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6 **Ecotoxicity assessment of ionic As(III), As(V), In(III) and Ga(III) species potentially**
7 **released from novel III-V semiconductor materials**
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ABSTRACT

III-V materials such as indium arsenide (InAs) and gallium arsenide (GaAs) are increasingly used in electronic and photovoltaic devices. The extensive application of these materials may lead to release of III-V ionic species during semiconductor manufacturing or disposal of decommissioned devices into the environment. Although arsenic is recognized as an important contaminant due to its high toxicity, there is a lack of information about the toxic effects of indium and gallium ions. In this study, acute toxicity of As(III), As(V), In(III) and Ga(III) species was evaluated using two microbial assays testing for methanogenic activity and O₂ uptake, as well as two bioassays targeting aquatic organisms, including the marine bacterium *Aliivibrio fischeri* (bioluminescence inhibition) and the crustacean *Daphnia magna* (mortality). The most noteworthy finding was that the toxicity is mostly impacted by the element tested. Secondly, the toxicity of these species also depended on the bioassay target. In(III) and Ga(III) were not or only mildly toxic in the experiments. *D. magna* was the most sensitive organism for In(III) and Ga(III) with 50% lethal concentrations of 0.5 and 3.4 mM, respectively. On the other hand, As(III) and As(V) caused clear inhibitory effects, particularly in the methanogenic toxicity bioassay. The 50% inhibitory concentrations of both arsenic species towards methanogens were about 0.02 mM, which is lower than the regulated maximum allowable daily effluent discharge concentration (2.09 mg/L or 0.03 mM) for facilities manufacturing electronic components in the US. Overall, the results indicate that the ecotoxicity of In(III) and Ga(III) is much lower than that of the As species tested. This finding is important in filling the knowledge gap regarding the ecotoxicology of In and Ga.

Keywords: III-V materials; Ecotoxicity; Arsenic; Gallium; Indium; Microbial toxicity

1. Introduction

III-V materials such as indium arsenide (InAs), gallium arsenide (GaAs) and indium gallium arsenide (InGaAs) are novel materials with high electron mobility, low power requirement and favorable optoelectronic properties (Dick et al., 2010; Vurgaftman et al., 2001; Yamaguchi et al., 2008). Because of these attractive properties, III-V materials are increasingly used in semiconductor manufacturing as light emitting diodes (LEDs), laser diodes, liquid crystal displays (LCDs), biosensors and microcircuits (Dayeh et al., 2009; Joyce et al., 2011). III-V materials are also finding extensive application in thin-film photovoltaic devices, thin film photovoltaic solar modules and other photovoltaic devices (Miles et al., 2005). It has been reported that the demand for Ga and In has increased significantly during the past 30 years (Lovik et al., 2015; White and Hemond, 2012). The world primary production of Ga in 2014, estimated at 440 metric tons, was four times higher than in 2010 (U.S. Geological Survey, 2015). This rapid growth has been mainly attributed to the higher content of GaAs in different electronic devices, especially smartphones, and increasing use of GaAs-based LEDs (Butcher and Brown, 2014). Global indium consumption in 2014 was about 1,500 metric tons. Increased indium consumption was reportedly driven by increased demand for LCD televisions in developing countries and for smartphones and tablets in developed countries (U.S. Geological Survey, 2015).

The extensive application of III-V materials may contribute to the release of hazardous materials through corrosion of decommissioned electronic and photovoltaic devices. Manufacturing activities could also potentially contribute to the environmental release of III-V materials. For example, chemical mechanical planarization (CMP) of thin films containing III-V

materials, such as GaAs and GaInAs, generates waste effluents that may contain particulate and ionic III-V species. Waste streams containing high levels of arsenic and other hazardous materials require treatment prior to discharge to surface water in order to meet environmental regulations. In the United States, the arsenic discharge limits for effluents produced by facilities manufacturing electronic components are 2.09 mg/L as one-day maximum and 0.83 mg/L as 30-day average concentration (USEPA, 2015). However, there are no current regulatory limits for the discharge of indium (In) and gallium (Ga). Chen and coworkers measured the concentration of As, Ga and In in the groundwater at sites in and around an industrial park (with 350 companies manufacturing integrated circuits) and found that the semiconductor industry in the region had impacted the groundwater (Chen, 2006), with average levels of As, Ga and In detected of 34.2, 19.3 and 9.3 $\mu\text{g/L}$, respectively. These values were significantly higher than those in clean groundwater offsite.

Arsenic and arsenic compounds are well-known for their high toxicity and carcinogenicity. There is a close relation between As toxicity and speciation, and inorganic As species are usually more toxic than many organic As forms to living organisms (Sharma and Sohn, 2009; Shaw et al., 2007; Sierra-Alvarez et al., 2004). The toxicity of trivalent arsenic (As(III) or arsenite) is related to its high affinity for sulfhydryl groups of biomolecules (Aposhian and Aposhian, 2006). The formation of these bonds leads to inhibition of crucial enzymatic functions within the cells (Sharma and Sohn, 2009). On the other hand, the microbial toxicity of arsenate (As(V)) is often the result of its potential for phosphate replacement. Consequently, As(V) inhibits enzymes that use phosphate and uncouples ATP formation, which ultimately results in depletion of cell energy (Rosen and Tamas, 2010). Compared to inorganic arsenic species, there is a lack of available information regarding the toxicity of In(III) and

Ga(III). In and Ga toxicity to humans is most evident from occupational exposure. Inhalation of In and Ga compounds such as indium tin oxide (ITO), indium phosphide (InP) and GaAs is linked to serious and sometimes fatal effects (Chen, 2007; Hines et al., 2013; Hoet et al., 2012; Nakano et al., 2014; Omae et al., 2011). So far only a very limited number of studies have evaluated the ecotoxicity of In(III) and Ga(III) (Jiang et al., 2015; Olivares et al., 2016; Zurita et al., 2007).

The objective of this study was to investigate the ecotoxicity of As(III), As(V), In(III) and Ga(III) species. To this end, two toxicity bioassays were performed, in which inhibition of bioluminescence activity in the marine bacterium, *Aliivibrio fischeri* (formerly known as *Vibrio fischeri*) and lethal effects on *Daphnia magna*, a multicellular aquatic microcrustacean, were tested. The Microtox test and acute toxicity bioassays with daphnids are used by municipalities and industries worldwide as standard methods to test the toxicity of chemicals and effluent streams. Both methods are accepted by organizations such as the U.S. Environmental Protection Agency (USEPA), the Organization for Economic Co-operation and Development (OECD) and the International Organization for Standardization (ISO) (Parvez et al., 2006; Rubinos et al., 2014; Ventura et al., 2012; Zhu et al., 2010; Zhu et al., 2009). In addition, two microbial assays, testing the impact of the various ionic species towards the methanogenic activity of an anaerobic mixed culture and on O₂ uptake by aerobic bacteria in wastewater treatment sludge. Methanogens and aerobic heterotrophic bacteria are microorganisms commonly found in natural environments that play important roles in matter and energy cycles (Madigan et al., 2014). These microorganisms are also widely used in wastewater treatment systems and, therefore, they are used as indicators in ecotoxicity studies (de Garcia et al., 2014; Ramos-Ruiz et al., 2016).

2. Materials and methods

2.1. Chemicals

Gallium(III) chloride (GaCl_3 , CAS# 13450-90-3, > 99.99%), sodium meta-arsenite (NaAsO_2 , CAS# 7784-46-5, $\geq 99\%$), sodium arsenate dibasic heptahydrate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, CAS# 10048-95-0, $\geq 98\%$) and citric acid ($\text{C}_6\text{H}_8\text{O}_7$, CAS# 77-92-9) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Indium(III) chloride tetrahydrate ($\text{InCl}_3 \cdot 4\text{H}_2\text{O}$, CAS# 22519-64-8, > 99.99%) was obtained from Strem Chemicals (Newburyport, MA, USA).

In(III) and Ga(III) have very low aqueous solubility in the neutral pH range (Wood and Samson, 2006). In order to avoid precipitation in the circumneutral bioassay media, the stock solutions of In and Ga were supplied with citrate at a molar ratio 1:3.75, metal:citrate. Citrate is a ubiquitous compound abundant in the natural environment and a common metabolite of living cells (Gamez et al., 2009). Citrate is also commonly used as a chelating agent in metal processing industries including semiconductor manufacturing (Thomas et al., 2000). Therefore, the use of citrate in this study is relevant to In(III) and Ga(III) exposure scenarios.

2.2. *Microtox* acute toxicity bioassay

Microtox is an *in vitro*, metabolic inhibition test system that uses a strain of a naturally bioluminescent marine bacterium named *Aliivibrio fischeri* that produces light as byproduct of cellular respiration. The toxicity of test chemicals can be recognized by the loss of luminescence level that results from cellular activity inhibition.

In the study, all samples were tested using a Microtox M500 analyzer following the Microtox acute toxicity test protocol (U.S. EPA, 2003). Microtox reagent (*A. fischeri*), reconstitution solution (ultra-pure water), osmotic adjusting solution (22% NaCl), and diluent (2% NaCl) were obtained from Fisher Scientific. Stock solutions, 6 mM As(III) and As(V) as well as 20 mM Ga(III) and In(III), were prepared prior to the experiments. The pH of each stock solution was adjusted to about 7.0 through addition of either diluted HCl or NaOH. In order to maintain the osmotic pressure of the samples, the stock solution was mixed with osmotic adjusting solution (10:1, v/v). Then nine dilutions were prepared by a 2-fold serial dilution using Microtox diluent. The concentration ranges used in this assay were: 0.01 to 2.7 mM for As(III) and As(V); and 0.04 to 9.10 mM for In(III) and Ga(III). Luminescent levels were tested after 0, 15 and 30 min of exposure. All tests were performed in duplicate; blank controls and samples with citrate alone were run in parallel. Percent microbial activity was calculated as described in the *Supplementary Information (SI) Section*.

2.3. Methanogenic toxicity bioassay

The methanogenic sludge was obtained from an anaerobic bioreactor treating brewery wastewater (Mahou, Guadalajara, Spain) and contained 7.92% of volatile suspended solids (VSS) per unit wet weight. Batch experiments were conducted in duplicate using glass serum flasks (160 mL) supplemented with inoculum (1.5 g VSS/L), and acetate-containing basal medium (25 mL) (described in the *SI Section*) (Liang et al., 2013). The flasks were sealed with butyl rubber stoppers and aluminum crimp seals. Next, the headspace was flushed with a mixture of N₂ and CO₂ (80:20, v/v) to create anaerobic conditions. All flasks were pre-incubated overnight to

ensure that the methanogens were adapted to experimental conditions. The following day, As (0-0.2 mM of As(III) or As(V)), In(III) (0-1.08 mM) or Ga(III) (0-2.50 mM) were added. Controls lacking toxicant addition and controls with citrate alone were run in parallel. Subsequently, the flasks were incubated in an orbital shaker (115 rpm) at $30 \pm 2^\circ\text{C}$. Methane (CH_4) production was determined periodically until 80% or more of the substrate in toxicant-free controls was depleted. The normalized microbial activity (NMA) was calculated as shown below:

$$NMA(\%) = \frac{\text{Maximum Specific Activity of Experimental Group}}{\text{Maximum Specific Activity of Control}} \times 100 \quad (1)$$

where the maximum specific activity was calculated from the slope of cumulative CH_4 production.

2.4. Aerobic toxicity bioassay

Aerobic return activated sludge (RAS) was obtained from a local municipal wastewater treatment plant (Tucson, AZ, USA). The VSS content of the sludge was 0.78% (wet weight). The assays were conducted in duplicate using glass serum flasks (160 mL) supplemented with basal medium (25 mL, *SI Section*) and RAS inoculum (0.5 g VSS/L) (Liang et al., 2013). The flasks were spiked with As (0-0.6 mM of As(III) or As(V)), In(III) (0-1.08 mM) or Ga(III) (0-1.78 mM), sealed and flushed with a mixture of $\text{He}/\text{CO}_2/\text{O}_2$ (60:20:20, v/v). Toxicant-free controls with and without citrate were run in parallel. The O_2 content in the headspace of the flasks was measured periodically until the O_2 consumption rate resembled that of the endogenous control without acetate. The NMA was calculated using Eq. 1, only in this case the maximum specific activity was calculated based on the slope of the O_2 consumption *versus* time plot.

2.5. *Daphnia* toxicity bioassay

The acute toxicity of Ga(III) and In(III) to *D. magna* was tested according to EPA Ecological Effects Test Guidelines (EPA, 1996). In the experiment, *D. magna* was exposed to Ga(III) or In(III) (0–5.0 mM) and the number of dead organisms was determined after 48 h of exposure. The assays were run in quadruplicate; controls were run in parallel. Arsenic toxicity data for *D. magna* are widely available in literature (Fan et al., 2015; He et al., 2016; Le et al., 2013; Wang et al., 2016), therefore As(III) and As(V) were not tested using this assay.

2.6. Analytical methods

The soluble concentration of As, In and Ga in liquid samples was measured using an inductively coupled plasma–optical emission spectrometry (ICP–OES) system (Optima 2100 DV from Perkin-Elmer, Shelton, CT, USA). The wavelengths used for As, In and Ga analyses were 193.696, 230.606 and 417.206 nm, respectively.

The CH₄ and O₂ content in headspace samples were measured using a Hewlett Packard 5890 Series II gas chromatograph (Agilent Technologies, Palo Alto, Ca, USA) with He as the carrier gas. CH₄ was determined using a flame ionization detector and a Restek Stabilwax-DA column (30 × 0.35 mm, ID 0.25 µm). O₂ was analyzed using a thermal conductivity detector and a Carboxen[®]-1010 Plot column.

2.7. Statistical analysis

A four-parameter logistic function was used to fit the dose-response curves and calculate the respective IC₅₀ values with 95% confidence limits (details are described in the *SI Section*). All statistical analyses were performed using the software OriginPro 9.1 (OriginLab Corp., Northampton, MA, USA).

3. Results and discussion

3.1. *Microtox* acute toxicity bioassay

The dose response curves of *A. fischeri* exposed to As(III) and As(V) for 5, 15 and 30 min (Fig. 1) show that the presence of arsenic resulted in microbial activity inhibition. Also, the luminescence decreased with the increasing As concentrations and longer exposure time. As(III) was only mildly inhibitory at comparatively low concentrations (< 0.20 mM), while As(V) caused a rapid decrease of bioluminescence. After 15 min, 2.70 mM As(III) and As(V) led to 71.6% and 90.0% loss in light emission, respectively. For As(III), the 50% inhibition concentration (IC₅₀) after 30 min of exposure was 2.12 mM (with 95% confidence limits, 95%CL, reported in Table 1). In this assay, As(V) had higher toxicity than As(III), which is in agreement with previously published literature (Fulladosa et al., 2007; Rubinos et al., 2014). It has been reported that light production in *A. fischeri* through aerobic respiratory pathways requires ATP (Dunn, 2012). Therefore, the higher toxicity from As(V) may have been due to the blockage of ATP formation by As(V) replacing phosphate in biochemical reactions.

The relative luminescence activity of *A. fischeri* as a function of In(III), Ga(III) or citrate concentration is displayed in Fig. 2. Citrate-complexed In(III) and Ga(III) did not cause any

greater inhibition compared to samples exposed to citrate alone at the same concentration present in the corresponding In and Ga samples. Exposure to relative high concentrations of citrate resulted in mild inhibition (10.6% inhibition at highest concentration tested, 34 mM after 30 min exposure). No inhibition was observed with Ga(III) at concentrations up to 9.10 mM. High concentrations (4.50 and 9.10 mM) of In(III) only showed very limited inhibitory effect as the luminescence was about 5% lower than that from the samples with only citrate. In the experiments, luminescence inhibition was strongest at 5 min, thereafter, luminescence increased with longer exposure time. This trend is in contrast with the increased inhibitory effect observed for As(III) and As(V) with exposure time, as previously described. Some organic compounds (such as ethanol, methanol, isopropanol and acetonitrile) are reported to affect the general sensitivity in bioluminescence toxicity assay with *Vibrio harveyi* (Mariscal et al., 2003). In addition, citrate could be used as carbon source and substrate in *A. fischeri* metabolism (Dunn, 2012; Parvez et al., 2006) and consequently induce bioluminescence (Septer et al., 2015). Therefore, the elevated light emission may have resulted from the introduction of citrate in this assay.

Studies assessing the susceptibility of *A. fischeri* to Ga(III) and In(III) are widely lacking. We are only aware of one previous study testing the inhibitory impact of In(III) (supplied as $\text{In}(\text{NO}_3)_3$) to this bacterial species (Zurita et al., 2007). The reported IC_{50} values at 5 and 15 min were very low (0.06 and 0.04 mM, respectively), which is in stark contrast with the low inhibitory effects determined in the present study at concentrations as high as 9.10 mM (5% inhibition). Enhanced inhibition by Ga(III) and In(III) in the assays performed by Zurita *et al* could have been due to the use of a nitrate salt.

3.2. Methanogenic toxicity bioassay

Fig. S2 (*SI Section*) illustrates the time courses of methane production by an anaerobic sludge consortium exposed to increasing concentrations of the III-V species. The methanogenic toxicity was determined by comparing the maximum methane production rates in the samples exposed to the toxicants to that of the toxicant-free control (see *Section 2.3*). For example, the maximum specific activity was calculated from the slope of cumulative CH₄ production at 46 h in the experiments with As(III) (Fig. S2).

Fig. 3 shows the normalized methanogenic activity with respect to the concentration of As(III), As(V), In(III) and Ga(III) in the various bioassays. The results indicate that both As(III) and As(V) were not toxic at the lowest concentration tested (0.005 mM), however, toxicity was observed at higher concentrations. Both As species caused similar inhibition responses and more than 95% methanogenic inhibition was observed at 0.20 mM. The IC₅₀ value calculated for both As(III) and As(V) in this bioassay was about 0.02 mM. The inhibition results obtained for As(III) are in close agreement with those reported in a previous study using a similar experimental protocol (Rodriguez-Freire et al., 2015). On the other hand, the acetoclastic methanogens in the inoculum utilized in this study were more sensitive to As(V) than previously reported by Sierra-Alvarez and coworkers for a methanogenic mixed culture (IC₅₀ > 0.50 mM) (Sierra-Alvarez et al., 2004). In the latter study, anaerobic sludge treating recycled paper wastewater was used and the period of exposure was shorter (8-24 h). Since As(V) could undergo microbial reduction to As(III) under anaerobic conditions (Fendorf and Kocar, 2009; Islam et al., 2004), the concentration of the individual As species was tested. In this study, we found that only 10% of

As(V) was converted to As(III) after 100 h of exposure. Therefore, the enhanced toxicity was probably not due to a change in As speciation.

In the assays with citrate-complexed In(III) and Ga(III), the methanogenic activity only decreased by 22.7% and 29.4%, respectively, at the highest concentration tested (1.08 and 2.50 mM). The 20% inhibitory concentration (IC_{20}) values for In(III) and Ga(III) were 0.92 mM (95%CL= 0.63 to 1.08 mM) and 1.42 mM (95%CL= 1.27 to 1.59 mM), respectively, indicating that In(III) was slightly more inhibitory than Ga(III). Citrate is not inhibitory to methanogens at the concentrations present in these assays (Gamez et al., 2009).

3.3. Aerobic toxicity bioassay

The time courses of oxygen consumption by aerobic activated sludge exposed to increasing concentrations of As(III), As(V), In(III) and Ga(III) are summarized in Fig. S3 (*SI Section*). Dose-response curves for the various metal and metalloid species are shown in Fig. 4. In the experiments, aerobic sludge showed very high resistance to all the III-V species as there was no clear reduction of the oxygen consumption rate compared to the toxicant-free controls. Only when exposed to high concentrations of Ga(III) did a slight inhibition occur (26.7% at 1.80 mM). At the concentration used in these assays, citrate did not show any clear inhibitory effect on the aerobic sludge activity (data not shown).

The robustness of the mixed aerobic heterotrophic bacterial community from RAS is likely due to the high diversity of microorganisms in activated sludge enabling at least some cells to be tolerant. The fact that microorganisms tend to form flocs may also attribute to their high resistance to toxicants by avoiding direct exposure to III-V ionic species. Stasinakis et al. studied

the toxicity of inorganic As species on activated sludge respiration (Stasinakis et al., 2003). The inhibition was found to depend on As speciation and sludge age. The reported IC_{50} value for As(V) was 2.64 mM at sludge age of 10 days. This very high concentration, which is unlikely to occur in wastewater streams, is much larger than the highest concentration used in the present study (0.60 mM). On the other hand, the IC_{50} value calculated for As(III) in the same report was 0.14 mM, indicating a higher sensitivity of the mixed culture used by Stasinakis and coworkers compared to the present study where As(III) only had a negligible effect on aerobic heterotrophic bacterial activity at 0.60 mM. The age of the sludge utilized in the current study (13-18 days) (Schmitz et al., 2016) was considerably longer compared to that of the sludge utilized in the former study which could partially account for the different tolerance observed.

3.4. *Daphnia* toxicity bioassay

Fig. 5 shows the dose–response curves determined in acute toxicity bioassays with *D. magna* exposed to citrate-complexed In(III) and Ga(III) for 48 h. The results show that In(III) caused higher mortality than Ga(III). Mortality increased rapidly with increased In(III) concentration (90% lethal effect was observed at 1.25 mM) and then, tended to reach 100% gradually. For Ga(III), the dose–response curve was almost linear in the tested concentration range. Mortality was 40% at 1.25 mM and it reached 95% at 5 mM after 48 h of exposure. The 50% lethal concentration (LC_{50}) values for In(III) and Ga(III) were 0.50 mM and 3.40 mM, respectively (the 95% CL are reported in Table 1). These values indicate that In(III) and Ga(III) are much less toxic than As species (Table 1).

The toxicity of Ga(III) and In(III) to *D. magna* and other daphnids is poorly understood. We are only aware of a previous study that investigated the lethal impact of In(III) (supplied as In(NO₃)₃) on *D. magna* and reported a 24 h-LC₅₀ value of 0.23 mM (26.4 mg/L) (Zurita et al., 2007). However, this value is several orders of magnitude higher compared to the maximum solubility of In(III) at neutral pH and ambient temperature in the presence of chelating agents (low µg/L level; solubility product of In(OH)₃ = 1.3×10^{-34} (White and Hemond, 2012).

3.5. Comparison of As(III), As(V), In(III) and Ga(III) toxicity in different bioassays

The IC₂₀ and IC₅₀ values determined in the microbial toxicity bioassays as well as the LC₅₀ values obtained in the *D. magna* toxicity bioassays conducted in this study are summarized in Table 1. The results show that the toxicity mostly depends on the element utilized (As versus In and Ga) and secondarily is dependent on the target organism. Arsenic species showed clear inhibitory effects on different organisms. Acetoclastic methanogens were most sensitive to As inhibition and the IC₅₀ value determined for both As(III) and As(V) was very low, about 0.02 mM (1.5 mg/L). This concentration is lower than the maximum allowable one-day effluent discharge concentration (2.09 mg/L or 0.03 mM of total As) for facilities manufacturing electronic components, as discussed previously. Compared to As species, In(III) and Ga(III) ions were much less toxic. *D. magna* was the most sensitive organism to In(III) and Ga(III), among those utilized in the bioassays performed in this study with 48-h LC₅₀ values of 0.50 and 3.40 mM, respectively.

The ecotoxicity results reported here for Ga(III) and In(III) are generally in agreement with scant literature previously published for other aquatic species. Recent studies evaluating the

toxicity of Ga(III) in marine and brackish fish have shown that these metallic species are only inhibitory at moderate to high concentrations (96-h LC₅₀ values ranging from 0.28-0.78 mM) (Onikura et al., 2005; Yang and Chen, 2003). Toxic effects of Ga(III) and In(III) complexed with citrate were also recently evaluated in a study led by our laboratory using the embryonic zebrafish (*Danio rerio*) model (Olivares et al., 2016). The authors found that survival and developmental behavior of zebrafish embryos was not significantly affected by any of these metal ions at the highest tested concentration (0.90 mM). The embryonic zebrafish was also evaluated for developmental abnormalities associated with exposure to Ga(III) and In(III) and no effect was detected at concentrations up to 0.90 mM. Overall, our results represent the first comprehensive characterization of acute ecotoxicity effects of Ga(III) and In(III) ions towards daphnids and environmentally-relevant microbial populations. The results and insights from this study will contribute to fill a significant knowledge gap regarding the potential ecotoxicity implications of III-V ion contamination. The introduction of III-V materials in the manufacturing of electronic and photovoltaic devices is likely to increase environmental release of these metallic species.

4. Conclusions

This study demonstrates that the toxicity is mostly impacted by the element tested. Both As(III) and As(V) caused acute ecotoxicity, while In(III) and Ga(III) had either no or only mild acute inhibitory effects in different bioassays even at comparatively high concentrations, suggesting that short exposure to those ions might not pose a threat to aquatic organisms in the natural environment unless they are present at relatively high concentrations. A secondary

367 consideration was that the toxicity of As(III), As(V), In(III) and Ga(III) depended on the target
368 organisms utilized.

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513

514 **Table 1** Summary of IC₅₀ (microbial assays) and LC₅₀ (*Daphnia* assay) values for As(III), As(V),
515 In(III) and Ga(III). Values in the bracket show the 95% confidence limits.

	Microtox (30min)	Methanogen	Aerobic	<i>Daphnia</i> (48 h)
As(III), mM	1.20 (1.06 to 1.36)	0.021 (0.018 to 0.025)	> 0.60 ^a	0.05 ^b
As(V), mM	0.066 (0.065 to 0.068)	0.019 (0.018 to 0.021)	> 0.60 ^a	0.12 ^b
In(III), mM	> 9.10 ^a	> 1.08 ^a	> 1.08 ^a	3.40 (2.56 to 4.50)
Ga(III), mM	> 9.10 ^a	> 2.50 ^a	> 1.78 ^a	0.50 (0.39 to 0.65)

516 ^a The IC₅₀ was not reached at the highest concentration tested in this study.

517 ^b Reported by (He et al., 2016; Wang et al., 2016)

518

FIGURE CAPTIONS

Fig. 1. Dose-response curves for Microtox acute toxicity assays: relative light emission of *A. fischeri* at 5, 15 and 30 min of exposure as a function of the As(III) and As(V) concentration.

Fig. 2. Dose-response curves for Microtox acute toxicity assays: relative light emission of *A. fischeri* at 5, 15 and 30 min of exposure as a function of In(III) and Ga(III) concentration (**A**), and corresponding citrate concentration used for In and Ga stock solution preparation (**B**).

Fig. 3. Methanogenic activity of mixed anaerobic culture as a function of the concentration of As(III), As(V), In(III) and Ga(III).

Fig. 4. Inhibition of oxygen consumption activity by return activated sludge as a function of the concentration of As(III), As(V), In(III), and Ga(III).

Fig. 5. Percent *Daphnia magna* mortality in response to exposure to different concentrations of In(III) and Ga(III) after 48 hours of exposure.

Figure 1

Figure 2

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Figure 3

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Figure 4

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Figure 5