\ h}$	0.98 (0.08–1.23)	0.38
		$LC_{50/48\ h}$	0.20 (0.08–0.29)	0.08
	A. salina	$LC_{50/24\ h}$	49.32 (45.97–53.26)	19.17
	L. minor	$IC_{50/7\ d}$	3.96 (3.57–4.37)	1.54
[DDA] [H_2Cytr]	*V. fischeri*	$IC_{50/5\ min}$	1.40 (1.23–1.60)	0.73
		$IC_{50/15\ min}$	0.95 (0.85–1.03)	0.49
	D. magna	$LC_{50/24\ h}$	1.05 (0.94–1.15)	0.54
		$LC_{50/48\ h}$	0.23 (0.17–0.28)	0.12
	A. salina	$LC_{50/24\ h}$	30.07 (26.71–33.0)	15.57
	Pseudokirchneriella subcapitata	$IC_{50/72\ h}$	1.04 (0.93–1.15)	0.54
	L. minor	$IC_{50/7\ d}$	1.17 (1.13–1.22)	0.61
[$DDA]_2$ [HCytr]	*V. fischeri*	$IC_{50/5\ min}$	0.90 (0.77–1.04)	0.76
		$IC_{50/15\ min}$	0.61 (0.51–0.69)	0.51
	D. magna	$LC_{50/24\ h}$	0.34 (0.30–0.38)	0.29
		$LC_{50/48\ h}$	0.07 (0.03–0.11)	0.06
	A. salina	$LC_{50/24\ h}$	7.86 (6.76–9.19)	6.63
	P. subcapitata	$IC_{50/72\ h}$	0.55 (0.51–0.60)	0.47
	L. minor	$IC_{50/7\ d}$	0.69 (0.66–0.71)	0.58
[$DDA]_3$ [Cytr]	*V. fischeri*	$IC_{50/5\ min}$	0.51 (0.4–0.63)	0.60
		$IC_{50/15\ min}$	0.34 (0.28–0.41)	0.40
	D. magna	$LC_{50/24\ h}$	0.07 (0.05–0.08)	0.08
		$LC_{50/48\ h}$	0.04 (0.02–0.6)	0.04
	A. salina	$LC_{50/24\ h}$	6.15 (5.03–8.07)	7.18
	P. subcapitata	$IC_{50/72\ h}$	0.59 (0.51–0.67)	0.69
	L. minor	$IC_{50/7\ d}$	0.46 (0.43–0.48)	0.53

Note: Values in bold were used for toxicity classification.
IC_{50}, inhibitory 50% concentration; LC_{50}, lethal 50% concentration; QAILs, quaternary ammonium ionic liquids.

Table 3
Toxicity classification criteria of chemicals in relation to their harmfulness to aquatic biocenoses according to EU [35]

LC $(EC)_{50}$, mg/L	Toxicity classification
<0.1	Extremely toxic
0.1–1	Very toxic
1–10	Toxic
10–100	Harmful
>100	Not toxic

LC_{50}, lethal 50% concentration; EC, effective concentration.

Taking into account that the tested QAILs were synthesized in order to be used as components of biocidal formulations, they could constitute a threat to non-target bacteria and fungi in the environment. Unfortunately, the literature is limited in information on the toxicity of these compounds to marine species [37]. In this study, *V. fischeri* marine bacteria were proved to be sensitive to QAILs. The toxicity depended on the number of DDA cations in a molecule, as well as on the concentration and exposure time. The mechanism of ILs toxicity toward bacteria is based on the interruption of the proton flow in the membranes, inhibition of the cell respiratory system, replication disturbance, and loss of membrane integrity, which leads to the leakage of intracellular components, lysis, and denaturation of the cell's inner material [38]. *A. salina* was the least sensitive to the tested QAILs. This species had already been proved to be less sensitive to similar compounds than to others—for example, in toxicity tests of selected QACs toward *V. fischeri*, *T. thermophila* and *Spirostoum ambiguum* [39] and of imidazolium tetrafluoroborates toward *Thamnocephalus platyurus*, *Brachionus calyciflorus* and *Brachionus plicatilis* as well (in contrast to popular organic solvents like trichloromethane, benzene, methylbenzene, methanol, acetonitrile, 2-propanole, and ethanole) [40]. Besides the fact that *A. salina* was the least sensitive to the tested QAILs, the rule of higher toxicity with more DDA cations in the molecule is obvious in this species just like in other test organisms from this work. Algae play an important role in the balance of ecosystems, being the first trophic level which provides organic compounds and oxygen [41]. It is believed that algae are more sensitive to QACs than fish and crustaceans are [42]. The presented results prove that *P. subcapitata* are sensitive to QAILs, nevertheless not as much as other species, like *D. magna* or *V. fischeri*.

As already mentioned above, the toxicity of ILs is believed to be stronger with longer alkyl substituents. Nevertheless, there is a lack of information in the literature on how the toxicity changes with a varying number of substituents or cations. Table 4 presents the available data on the toxicity of compounds whose function or structure is similar to the tested QAILs in *D. magna*, *V. fischeri*, and *P. subcapitata*. ILs belonging to different classes and those containing alkyl substituents of 8–16 carbon atoms were selected for the comparison. Fungicides, which are a distinct class of chemicals, were also chosen, as the tested QAILs are used along with them in biocidal formulations or as their replacements. The QAILs which were the subject of this study contain two, four, or six alkyl chains. They were comparably or more toxic in *V. fischeri* and *D. magna* than QACs containing one alkyl chain of 12 to even 16 carbon atoms and in a few cases also a benzyl group. All of the tested QAILs were much more toxic than IMILs and pirrolidinium ILs with one alkyl chain of eight carbons in *V. fischeri*. IMILs are believed to be more toxic than QAILs [43]. Nevertheless, IMILs with one chain of 10 and 14 carbon atoms were comparably or less toxic in *V. fischeri* and *D. magna*, respectively, than the tested QAILs with two alkyl chains. $[DDA]_2$ [HCytr] was even twice as toxic in *D. magna* as IMIL with one alkyl chain of 12 carbon atoms. The available literature is not rich in information on the toxicity of ILs, pesticides, or surfactants similar to the tested QAILs toward *P. subcapitata*. Pretti et al. [46] tested two commercially available QAILs named Ammoeng 100 and Ammoeng 130. The former is a mixture of forms containing alkyl chains of different lengths attached to a positively charged nitrogen atom. Its structure contains four different substituents: one chain obtained from coconut oil, two other chains of 6–16 carbon atoms with an etoxyl group, and one methyl group. The chemical name of Ammoeng 130 is ethylmethyldistearylammonium chloride. Ammoeng 130 was less toxic toward algae than the QAILs tested herein regardless of the presence of two attached stearyl chains. The toxic effect of Ammoeng 100 cannot be compared at the micromole scale, as there is no information given on the exact molecular mass of the compound, although the effect expressed in mg/L is close to the effects calculated for the three tested QAILs. The tested QAILs were less toxic toward *P. subcapitata* than benzalkonium chloride and ammonium bromide with one chain of 14 carbon atoms. All of the tested QAILs were more toxic than the presented fungicides toward *P. subcapitata* and *D. magna*.

In summary, the results of this study in comparison with the data in Table 4 indicate that not only the length, but also the number of the substituents has an impact on the toxicity of ILs. This effect is probably based on a stronger ability of compounds with more alkyl chains in one molecule to disturb biological membranes than that of compounds with only one alkyl chain which can be less harmful or act on a smaller surface of the cell membrane. Thus, the more alkyl chains in the molecule, the higher the ability of ILs to penetrate and disturb biological membranes.

3.2. Enzyme activity measurements

$[DDA]_3$ [Cytr] changed the activities of antioxidant enzymes in *D. magna* and *L. minor* after treatment with 25% and 50% effective concentrations (Fig. 1). Generally, this compound caused decreased activity of CAT and increased activity of SOD in both test organisms. There was no significant change in the activity of GST. A PC also produced similar effects between two tested species. The results of this study show that $[DDA]_3$ [Cytr] significantly upregulated SOD activity both in *D. magna*, as well as in *L. minor*, which indicates oxidative stress. The strength of the effect increased with the increasing toxicant concentration. Low concentrations of xenobiotics can lead to an increased activity of antioxidant defense systems in aquatic organisms [27]. On the other hand, the activity of CAT was changed insignificantly. CAT activity was decreasing with increased IL concentration in both organisms with the exception of LC_{25} treatment in *D. magna*, which resulted in increased activity. The activity of GST was

Table 4
Toxicity of compounds of a similar structure or function to the tested QAILs toward different aquatic species

Compound	Class	Organism	Toxic effect			Reference
			Type	Value		
				mg/L	μM	
Didecyldimethylammonium chloride	QAC	*Vibrio fischeri*	$IC_{50/30\ min}$	0.24	0.78	[44]
Dimethylditetradecylammonium bromide		*Pseudokirchneriella subcapitata*	$IC_{50/72\ h}$	0.02	0.04	[4]
		Daphnia magna	$LC_{50/48\ h}$	0.02	0.04	
Dodecyltrimethylammonium bromide		*D. magna*	$LC_{50/24\ h}$	0.37	1.2	[45]
		D. magna	$LC_{50/24\ h}$	0.38	1.23	[44]
Tetradecyltrimethylammonium bromide		*V. fischeri*	$IC_{50/30\ min}$	0.28	0.83	
		D. magna	$LC_{50/24\ h}$	0.09	0.27	[45]
		D. magna	$LC_{50/24\ h}$	0.14	0.42	[44]
Hexadecyltrimethylammonium bromide		*V. fischeri*	$IC_{50/30\ min}$	0.63	1.73	
		D. magna	$LC_{50/24\ h}$	0.06	0.16	[45]
			$LC_{50/24\ h}$	0.13	0.36	[44]
Dodecylbenzyl dimethylammonium		*V. fischeri*	$IC_{50/30\ min}$	0.18	0.47	
		D. magna	$LC_{50/24\ h}$	0.13	0.34	
Tetradecylbenzyldimethylammonium chloride		*V. fischeri*	$IC_{50/30\ min}$	0.15	0.41	
		D. magna	$LC_{50/24\ h}$	0.13	0.35	
Hexadecylbenzyldimethylammonium chloride		*V. fischeri*	$IC_{50/30\ min}$	0.55	1.39	
		D. magna	$LC_{50/24\ h}$	0.22	0.56	
Benzalkonium chloride		*V. fischeri*	$IC_{50/30\ min}$	0.5	1.42	
		P. subcapitata	$IC_{50/72\ h}$	0.04	0.12	[4]
		D. magna	$LC_{50/48\ h}$	0.04	0.12	
Ammoeng 100		*P. subcapitata*	$IC_{50/72\ h}$	0.12	*	[46]
Ammoeng 130		*P. subcapitata*	$IC_{50/72\ h}$	0.83	1.4	
Octylmethylimidazolium bromide	IMIL	*V. fischeri*	$IC_{50/15\ min}$	39.26	143.18	[47]
		D. magna	$LC_{50/48\ h}$	0.95	3.45	[48]
Dimethyloctylimidazolium bromide		*V. fischeri*	$IC_{50/15\ min}$	16.56	57.25	[47]
Decylmethylimidazolium bromide		*D. magna*	$LC_{50/48\ h}$	0.15	0.49	[48]
Decylmethylimidazolium chloride		*V. fischeri*	$IC_{50/15\ min}$	0.15	0.59	[49]
Decylmethylimidazolium tetrafluorobromide		*V. fischeri*	$IC_{50/15\ min}$	0.2	0.66	
Dodecylmethylimidazolium bromide		*D. magna*	$LC_{50/48\ h}$	0.05	0.15	[48]
Tetradecylmethylimidazolium chloride		*V. fischeri*	$IC_{50/15\ min}$	0.22	0.71	[49]
Octylmethylpirrolidinium bromide	PirrIL	*V. fischeri*	$IC_{50/15\ min}$	39.26	141.08	[47]
Octylmethylpiperidinium bromide	PipIL	*V. fischeri*	$IC_{50/15\ min}$	23.55	80.57	
Ethyloctylpiperidinium bromide		*V. fischeri*	$IC_{50/15\ min}$	19.09	62.32	
Climbazole	Azole fungicide	*P. subcapitata*	$IC_{50/72\ h}$	1.2	4.1	[50]
		D. magna	$LC_{50/48\ h}$	15.99	54.62	
Tebuconazole		*D. magna*	$LC_{50/24\ h}$	56.83	184.62	[51]
		D. magna	$LC_{50/48\ h}$	40.1	130.27	

* Not possible to calculate.
IC_{50}, inhibitory 50% concentration; IMIL, imidazolium ionic liquid; LC_{50}, lethal 50% concentration; PipIL, piperidinium IL; PirrIL, pirrolidinium IL; QAC, quaternary ammonium compound; QAILs, quaternary ammonium ionic liquids.

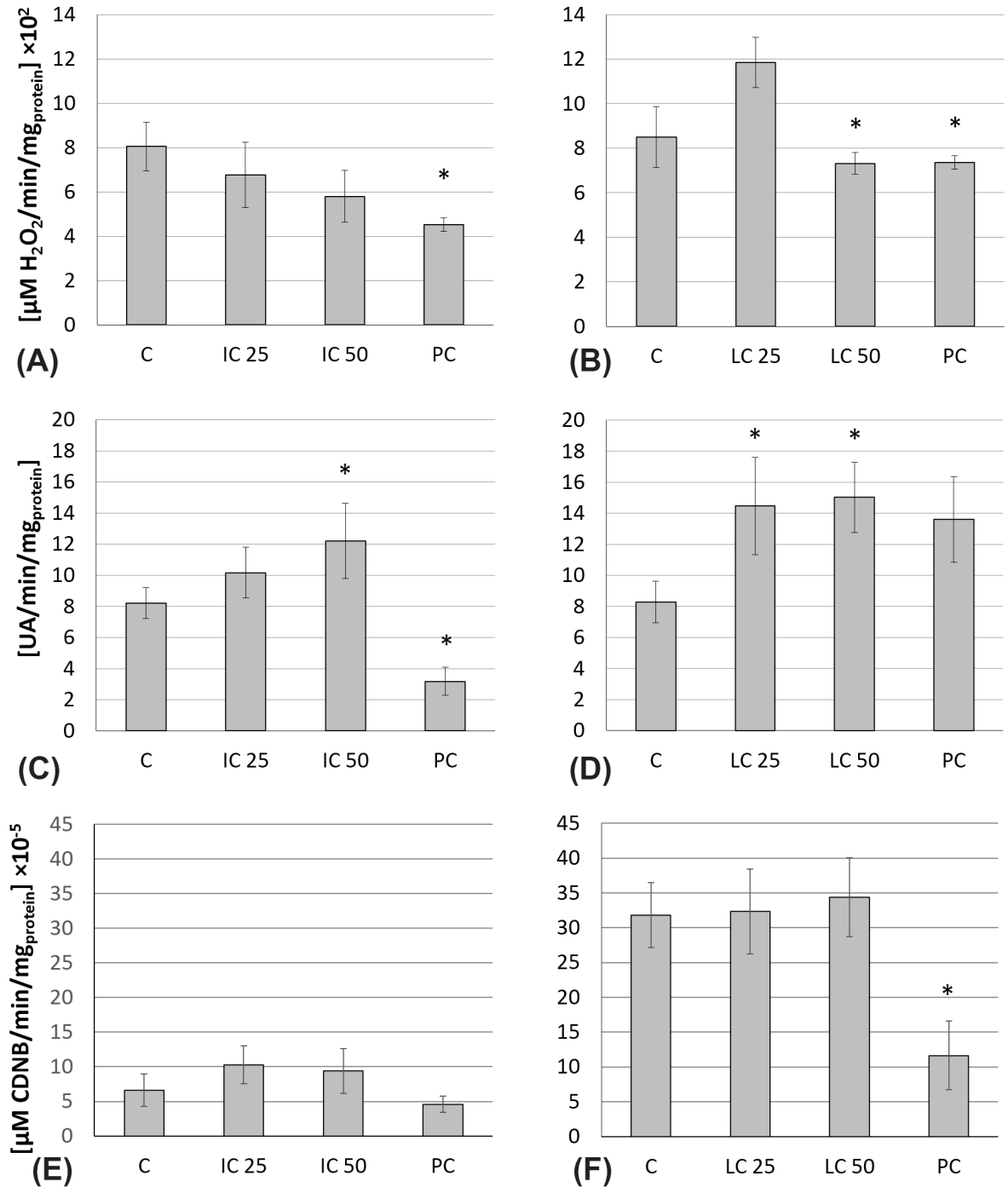

Fig. 1. The activities of ((A), (B)) CAT, ((C), (D)) SOD, and ((E), (F)) GST in *L. minor* ((A), (C), and (E)) and *D. magna* ((B), (D), and (F)). Data are presented as means ± standard deviation. Asterisks denote results statistically different from controls ($p < 0.05$). C—control, PC—positive control sample, IC_{25}—inhibitory 25% concentration, IC_{50}—inhibitory 50% concentration, LC_{25}—lethal 25% concentration, and LC_{50}—lethal 50% concentration.

not changed. According to Wang et al. [53], who tested SDS toxicity in the mouse's primary hepatocytes, enzyme activity may return rapidly to the normal level after exposure to a low concentration surfactant, which increases its molecular flexibility. If the surfactant concentration is high, it leads to the damage of the enzyme structure and the inhibition of its activity. ILs have already been proved to change the activities of antioxidant enzymes in daphnids [48] and macrophytes [54], and QACs to cause protein denaturation [55–58].

It was proved that SDS, which is a popular anionic surfactant containing a long alkyl chain, can cause oxidative stress in *Mytilus galloprovencialis* and change the activity of antioxidant enzymes. The toxic action of this compound involves cellular membrane disruption [52]. It is possible that

the molecular mechanism of SDS and ILs toxicity is therefore very similar. The results of the present study show that $[DDA]_3$ [Cytr] significantly upregulated SOD activity both in *D. magna*, as well as in *L. minor*, which indicates oxidative stress. The effect was stronger with increased toxicant concentration. Low concentrations of xenobiotics can lead to an increased activity of antioxidant defense systems in aquatic organisms [27]. On the other hand, the activity of CAT was changed insignificantly. CAT activity was decreasing with increased IL concentration in both organisms with the exception of LC_{25} treatment in *D. magna*, which resulted in increased activity. The activity of GST was not changed. According to Wang et al. [53], who tested SDS toxicity in the mouse's primary hepatocytes, enzyme activity may return rapidly to the normal level after exposure to a low concentration of the surfactant, which increases its molecular flexibility. If the surfactant concentration is high, it leads to the damage of the enzyme structure and the inhibition of its activity. ILs have already been proved to change the activities of antioxidant enzymes in daphnids [48] and macrophytes [54], and QAC to cause protein denaturation [55–58].

4. Conclusion

All of the tested QAILs are highly toxic to five aquatic organisms. The toxicity is higher with more DDA cations in the molecule and is less dependent on the anion type. The results obviously indicate that the presence of any of the five tested QAILs could pose a serious threat to aquatic ecosystems by disturbing the trophic chain. The presented results suggest that the toxic mechanism of QAILs' action in aquatic organisms may involve oxidative stress. The popularity of ILs is increasing due to their special features, involving their "designable" character, low vapor pressure, inflammability, extraordinary solving properties, and many more. They will be applied in more processes with time, entering the environment in ever higher quantities. Thus, it is very important to understand their environmental impact, especially for those producers who wish to obtain both safe and effective formulations.

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