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Clean energy, but contaminated waters?

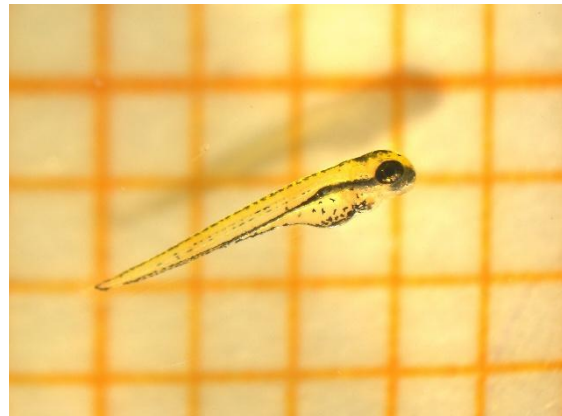
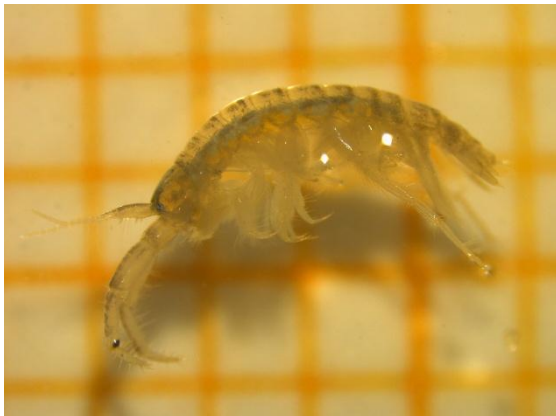
Assessing the effects of indium and gallium emissions from offshore wind farms on *Corophium volutator* and *Danio rerio*

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Abstract

Offshore wind farms (OWFs) are rapidly expanding as part of the global energy transition, yet the ecotoxicological consequences of their chemical emissions remain poorly understood. Sacrificial anodes used for cathodic protection in turbine foundations release the technology-critical elements indium (In) and gallium (Ga) into marine environments. While other OWF-derived metals, including aluminium, zinc, copper, cadmium, and lead, have been extensively studied, ecotoxicological data on In and Ga remain scarce. This study assessed the effects of In and Ga on two model organisms: the marine sediment-dwelling amphipod *Corophium volutator*, exposed to metal-spiked sediment over 28 days, and the freshwater zebrafish *Danio rerio*, exposed to waterborne In and Ga separately over 120 hours post-fertilization.

Neither species showed significant mortality at the tested concentrations. In *C. volutator*, the specific growth rate was significantly elevated at the highest concentration, potentially indicating hormesis. Concentration-dependent accumulation of both metals was demonstrated in *C. volutator* tissue and sediment. Indium partitioned almost completely to the sediment phase, remaining below the detection limit in overlying water across all treatments. Biota-sediment accumulation factors (BSAFs) were significantly higher for In than Ga, indicating disproportionate bioavailability of In despite its lower dissolved concentrations. In *D. rerio*, both metals accumulated in a concentration-dependent manner. Gallium remained predominantly dissolved (>80%) and displayed a clear monotonic dose-response in larval tissue. Indium, by contrast, was largely particulate (<2% dissolved), which limited waterborne exposure. A novel finding was a potential inverse relationship between In exposure concentration and Ga accumulation in In-exposed larvae, suggesting inhibition of Ga uptake by In, possibly via shared iron transport pathways. Morphological abnormalities in *D. rerio* were rare and not reliably attributable to metal exposure.

These results indicate that the primary ecological risk of OWF-derived In and Ga lies in the sediment, with benthic organisms likely facing greater metal exposure than pelagic organisms. This study provides a foundation for future risk assessment and highlights the need to include sediment quality monitoring in environmental risk and management frameworks for OWFs.

Keywords: Offshore wind farms, indium, gallium, *Corophium volutator*, *Danio rerio*, marine pollution, sediment contamination, bioaccumulation

Lay abstract

Offshore wind farms generate clean electricity, but the steel structures they are built on require protection against corrosion in seawater. To achieve this, blocks of metal alloy called sacrificial anodes are attached to the foundations and gradually dissolve. This way, metals are released into the surrounding water and sediment. Two of these metals, indium and gallium, are the focus of this study. Although they are widely used in modern technologies, very little is known about their effects on aquatic life.

This research tested whether these metals are harmful to two small aquatic animals: the European mud scud, a tiny shrimp-like creature that mostly lives buried in estuarine mud, and embryos of the zebrafish, commonly used as a model vertebrate for understanding the effect of aquatic pollutants. Both species were exposed to increasing concentrations of indium and gallium under controlled laboratory conditions.

Neither animal died because of the tested concentrations, and the zebrafish embryos developed normally into larvae. However, both species accumulated the metals in their bodies in proportion to the concentration they were exposed to. This means that the metals were absorbed and retained despite causing no visible harm. Interestingly, the mud scuds and the sediment both accumulated indium so efficiently that almost none remained in the water. This suggests that sediment-dwelling animals living near offshore wind farms could be silently accumulating these metals over time, even when water measurements would suggest no contamination. The results call for monitoring programmes around offshore wind farms to include regular analysis of both the water and the sediment.

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Table of contents

Abstract	3
Lay abstract	4
Acknowledgements	5
Table of contents	6
List of abbreviations.....	8
Chemical species.....	8
List of figures and tables	9
1. Introduction.....	11
1.1 Chemical emissions from OWFs	11
1.2 Indium and gallium	12
1.2.1 Physical and chemical properties.....	12
1.2.2 Environmental occurrence	13
1.2.3 Ecotoxicology	14
1.3 Model organisms.....	15
1.3.1 <i>Corophium volutator</i>	15
1.3.2 <i>Danio rerio</i>	16
1.3.3 Empirical toxicity data	17
1.4 Objectives.....	19
2. Materials & methods	22
2.1 <i>Corophium volutator</i>	22
2.1.1 Sampling of sediment and test organisms.....	22
2.1.2 Chronic stress exposure (28 days)	24
2.1.3 Analysis of the samples	26
2.1.4 Reference toxicant test.....	27
2.2 <i>Danio rerio</i>	28
2.2.1 Preparation of the different media	28
2.2.2 Experimental design.....	30
2.2.3 Scoring of mortality and renewing the media.....	31
2.3 Statistical analysis	32
2.3.1 <i>Corophium volutator</i>	32
2.3.2 <i>Danio rerio</i>	33
2.4 Ethical statement	33

3. Results	34
3.1 <i>Corophium volutator</i>	34
3.1.1 Survival and growth	34
3.1.2 Metal accumulation	37
3.1.3 Reference toxicant test	41
3.2 <i>Danio rerio</i>	41
3.2.1 Survival and hatching	41
3.2.2 Metal accumulation	43
3.2.3 Morphological abnormalities	48
4. Discussion	49
4.1 <i>Corophium volutator</i>	49
4.1.1 Survival and growth	49
4.1.2 Metal accumulation	50
4.2 <i>Danio rerio</i>	52
4.2.1 Survival and hatching	52
4.2.2 Metal accumulation	52
4.2.3 Morphological abnormalities	55
4.3 Comparative evaluation and ecological relevance	55
5. Conclusion	57
6. Bibliography	59
Appendix	65

List of abbreviations

BSAF – biota-sediment accumulation factor	LL2.2 – two-parameter log-logistic model	TAN – total ammoniacal nitrogen
CRM – critical raw material	LOD – limit of detection	TCE – technology-critical element
df – degrees of freedom	NOEC – no observed effect concentration	TNF- α – Tumour Necrosis Factor- α
drc – dose-response curve	OECD – Organisation for Economic Co-operation and Development	WGS 84 – World Geodetic System 1984
dw – dry weight	OWF – offshore wind farm	ZFET – Zebrafish Embryo Toxicity Test
ES – embryo solution	ppt – parts per thousand	ρ – rho
EZRC – European Zebrafish Research Center	PSA 78 – Practical Salinity Scale 1978	χ^2 – chi-squared
hpf – hours post fertilisation	ROS – reactive oxygen species	
(HR-)ICP-MS – (high-resolution) inductively coupled plasma mass spectrometry	SD – standard deviation	
LC ₅₀ – lethal concentration 50%	SGR – specific growth rate	

Chemical species

Ag – silver	Ga(OH) ₄ ⁻ – gallate	Na ₂ [Fe(CN) ₅ NO].2H ₂ O – sodium nitroprusside dihydrate
Al – aluminium	HCl – hydrochloric acid	NaHCO ₃ – sodium bicarbonate
As – arsenic	Hg – mercury	NaOCl – sodium hypochlorite
CaCl ₂ .2H ₂ O – calcium chloride dihydrate	HNO ₃ – nitric acid	NaOH – sodium hydroxide
C ₇ H ₅ NaO ₃ – sodium salicylate	In – indium	Na ₂ SO ₄ – sodium sulphate
Cd – cadmium	InCl ₃ – indium(III) chloride	NH ₃ – ammonia
Co – cobalt	In(NO ₃) ₃ – indium(III) nitrate	NH ₄ ⁺ – ammonium
Cr – chromium	In(OH) ₃ – indium(III) hydroxide	NH ₄ Cl – ammonium chloride
Cu – copper	ITO – indium tin oxide	Ni – nickel
DCA – 3,4-dichloroaniline	KCl – potassium chloride	Pb – lead
F – fluorine	Li – lithium	Sb – antimony
Fe – iron	MgCl ₂ .6H ₂ O – magnesium chloride hexahydrate	-SH – sulfhydryl group
Ga – gallium	Mn – manganese	Sn – tin
GaAs – gallium arsenide	Na ₃ C ₆ H ₅ O ₇ – sodium citrate	V – vanadium
GaCl ₃ – gallium(III) chloride	NaCl – sodium chloride	Zn – zinc
GaN – gallium nitride		ZnS – zinc sulphide

List of figures and tables

Figure 0 (title page) – Photographs of a *C. volutator* individual (left) and *D. rerio* individual (right).

Figure 1 – The experimental setup of the *C. volutator* experiment, illustration on the left made using BioRender.

Figure 2 – The experimental setup of the *D. rerio* experiment, illustration on the left made using BioRender.

Figure 3 – Amount of surviving individuals of *C. volutator* per treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 4 – Absolute growth in grams per individual of *C. volutator* per treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 5 – Specific growth rate in grams over 28 days per individual of *C. volutator* per treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 6 – Relative growth in percentage, compared to the mean value of the control treatment, per individual of *C. volutator* per treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 7 – Indium concentrations in individuals of *C. volutator* and in the sediment in µg per g dry weight (dw), for each treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 8 – Gallium concentrations in individuals of *C. volutator* and in the sediment in µg per g dry weight (dw), for each treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 9 – Mean BSAF values for gallium and indium per *C. volutator* treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Figure 10 – Dose-response curve showing the NH_4^+ -concentration in mg per litre and the percentual mortality of the reference toxicant test for *C. volutator*, including the LC_{50} (n = 2).

Figure 11 – Percentage of surviving *D. rerio* individuals per treatment (n = 9, for ES n = 36). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

Figure 12 – Hatching of *D. rerio* individuals over time in all treatments (n = 9, for ES n = 36). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

Figure 13 – Indium accumulation in µg per individual of indium-exposed *D. rerio* larvae per treatment (n = 9, for ES n = 36). In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

Figure 14 – Gallium accumulation in µg per individual of gallium-exposed *D. rerio* larvae per treatment (n = 9, for ES n = 36). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

Figure 15 – Dose-response curve for gallium accumulation in µg per individual of gallium-exposed *D. rerio* larvae for the different treatments (n = 9, for ES n = 36). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

Figure 16 – Gallium accumulation in µg per individual of indium-exposed *D. rerio* larvae per treatment (n = 9, for ES n = 36).

Figure 17 – The dissolved fraction in percentage of the total fraction for all *D. rerio* treatments of both gallium and indium. Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

Figure 18 – Water concentrations in mg per litre for all indium treatments, showing both the filtered and non-filtered samples, and the background values measured in the non-filtered ES (dashed line) for the *D. rerio* experiment. In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

Figure 19 – Water concentrations in mg per litre for all gallium treatments, showing both the filtered and non-filtered samples, and the background values measured in the non-filtered ES (dashed line) for the *D. rerio* experiment. Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

Figure 20 – Different stages of *D. rerio*, photographed at approximately 0, 24, 48, 72, 96, and 120 hpf (left-to-right and top-to-bottom).

Table 1 – Mean BSAF values and standard deviations for indium and gallium per *C. volutator* treatment. T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Table 2 – Estimated LC_{50} , LC_{90} , and LC_{95} values in mg NH_4^+ per litre, with their upper and lower limits, of the reference toxicant test for *C. volutator*.

Appendix 1 – Surviving and dead organisms of *C. volutator* per experimental unit, including their total weight in grams at the end of the experiment.

Appendix 2 – The mean natural concentrations and standard deviations of fifteen metals (excluding indium and gallium, since they are already mentioned in the main text) in µg per gram of sediment at the *C. volutator* sampling site.

Appendix 3 – The concentrations of twelve metals (excluding indium and gallium, since they are already mentioned in the main text) in µg per litre in the water samples of the *C. volutator* experiment, over time in the different treatments.

Appendix 4 – Reference toxicant test results per treatment for *C. volutator*, showing the surviving and dead organisms per concentration in mg NH₄⁺ per litre.

Appendix 5 – Metal content in µg per individual of *D. rerio*, shown per treatment.

Appendix 6 – Concentrations of indium (left) and gallium (right) in mg per litre, for both non-filtered and filtered water samples per treatment of *D. rerio* larvae.

Appendix 7 – Example of a scoring sheet for *D. rerio* larvae at 120 hpf (indium T6).

1. Introduction

Offshore wind energy is an important part of the international turn to cleaner energy. On a global scale, it went up from 7.18 gigawatts in 2013 to 72.66 GW in 2023 and is projected to reach 1.000 GW by 2050 (Satymov et al., 2025). The climate benefits compared to fossil fuels are compelling, but the ecological consequences of the infrastructure needed should also be considered. Offshore wind farms (OWFs) are often preferred over their onshore counterparts, for several reasons. Examples are higher and more consistent wind speeds or reduced land-use conflicts (Kaldellis & Apostolou, 2017). Despite these advantages, questions are raised about the environmental impact (Hengstmann et al., 2025). While it is a low-carbon technology, potential environmental issues are posed by the OWFs, like the release of chemical substances from the structures during their installation, operation, and decommissioning. This could affect marine organisms or ecosystems in yet unknown or poorly understood ways. In this thesis, the effects of two technology-critical trace metals associated with sacrificial anodes used in OWFs, gallium (Ga) and indium (In), on two species, the European mud scud *Corophium volutator* (Pallas, 1766) and the zebrafish *Danio rerio* (Hamilton-Buchanan, 1822), are studied.

1.1 Chemical emissions from OWFs

Offshore wind turbines are complex structures which comprise of various parts, including foundations, generator systems, rotor blades, and submarine cabling networks (Rueda-Bayona et al., 2022). All these structures have the potential to introduce chemical substances into the surrounding environment through distinct pathways, including leaching, corrosion, cathodic protection, anti-fouling and -corrosion coatings, and through the release of lubricants and hydraulic fluids (Hengstmann et al., 2025).

Sacrificial anodes are electrochemically active metal alloys, mostly zinc-aluminium-indium (Zn-Al-In), that preferentially corrode. This process donates electrons to the protected structure. Indium serves here as a critical alloying element that prevents passivation of the Al and Zn surface (Kirchgeorg et al., 2018). Other trace elements, including cadmium (Cd), manganese (Mn) and Ga, can remain as impurities from manufacturing. One monopile foundation of an offshore wind turbine carries several tonnes of sacrificial anode material. The rate at which these sacrificial anodes dissolve is substantial. It has been estimated that an OWF of 150 turbines releases approximately 9 to 14 kg In and 5 to 8 kg Ga over a five-year operational period (Reese et al., 2020; Ebeling et al., 2023). Field monitoring at OWFs in the German Bight

confirmed these elevated concentrations of In and Ga near the infrastructure (Ebeling et al., 2025).

Apart from the sacrificial anodes, other components of the OWFs can also be a source of In and Ga. Sophisticated electronic systems like converters, inverters, and semiconductors are examples of this, which contain technology-critical elements (TCEs) like In and Ga (Bobba et al., 2020). Indium is present in indium tin oxide (ITO) thin films, which are used in display technologies and photovoltaic components in monitoring and communication systems. Gallium is a key constituent of gallium arsenide (GaAs) and gallium nitride (GaN) semiconductors, which are used in power conversion systems, but also in Ga-based phosphors in LED systems, used for navigational or warning lights on the offshore infrastructure.

Even though the quantities of In and Ga in these technologies are small, the cumulative load across an OWF may represent a significant source of these elements to the environment. Since recycling pathways for In and Ga from OWFs are not well established, significant fractions can enter the environment during decommissioning. Klein et al. (2022) provided a comprehensive determination of preliminary reference values for TCEs like In and Ga in uncontaminated North Sea sediments, against which future monitoring data can be benchmarked. Nevertheless, the ecotoxicological consequences and behaviour of In and Ga released from OWFs are still knowledge gaps that this thesis seeks to address.

1.2 Indium and gallium

1.2.1 Physical and chemical properties

Indium (In; atomic number 49) is a post-transition metal (Schwarz-Schampera, 2014). It belongs to group 13 of the periodic table, and has an atomic mass of 114.82 g/mol. Under environmentally relevant conditions, it mainly occurs as In^{3+} , its trivalent oxidation state. Under highly reducing conditions, the monovalent species In^+ occurs as well. The metal is characterized by a low melting point of 156.6 °C, and a high malleability. Its geochemical behaviour is intermediate between the lithophile and chalcophile groups. It is commonly associated with sphalerite (zinc sulfide; ZnS) and other sulphide ore deposits, from which it is predominantly produced as a by-product of Zn refining.

Gallium (Ga; atomic number 31) is also a post-transition metal from group 13 of the periodic table (Butcher & Brown, 2014). It has an atomic mass of 69.72 g/mol, and almost exclusively

occurs as Ga^{3+} . It has an even lower melting point of 29.76 °C. Ga is a dispersed element in the Earth's crust, with an average abundance of around 18 mg/kg. It is primarily recovered as a by-product of Al smelting and Zn ore processing.

The European Commission classifies both In and Ga as critical raw materials (CRMs), due to their strategic economic importance, combined with high supply concentration risk and limited substitutability in key technological applications like OWFs (Bobba et al., 2020). Approximately 1,100 tonnes In and 900 tonnes Ga were produced globally in 2025, which is several orders of magnitude smaller than those of other metals like Cu and Zn (USGS, 2026).

1.2.2 Environmental occurrence

Even though the natural background concentrations of In and Ga vary across regions and depths, both tend to be low in aquatic systems. Coastal and estuarine environments may show relatively higher concentrations, due to riverine inputs, resuspension of contaminated sediments, and anthropogenic sources like urban runoff and industrial effluents. According to Batley & Campbell (2022), in uncontaminated rivers dissolved In concentrations range from approximately 0.003 to 0.05 ng/L, while dissolved Ga concentrations range from 1 to 120 ng/L. Uncontaminated marine waters show In occurring at approximately 0.006 to 0.5 ng/L, and Ga at levels below 3 ng/L. Due to these low background levels, a large disparity between environmentally relevant and ecotoxicologically active concentrations exists (Yang & Chen, 2017). This may suggest that these metals do not pose acute risks. However, at a local scale the exposure could be high enough to pose an environmental risk.

In natural waters, both In and Ga exhibit complex speciation behaviour (Yandem & Jabłońska-Czapla, 2024). This has an impact on their bioavailability and toxicity. In seawater, it is currently assumed that In^{3+} mainly occurs as its indium(III) hydroxide ($\text{In}(\text{OH})_3$) species (Mejías et al., 2025). Free In^{3+} presence is much lower under marine conditions. In these conditions, Ga^{3+} mainly exists as gallate ($\text{Ga}(\text{OH})_4^-$) (Savenko & Savenko, 2010). This alters its partitioning behaviour between dissolved and particulate phases compared to fresh or estuarine waters.

1.2.3 Ecotoxicology

1.2.3.1 Mechanisms

The mechanisms through which trivalent metal cations like In^{3+} and Ga^{3+} exert cellular toxicity are previously researched (Jiang et al., 2015; Schulze et al., 2023). They include the displacement of essential metal cofactors like Zn^{2+} , Fe^{3+} , and Mn^{2+} from metalloenzyme active sites, and the interaction with sulfhydryl (-SH) groups of proteins and enzymes. Other mechanisms are the induction of oxidative stress through Fenton-type reactions or the depletion of antioxidant defences, disruption of the membrane integrity, and interference with signal transduction cascades and nuclear receptor function.

Both In^{3+} and Ga^{3+} share the coordination chemistry and ionic radius of Fe^{3+} sufficiently to substitute iron at biological active sites (Schulze et al., 2023). This hypothesis of iron mimicry has been proposed as a primary explanation for the toxicological potency of Ga^{3+} in particular (Ikhazuagbe et al., 2025). The pathway that appears to be affected the most is the ribonucleotide reductase. The chemical mimicry of Fe by Ga has been used therapeutically as anti-cancer and antibacterial agents, against for example iron-dependent pathogens like *Pseudomonas aeruginosa* (Chitambar, 2010). In the case of In, most mechanistic toxicity studies have focused on occupational exposure in the ITO manufacturing sector (Tsai et al., 2020). Here, inhalation of In-containing particles is associated with lung diseases, for example pulmonary fibrosis. Apart from that, indium(III) chloride can induce apoptosis and generate reactive oxygen species (ROS), at least in macrophage cell lines derived from the house mouse (*Mus musculus*).

1.2.3.2 Aquatic and sediment toxicity

Indium and Ga are among the least toxicologically characterized elements relevant to marine pollution. Ecotoxicological data for these metals in aquatic systems are very limited compared to other pollutants like Cu, Cd, or Hg. Available literature heavily relies on single-species acute exposure tests with a restricted taxonomic range, often on terrestrial species.

The sediment-associated toxicity data for both In and Ga is also very sparse. Both elements know a strong partitioning to sediments (Ringerling et al., 2018; Yandem & Jabłońska-Czapla, 2024), which is the preferred habitat of *Corophium volutator*. Datasets on this topic are lacking, and cause a gap in risk assessment. Without knowledge on the relationship between the total sediment concentration, the porewater concentration, and the biological exposure concentration

of these two metals, establishing meaningful sediment quality guidelines and predicting the ecological impacts from OWFs in regards to In and Ga toxicity is barely achievable.

1.2.3.3 Mixture toxicity

The benthic and pelagic organisms living near OWFs are not exposed to single, isolated metals, but rather to complex mixtures of co-released substances. The dissolution of sacrificial anode alloys simultaneously introduces Zn, Al, In, Ga, and trace quantities of other alloy constituents like Cu, Fe, and Mn into the environment (Kirchgeorg et al., 2018). The ecological consequences of multi-metal exposure are not predictable from single-substance toxicity data, as metals may interact through additive, synergistic, or antagonistic mechanisms (Tchounwou et al., 2012).

Synergistic interactions are of particular concern. When two metals share the same uptake pathway or molecular target, combined toxicity may exceed the sum of the individual effects. This implies that relatively low and harmless environmental concentrations of individual metals can still produce adverse outcomes in combination. Conversely, antagonistic interactions can for example occur when multiple metals compete for the same biological ligand, meaning their combined effect is less harmful compared to their individual effect. For In and Ga, almost no toxicity data with co-occurring metals exist in published literature, causing a knowledge gap for risk assessment. This thesis addresses single-substance toxicity and mixture toxicity (solely with In and Ga) as a first step towards the eventual assessment of the complete mixture effects.

1.3 Model organisms

1.3.1 *Corophium volutator*

The European mud scud *Corophium volutator* (Crustacea; Amphipoda) is a small, tube-dwelling amphipod crustacean, which is widely distributed throughout inter- and subtidal estuarine mudflats of the Atlantic coasts of both Europe and North America (Meadows & Reid, 1966). It represents one of the most abundant macroinvertebrate species in many intertidal mudflat communities, with population densities reaching up to 100,000 individuals per square meter (De Backer et al., 2009). *C. volutator* plays a crucial role as a key trophic link between microphytobenthos and higher consumers like migratory shorebirds and benthic-feeding fish.

The species inhabits U-shaped burrows, which are constructed in the upper five centimetres of fine cohesive sediment, through which it pumps water for feeding and respiration (Farrell et al.,

2022). Sediment permeability and oxygen penetration are enhanced by this bioturbation activity, which contributes to nutrient cycling and contaminant redistribution in intertidal sediments. This lifestyle causes *C. volutator* to be exposed to contaminants via various pathways. Either through dissolved-phase waterborne exposure, sediment ingestion, or dietary routes, given its contact with both the sediment matrix and the overlying water. This makes the species a particularly interesting indicator of sediment contamination.

Corophium volutator is a selective deposit feeder, ingesting sediment particles predominantly smaller than 160 µm in diameter and selecting the particles with a high organic carbon content (Nielsen & Kofoed, 1982). This feeding selectivity has direct implications for metal exposure via the dietary route. Fine-grained, organic-rich particles exhibit the highest affinity for trace metal adsorption (Lee et al., 2024). The species mostly feeds on these particles and therefore has a relatively higher exposure to metal concentrations compared to the surrounding sediment.

Numerous international regulatory frameworks and protocols have adopted the species as a standard test organism for the assessment of sediment toxicity (Scarlett et al., 2007). Due to its sensitivity, ecological relevance, and relative ease of laboratory culture, *C. volutator* is an ideal test species for investigating the ecotoxicology of contaminants from OWFs.

1.3.2 *Danio rerio*

The zebrafish *Danio rerio* (Teleostei; Cyprinidae), a freshwater cyprinid native to South Asia, is one of the most extensively used vertebrate model organisms in biology and biomedical research (Spitsbergen & Kent, 2003). Over the past decades, it became established as an important model in aquatic ecotoxicology. Its rapid embryonic development, transparency during early life stages, large clutch sizes, external fertilisation, and well-annotated genome made it suited for toxicological research in an aquatic context (Scholz et al., 2008).

The zebrafish embryo toxicity test (ZFET; OECD Test Guideline 236) has been adopted as an internationally standardised regulatory ecotoxicology test, applicable to the acute toxicity screening of chemicals in the aquatic environment (OECD, 2025). The fertilised egg, developing embryo and larva are monitored using several morphological and functional endpoints, including coagulation, somite formation, heartbeat, spontaneous movement, and morphological malformations at 24, 48, 72, 96, and 120 hours post-fertilization (hpf). Due to the transparency of the embryos, direct and non-invasive assessment of organ development, including heart, brain, notochord, yolk sac, and vasculature, is possible.

Danio rerio can be used to investigate acute, developmental, neuro- and cardiovascular toxicity of dissolved metals and metal complexes under controlled waterborne exposure conditions (Manna & Firdous, 2025). Given the relevance of teleost fish in the marine food web, the developmental sensitivity of early life stages to chemical stress, and the mechanistic insights that the zebrafish model can provide, the species is relevant to assess chemical emissions of OWFs.

The emergence of OWFs in freshwater bodies is another main reason to include *D. rerio* as a test organism in this study. OWFs are historically seen as synonymous with marine environments, but in recent years freshwater installations, for example on Lake IJssel in the Netherlands and Lake Vänern in Sweden, are being built as well (Radowitz, 2020). With the intensification of the energy transition towards renewable energy, freshwater wind farms are expected to increasingly appear. The chemical emissions known from marine environments may also have an effect in freshwater environments, but the ecotoxicological database in this context, especially related to In and Ga, is even more limited than in the marine context.

1.3.3 Empirical toxicity data

1.3.3.1 *Corophium volutator*

Research examining the toxicity or bioaccumulation of In and Ga in *C. volutator* is extremely limited. One study investigated the ecotoxicological impacts of cathodic protection by Al-Zn-In sacrificial anodes on marine organisms including *C. volutator* (Bell et al., 2020). While exposure to dissolved galvanic anode material did not produce significant acute toxicity, an interesting observation was made when looking at bioaccumulation. In the presence of sediment, no significant metal uptake took place, but in the absence of sediment, all three metals were accumulated by the species. Indium even exhibited the highest relative enrichment in the tissue, despite constituting only a minor fraction of the node alloy composition. This suggests that In is disproportionately bioavailable and -accumulable for *C. volutator*.

A comparative context can be generated with previous studies in which *C. volutator* is exposed to other metals. One study demonstrated that acute Cd toxicity can vary with season, with LC₅₀ values being higher in winter than in summer (Kater et al., 2000). Another study showed that elevated waterborne Cu concentrations caused significant increases in bioaccumulation of Cu, and reduced egg production (Eriksson & Weeks, 1994). Mortality increased at low oxygen concentrations, suggesting that metal toxicity in this species can be different depending on other co-occurring environmental stressors. A study looking at the effects of different Zn

concentrations over a 100-day period, showed that only the highest concentration significantly impaired growth and reduced longevity in *C. volutator* (Conradi & Depledge, 1999). This suggests a considerable degree of tolerance to the metal.

1.3.3.2 *Danio rerio*

Very few published studies have investigated the toxicity of In and Ga to *D. rerio*. One study conducted a high-throughput, multi-endpoint in vivo developmental and behavioural assay using *D. rerio* embryos (Olivares et al., 2016). The embryos were exposed to a range of In^{3+} -citrate and Ga^{3+} -citrate concentrations. This study showed neurobehavioural effects at concentrations that survival-based endpoints would classify as non-toxic. Another study focused on the effects of In toxicity alone, by exposing *D. rerio* embryos and liver cells to indium(III) nitrate ($\text{In}(\text{NO}_3)_3$) and ITO (Brun et al., 2014). Endoplasmatic reticulum stress and oxidative stress were observed, pro-apoptotic genes were upregulated, and tumour necrosis factor- α (TNF- α) was activated. The effects in the isolated liver cells were more pronounced than in the embryos, suggesting that the chorion and developing tissue architecture provide protection for the embryos.

A study provided acute toxicity data for both In and Ga, by assessing 96-hour LC_{50} values in *D. rerio* (Yang & Chen, 2017). They ranged from 12.84 to 18.35 mg In/L, and from 7.25 to 11.42 mg Ga/L. These LC_{50} values are several orders of magnitude above ambient environmental concentrations, but they established a toxicological baseline which can be used for the design of exposure studies.

In many metal toxicity studies using *D. rerio* embryos, In and Ga are not included. Such studies can, however, still provide valuable comparative context for interpreting endpoints. One study characterised the acute toxicity of six trace metals, namely As, Cd, Hg, Pb, Cu, and Zn, on *D. rerio* embryos (Fasmina et al., 2021). A range of (sub-)lethal endpoints were documented, including coagulation of the fertilised egg, absence of somite formation, lack of tail detachment, absence of heartbeat, growth retardation, yolk sac and pericardial oedema, haemorrhages, and scoliosis. This endpoint repertoire is relevant to the experimental design of the present study, as it can be used as a framework for characterising morphological and developmental effects of In and Ga.

1.4 Objectives

This introduction has shown substantial gaps in the scientific literature related to ecotoxicology and chemical pollution related to offshore wind energy. Despite the exponential growth of OWFs, mainly in European waters, the ecotoxicological significance of metals, particularly TCEs like In and Ga, released from offshore wind infrastructure has not been fully addressed. Most studies present physical effects like underwater noise or altered wind patterns, mainly reported for birds, mammals, and the broader ecosystem structure (Degraer et al., 2022). The few studies on environmental monitoring mainly focus on already well-researched substances like Cu and Zn, maintaining the knowledge gap.

The German OffChEm project identified Al, Zn, Pb, Cd, In, and Ga as elements of interest for assessing potential emissions from OWFs, with In and Ga proposed as tracers since they have no major natural marine sources (Reese et al., 2020). Neither In nor Ga is listed as a priority (hazardous) substance under the EU Water Framework Directive (2000/60/EC) or the EU Marine Strategy Framework Directive (2008/56/EC). Environmental Quality Standards for these elements in marine waters or sediments do not exist at the EU level. If contamination from OWFs occurs at ecotoxicologically significant levels, current monitoring and permitting frameworks would be inadequate to identify or respond to the problem.

This thesis aimed to address three research objectives. First, mortality and growth. Second, the accumulation of metals. Third, the scoring of morphological characteristics. The following research questions and null hypotheses shaped this thesis:

1. What are the effects of a waterborne mixture of In and Ga on the survival and growth of *C. volutator* under sediment-spiked exposure conditions?

Exposure to increasing concentrations of a mixture of In and Ga will result in concentration-dependent increases in mortality and reductions in growth in *C. volutator*.

2. What are the effects of waterborne In and Ga on the survival and development of *D. rerio* eggs, embryos, and larvae?

Exposure to increasing concentrations of both In and Ga will result in concentration-dependent increases in embryo mortality and impairment of larval growth in *D. rerio*. Gallium is expected to be more potent, based on previously reported LC₅₀ values.

3. Does *C. volutator* accumulate In and/or Ga in its tissues following exposure, and is this accumulation concentration-dependent?

Corophium volutator will accumulate In and Ga in a concentration-dependent manner over the exposure period. Indium is expected to show high relative bioaccumulation, based on a previous study.

4. Does *D. rerio* accumulate In and/or Ga in its tissues following exposure, and is this accumulation concentration-dependent?

Danio rerio will accumulate In and Ga in a concentration-dependent manner over the exposure period.

5. Does metal accumulation in *C. volutator* tissue correlate with metal partitioning in the surrounding sediment, and does sediment act as a significant sink modifying bioavailable exposure concentrations?

Metal accumulation in *C. volutator* tissue will correlate with metal partitioning in the surrounding environment. The presence of sediment will reduce the dissolved metal concentrations in the overlying water column, through absorption to fine particles and organic matter. Waterborne exposure is therefore reduced, and tissue accumulation is lowered, as compared to water-only exposure.

6. What morphological abnormalities are induced in developing *D. rerio* eggs, embryos, and larvae by exposure to various concentrations of In and Ga, and do the type and severity of malformations progress over the 120-hour period?

Concentrations of both metals will induce a concentration- and time-dependent increase in the frequency and severity of morphological abnormalities in *D. rerio* eggs, embryos, and larvae. Examples are yolk sac and pericardial oedema, tail and spinal deformities, and impaired cardiac function.

7. Are specific developmental stages or organ systems of *D. rerio* more sensitive to In or Ga exposure?

The period between 24 and 72 hpf, when organogenesis, somitogenesis, and cardiovascular system development take place, forms a window of heightened sensitivity for *D. rerio*. During this period, morphological endpoints will show the steepest concentration-response relationships.

2. Materials & methods

2.1 *Corophium volutator*

The protocol used here was adapted from Thain & Roddie (2001) and Schipper et al. (2006), with slight modifications. Test organisms of the species *Corophium volutator*, the European mud scud, were used. The experiment consisted of four treatments, one control and three with different metal concentrations, with seven replicates each, corresponding to 28 exposure units (replicates). Each replicate contained twenty individuals of *C. volutator* exposed to natural sediment spiked with the metals indium (In) and gallium (Ga). Sediment spiking was achieved by dissolving the respective concentration of metals in 3 L of artificial seawater and mixing. To achieve a relative equilibrium in metal concentrations between the overlaying water and the sediment, the exposure units were kept for 24 hours before exposure of the organisms. The organisms were exposed for 28 days, with water exchange after every two or three days, and feeding the day after. The endpoints studied included survival, absolute & specific growth, and metal accumulation in the organic tissue. Additionally, metal concentrations in the water and sediment were measured.

2.1.1 Sampling of sediment and test organisms

Sampling was conducted on 28 October 2025 near the Dutch city of Goes at the Eastern Scheldt (51°31'55"N, 3°55'58"E, using WGS 84). This is an intertidal area where *C. volutator* is known to be present (De Borger et al., 2020). Organisms were collected by scooping approximately 5 to 10 cm of sediment using a shovel, and were carefully sieved through different sieves (1000, 500, and 250 µm). Field variables were measured with the HQ 30D Flexi Digital Multi-Parameter Meter Kit (Hach, Loveland, Colorado, United States), and showed a temperature of approximately 13.2 °C and a conductivity of approximately 44.9 mS/cm. Using the Practical Salinity Scale 1978 (PSA 78; Lewis & Perkin, 1981), a salinity of around 34 ppt was found. Subsequently, different sizes of *C. volutator* remained on the sieve and were placed, together with some sediment and seawater, in polyethylene containers for transport to the laboratory on Campus Groenenborger, University of Antwerp. There, the mud scuds were separated from the sediment and selected according to size, respectively individuals retained on the 1000, 500, and 250 µm sieve. The different sizes were placed in different containers, with a two cm layer of sieved (250 µm) sediment collected at the sampling site and gently aerated using Pasteur

pipettes connected to plastic air tubes. During a three-week acclimation period, a mixture of artificial seawater and natural seawater collected at the sampling site was used to acclimate the organisms, before fully keeping them in artificial seawater to ensure a gradual transition and to favour an ideal salinity and water quality adjustment. The artificial seawater was made by dissolving 35.75 g of sea salt (Aquaforest, Brzesko, Poland) per 1 litre of deionized water (EUROWATER Technologies, Grundfos Water Treatment Germany GmbH, Germany), reaching a final salinity of 32 ppt. The mud scuds were acclimatized in a climate chamber for three weeks under the same conditions as during the experiment. Some organisms were used to determine the exact species, using certain characteristics to differentiate them from similar species like *C. arenarium* and *C. multisetosum* (Barnes, 1994). The variables, which were measured in the water, remained within the following limits during the exposure period: temperature (15 ± 2 °C), salinity (24-36 ppt), oxygen (>50%), pH (7-9), and ammonium (<5 mg/L), as mentioned in the protocol by Schipper et al. (2006). Furthermore, a photoperiod of 12 hours of light followed by 12 hours of darkness was followed, and the light intensity was monitored (broad spectrum; 24 hours; 500-1000 lux).

2.1.1.1 Water and sediment metal analysis

Three subsamples of the collected sediment were analysed on the background metal content. The samples were dried at room temperature, after which around 200 mg wet weight was placed in pre-weighed 50 mL-tubes. Following this, the samples were placed in an oven (IP60-U Standard Incubator, LTE Scientific, Oldham, United Kingdom) for 48 hours at 60 °C. After this period, the samples were placed in a desiccator for 30 minutes to come to room temperature. Then the dry weight of each sample was measured.

The sample digestion was done by adding 3 mL HCl (37 %) and 1 mL HNO₃ (69 %) of high purity grades, and they were kept at room temperature for over 12 hours. Then, the sample tubes were placed in a hot block (Environmental Express HotBlock SC154 Digestion System, Cole-Parmer, Vernon Hills, Illinois, United States) under a fume hood, without the caps at a working temperature of 115 °C for 60 minutes. Milli-Q water (IQ Element water purification unit & IQ 7003 water purification system, Merck KGaA, Darmstadt, Germany) was added to the 40 mL mark. The tubes were closed tightly and stored until analysis. The same procedure was followed for three blanks with Milli-Q water, and three samples of reference material. The reference material was channel sediment BCR-320R (Held et al., 2006). The fish feed given to *C.*

volutator during the experiment was analysed in the same way as well, by taking three samples. This time fish muscle ERM-BB422 was used for three reference samples (Snell et al., 2012a). After this, 2 mL was taken from all samples, and this was further diluted four times by adding 6 mL of Milli-Q water. These samples were then analysed with a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS; Thermo Fisher Scientific, Bremen, Germany). Seventeen metals were analysed in the sediment and feed samples (Li, Sb, V, Cr, Mn, Fe, Co, Ni, Sn, Ag, Cd, Pb, Al, Cu, Zn, Ga, and In), and fourteen in the water samples (Cd, Pb, Cr, Co, Ni, Zn, As, Ag, Al, Mn, F, Cu, Ga, and In).

2.1.2 Chronic stress exposure (28 days)



Figure 1: The experimental setup of the C. volutator experiment, illustration on the left made using BioRender.

Organisms were exposed in a climate chamber at 15 ± 2 °C. Approximately 200 mL (two cm) of sieved sediment was placed in polypropylene 700 mL beakers. Four different treatments were used, including a control treatment, with seven containers each (28 exposure containers in total). To each beaker, 600 ± 10 mL of artificial seawater (spiked with the previously mentioned increasing levels of In and Ga) was added, while avoiding disturbance of the sediment. Each beaker was gently aerated with a Pasteur pipette and covered with a lid to minimize water loss through evaporation. This experimental setup is shown in figure 1. To establish an equilibrium, the containers were stored in the climate chamber for approximately 24 hours (overnight). After this, a 9.5 mL water sample was taken from each replica for metal analysis, and a 5 mL water sample for ammonium analysis. The boundary conditions listed in the guidelines were measured in each container at this point, namely oxygen concentration, pH, temperature, and salinity.

2.1.2.1 Starting the experiment

After a two-week acclimation, the mud scuds were taken from the reference sediment with the use of a 500 µm sieve. Thereafter, they were rinsed with artificial seawater and counted. Twenty organisms of a similar size (approximately 6 to 7 mm) were added to each prepared container. To achieve the four different treatments, the artificial seawater was spiked with various amounts of In and Ga. The first treatment had 0.38 µg In/L and 2.34 µg Ga/L. The second treatment had 4.19 µg In/L and 25.8 µg Ga/L. The third treatment had 42.3 µg In/L and 260 µg Ga/L. The control treatment only had the background levels of In and Ga, and therefore represents the current nominal concentration of these two emergent metals from the collection location. The treatments T1, T2, and T3 roughly show an exponential increase of the natural levels in water (respectively 10 times, 100 times, and 1000 times). This is based on the FOREGS Geochemical Atlas of Europe, which mentions natural background levels in water between <0.002 µg/L and 0.015 µg/L for indium and between <0.002 µg/L and 0.17 µg/L for gallium (Salminen et al., 2005). The different concentrations were prepared using stock solutions of indium(III) chloride (InCl₃) and gallium(III) chloride (GaCl₃), mixed with artificial seawater. The mixtures were then stored in polyethylene buckets, and before exposing the *C. volutator* to the concentrations, they were shaken for 10 minutes at 100 rpm on the 3017 Orbital Shaker (GFL, Lauda-Königshofen, Germany). The aeration of the beakers was done using a glass Pasteur pipette, and to allow excess air to escape a small hole was made in the lid of the container. All containers stayed in the climate chamber for 28 days at a temperature of 15 ± 2 °C. They were randomly placed within the space of the experiment.

All water quality variables (pH, ammonia, oxygen concentration, temperature, and salinity) were recorded every two or three days, and spot checks were conducted in between. The aeration was also monitored. The pH was measured using the FiveEasy F20 Standard Benchtop pH/mV Meter (Mettler Toledo, Greifensee, Switzerland). Total ammonia content was measured using a Tetra Test NH₃/NH₄⁺ (Spectrum Brands Inc., Middleton, Wisconsin, United States). Oxygen concentration and temperature were measured with the ProfiLine Oxi 3310 Portable Dissolved Oxygen Meter (WTW, London, United Kingdom). Salinity was measured by using the S/Mill-E Handheld Salinity Refractometer (ATAGO, Tokyo, Japan). Every two to three days, 9.5 mL water samples from each container were collected for further analysis. This happened prior to water exchange, which was done using a siphon to minimize disturbance of the sediment. The water samples taken for determination of the metal concentration in the water

over the course of the experiment were acidified by adding 0.5 mL HNO₃ (69%). Correspondingly, they were stored at -20 °C until further analysis. A day after taking these samples, the mud scuds were fed. Each container received 12 mg/L of fish feed (Granured Nature Cichlidenfutter, Sera, Heinsberg, Germany) per feeding moment. Initially at the first feeding moment they were fed with twice the amount of feed (24 mg/L), but due to relatively high ammonia concentrations (approximately 1.5 mg/L) this amount was halved.

2.1.2.2 Ending the experiment

After 28 days, the last water samples for both metal and ammonia analysis were collected from each container, and the water quality variables were measured for the last time. The container content was sieved with a 500 µm sieve, and all organisms were collected. Organisms that did not move within ten seconds after touching them, or were not found, were considered dead. Average mortality in the bioassay was assessed. The wet weight of the animals was measured immediately after to determine absolute growth and specific growth rate. Dry weight was measured later, after placing the samples in the oven for 48 hours at 60 °C and in a desiccator for 30 minutes. Sediment samples were taken from all containers, and stored at -20 °C. The same was done with the pooled *C. volutator* samples of each container.

2.1.3 Analysis of the samples

The frozen water samples were defrosted and thereafter used for metal analysis. Only the samples from day 1, day 14 and day 28 were analysed, since no large differences were expected. One mL of each 14 mL-tube per treatment was pooled together. This pooled sample was diluted ten times, so 1 mL was taken and added to 9 mL of acidified Milli-Q-water (1% HNO₃; to maintain similar level of acidity as the sample). This is done to make the metals in the samples homogeneous. These twelve samples were then analysed using ICP-MS.

The 5 mL water samples for ammonia analysis were taken out of the freezer. Both the samples from day 1 and day 28 of the experiment were analysed concurrently. The method used for this was the salicylate-hypochlorite method with slight modifications (Bower & Holm-Hansen, 1980; Le & Boyd, 2012). The salicylate solution was made by dissolving 440 g sodium salicylate (C₇H₅NaO₃) and 0.28 g sodium nitroprusside dihydrate (Na₂[Fe(CN)₅NO].2H₂O) in 1 L ammonia-free Milli-Q water. The alkaline citrate solution consisted of 18.5 g sodium hydroxide (NaOH) and 100 g sodium citrate (Na₃C₆H₅O₇), dissolved in 1 L Milli-Q water. Both

solutions were kept at 5 °C in brown bottles, the one with the alkaline citrate solution covered with aluminium foil to shield it from light. The alkaline hypochlorite solution was composed of 10 mL sodium hypochlorite (5% NaOCl) in the self-prepared 90 mL alkaline citrate solution. Reagent grade seawater was made using 24.09 g sodium chloride (NaCl), 10.91 g magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), 4.02 g sodium sulphate (Na_2SO_4), 1.5 g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.68 g potassium chloride (KCl), and 0.20 g sodium bicarbonate (NaHCO_3), all dissolved in 1 L Milli-Q water, leading to a salinity of 34 ppt. The stock total ammonia nitrogen standard solution consisted of 3.82 g ammonium chloride (NH_4Cl) dissolved in 1 L of reagent grade seawater. This led to 1000 mg/L total ammoniacal nitrogen (TAN; this represents the sum of ammonium (NH_4^+) and ammonia (NH_3)). With this solution a standard solution was made, with concentrations ranging from 0.0 to 0.5 mg/L TAN.

To each 5 mL sample, 1.2 mL salicylate catalyst solution was added. This was mixed, and then 2.0 mL alkaline hypochlorite solution was added and mixed again. These samples were then incubated in the dark for 60 minutes at room temperature. After this period, the absorbance was measured at 640 nm, using a spectrometer (Spectronic 20 Genesys, Helsingborg, Sweden). The concentrations of ammonia were calculated using the formula $y = mx + c$, with y = absorbance, m = slope of the curve, c = intercept of y , and x = ammonia concentration (mg/L).

Following the same procedure as mentioned earlier (see section 2.1.1.1), the sediment from each container was analysed using ICP-MS. Three blanks and three reference samples were analysed as well. A pooled sample containing all the surviving mud scuds from each treatment were also analysed this way, again using three blanks and three samples with reference material. This time mussel tissue ERM-CE278k was used (Snell et al., 2012b).

2.1.4 Reference toxicant test

A reference toxicant, an ammonium chloride (NH_4Cl) solution, was used as a quality control to check the sensitivity of the mud scuds. This happened for 72 hours during the main experiment. 28.9 grams of NH_4Cl was dissolved in 1 L of Milli-Q water, leading to a concentration of 10 g/L NH_4^+ . Twelve containers were used, each containing twenty mud scuds, 500 mL of artificial seawater, and no sediment. A concentration series with six different concentrations was used (0, 36, 72, 110, 180, and 360 mg NH_4^+ /L), as described by Schipper et al. (2006). This was done in duplicate. The solution was added, and pH, temperature, oxygen content, salinity, and ammonium content were measured. Aeration happened in the same way as during the main

experiment, with a glass Pasteur pipette and a small hole in the lid. After 72 hours, the surviving and dead mud scuds were counted, and a 72h-LC₅₀ value was calculated. Ideally, this was in the range of 36 to 220 mg NH₄⁺/L.

2.2 *Danio rerio*

Fertilised zebrafish (*Danio rerio*) embryos (EZRC F1; European Zebrafish Resource Center, first generation of offspring; 1 hour post fertilisation (hpf)) were obtained from the Zebrafishlab at Campus Drie Eiken, University of Antwerp. The embryos were exposed to different concentrations of Ga and In for 120 hours. Every 24 hours, key indicators of lethality were monitored. These included coagulation of embryos, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and lack of heartbeat. From 0 to 72 hpf, organisms are referred to as embryos; thereafter as larvae (Elizalde-Velázquez & Herrera-Vázquez, 2023). At the end of the exposure period, acute toxicity was determined based on the recorded observations, and the LC₅₀ was calculated. The different stages in the embryonic development of the zebrafish used here were as described by Kimmel et al. (1995). The experiment was conducted in accordance with OECD Test Guideline 236 (Fish Embryo Acute Toxicity Test) (OECD, 2025).

2.2.1 Preparation of the different media

2.2.1.1 Embryo solution

The embryo solution (ES) was prepared by dissolving Instant Ocean Sea Salt (Instant Ocean, Blacksburg, Virginia, United States) in deionized water (IQ Element water purification unit & IQ 7003 water purification system, Merck KGaA, Darmstadt, Germany) to reach an electrical conductivity of 480 to 500 µS/cm (OECD, 2025). For this, approximately 60 mg salt/L was added to the deionized water (Westerfield, 2000). The ES was stirred with a magnetic stirrer (Multistirrer Digital, Velp Scientifica, Usmate, Italy) to ensure uniform mixing. The electrical conductivity of the ES was approximately 494 µS/cm, which was within the recommended range. Conductivity was measured using a HQ 30D flexi (Hach Company, Loveland, Colorado, United States). The pH was adjusted to 7.39 by adding small volumes of a 30 g/L NaHCO₃ solution or dilute 0.5 M HCl and was measured using a FiveEasy™ pH / mV bench meter (Mettler Toledo, Greifensee, Switzerland). The ES was used as a negative control and as the dilution water for the other media. The final ES was kept in a clearly labelled 5 L brown glass

bottle, and placed in the exposure incubator (EB1E Refrigerated incubator, Snijders Scientific, Tilburg, the Netherlands) at a temperature of 26 °C. Additionally, the water quality variables fell within the recommended ranges for electrical conductivity, pH, and temperature; respectively 150 to 2,000 µS/cm, 6.5 to 8, and 24 to 31 °C (Porteus et al., 2024). These ranges are relatively broad, but consistent with the wide variation in water chemistry that zebrafish are used to in their natural environment.

2.2.1.2 Indium

Six concentrations of In were tested, namely 0.06 mg/L, 0.6 mg/L, 1.2 mg/L, 6.0 mg/L, 30 mg/L, and 90 mg/L. In was added as an aqueous indium(III) chloride (InCl₃) stock solution. To account for the difference in molar mass between In and InCl₃, respectively 114.82 g/mol and 221.17 g/mol, a conversion factor of 1.93 was applied. Therefore, the corresponding concentrations of InCl₃ were 0.116 mg/L, 1.16 mg/L, 2.32 mg/L, 11.6 mg/L, 57.9 mg/L, and 173.7 mg/L. Stock solutions were prepared in ES and diluted to a final volume of 500 mL per treatment. The conductivity and pH of all solutions were measured as described for the ES. All solutions were stored in the exposure incubator at 26 °C until use.

2.2.1.3 Gallium

Six concentrations of Ga were tested, namely 1.0 mg/L, 5.0 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, and 90 mg/L. Ga was added as an aqueous gallium(III) chloride (GaCl₃) stock solution. To account for the difference in molar mass between Ga and GaCl₃, respectively 69.72 g/mol and 176.07 g/mol, a conversion factor of 2.53 was applied. Therefore, the corresponding concentrations of GaCl₃ were 2.53 mg/L, 12.65 mg/L, 50.6 mg/L, 101.2 mg/L, 151.8 mg/L, and 227.7 mg/L. Stock solutions were prepared in ES and diluted to a final volume of 500 mL per treatment. The conductivity and pH of all solutions were measured as described for the ES. All solutions were stored in the exposure incubator at 26 °C until use.

Although environmental concentrations are lower for both In and Ga, higher concentrations were included to enable LC₅₀ determination.

2.2.1.4 Positive control

The last medium was 3,4-dichloroaniline (DCA), used as a positive control. This ensured that embryo viability, water quality, and experimental sensitivity were adequate. A solution of 4.0

mg DCA/L was prepared. This provided a consistent benchmark with predictable (non-)lethal effects for comparing results across different studies or laboratories (Schiwy et al., 2020).

500 mL of the DCA solution was prepared with the ES as the dilution water, following the OECD guidelines. It was kept in the exposure incubator at a temperature of 26 °C. Exposure typically causes >30% mortality in zebrafish embryos within 96 hours. Beyond just killing the embryos, it also induces characteristic malformations, such as spinal malformations, oedema, and tail deformities.

2.2.2 Experimental design

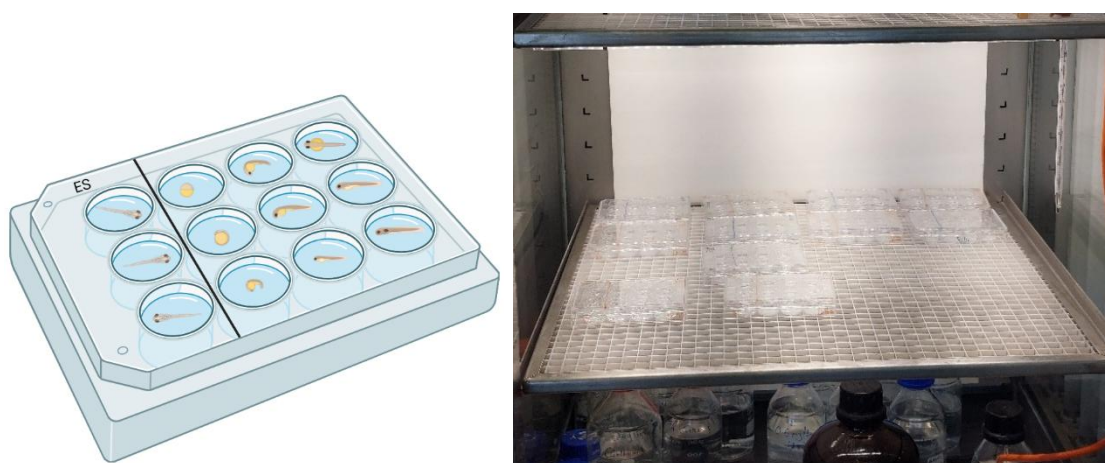


Figure 2: The experimental setup of the D. rerio experiment, illustration on the left made using BioRender.

Embryos were exposed to the respective test solutions in 12-well plates (Cell Culture Multiwell Plate, Greiner Bio-One, Kremsmünster, Austria). Sixteen plates were prepared, three negative controls with ES, one positive control with DCA, and six plates for the different treatments of both In and Ga. All plates had one internal control row (with three wells), where only ES was added. The other nine wells contained the treatment ($n = 9$). Plate layouts were labelled accordingly. Every well contained 2.5 mL of medium, and one zebrafish embryo. This experimental setup is shown in figure 2. From each concentration, a 9.5 mL water sample was collected for metal analysis. To this, 0.5 mL HNO_3 (69%) was added, and the samples were stored in a freezer at -20 °C until analysis. All the plates, covered with their lids, were put in the exposure incubator.

2.2.3 Scoring of mortality and renewing the media

From 0 to 96 hpf, the mortality was scored daily for each embryo or larva based on four parameters: coagulation, lack of somite formation, non-detachment of the tail and the absence of a heartbeat. Heartbeat was determined by counting the number of observed pulsations of the heart for ten seconds. For this, a counter was used (1×5 Multiple-Tally Counter, The Denominator Company, Waterbury, Connecticut, United States). Where the visibility was poor due to precipitation or because the organism was in a certain position, only the presence of the heartbeat was determined. At the 120 hpf mark, also the hatching status and swim bladder inflation were checked. This was done with a stereomicroscope, Leica S8 APO Microscope with Leica MC190 HD Microscope Camera and Leica KL300 LED (Leica Camera AG, Wetzlar, Germany), which made it possible to document this by making photos with the LAS EZ software (version 3.4.0). The temperature near the microscope was kept at 26 ± 2 °C by using an electric heater (DO7321F, Domo, Herentals, Belgium) and a thermocouple (Model 23609-232 Thermocouple, VWR, Radnor, Pennsylvania, United States). After the scoring, the medium was renewed with the respective exposure solutions, ES, or DCA, and the plates were put back in the exposure incubator.

2.2.3.1 Full morphological end scoring and euthanasia

Before the larvae were euthanised, a final scoring was done by visual inspection, including assessment of pectoral fins and pigmentation. Water samples from all treatments were also collected and prepared as stated above (section 2.2.2). This time, both filtered and non-filtered samples were taken. Sample filtration was achieved by using a filter (CHROMAFIL disposable syringe filters PET-45/25, Macherey-Nagel, Düren, Germany) with a membrane pore size of 0.45 µm to obtain the dissolved portion of the metal elements. After this, the zebrafish were put in ES for 20 minutes, to remove any metals attached to the fish. Then the fish were placed in 1.5 mL microcentrifuge tubes per treatment, and dried in the oven for 48 hours at 70 °C. After oven drying, fish samples were first placed in a desiccator for 30 minutes to cool and then the dry weight was recorded using a weighing scale (AT261 Analytical Balance, Mettler Toledo, Greifensee, Switzerland). Subsequently, the fish were stored in a freezer at -80 °C until analysis. Briefly, zebrafish were acid digested by adding 1 mL HNO₃ (69%) to the tubes. Thereafter, all tubes were placed in a sealed plastic box and placed in a microwave (MC455TBRCBB, Samsung, Seoul, South Korea) for 3 minutes at 100 watts, 6 minutes at 180 watts, and 3 minutes

at 300 watts. This method was adapted from previous research (e.g. Hirata et al., 2006). The samples were then transferred to 14 mL tubes, and 9 mL of Milli-Q water was added. After this, metal analysis took place using inductively coupled plasma mass spectrometry (ICP-MS). This method was also used to analyse the water samples.

2.3 Statistical analysis

The statistical analyses using the data gathered by the previously mentioned experiments were performed using R (version 4.4.2; R Core Team, 2024) within the RStudio environment (version 2026.01.2+418; Posit Team, 2026). The data was, where possible, assessed for normality with the Shapiro-Wilk test and for homogeneity of variances with the Levene's test, and summarized as means and standard deviations (SD). A significance level of $p < 0.05$ was used.

2.3.1 *Corophium volutator*

Survival, absolute growth, specific growth rate, and relative growth were analysed by doing either a one-way ANOVA followed by a Tukey's HSD test, or by a Kruskal-Wallis test followed by a Dunn's test with the Holm-Bonferroni method. This method provides greater statistical power than the standard Bonferroni correction, particularly with small group sizes. This way false positives were minimized (Haynes, 2013). Absolute growth was calculated by subtracting the original average weight of the final average weight per treatment. The specific growth rate (SGR) was calculated by subtracting the natural logarithm of the original average weight from the natural logarithm of the final average weight and dividing this by 28 (the amount of days the experiment took place). Relative growth was calculated by taking the mean weight of a treatment, dividing it by the mean weight of the control treatment and multiplying this by 100.

Differences in metal concentrations in sediment and organic tissue, and the biota-sediment accumulation factors (BSAFs) were analysed either by using a one-way ANOVA followed by a Tukey's HSD test, or by using a Kruskal-Wallis test followed by a Dunn's test with the Holm-Bonferroni method. When assumption checks indicated non-normality and unequal variances, a non-parametric approach was used.

The LC_{50} for the reference toxicant test was determined using the *drc* package in RStudio for analysis of dose-response curves. The percentage of mortality was calculated by dividing the number of dead individuals by the total number of individuals. A two-parameter log-logistic model (LL2.2) was fitted to the dose-response data using the *drm()* function with binomial error structure and logit link. The model assumed that the lower asymptote was fixed at 0, and the upper at 1. Median lethal concentration (LC_{50}), as well as LC_{90} and LC_{95} , were estimated using the *ED()* function. Confidence intervals (95%) were calculated using a method which derives intervals on the logarithmic scale and back-transforms them to the original concentration scale.

2.3.2 *Danio rerio*

To analyse if there was a significant difference in survival between the experimental treatments and the control treatment (ES), pairwise comparisons were performed using Fisher's exact test. This test was chosen due to the relatively small sample sizes and its suitability for categorical count data. To account for multiple comparisons across the twelve treatments, p-values were adjusted using the Benjamini-Hochberg procedure to control false discovery rate. Hatching was analysed using Kruskal-Wallis tests.

Metal concentrations per individual and from the water samples were analysed by calculating the Spearman's rank correlation coefficient, since in both cases only one value per treatment could be used for the analysis. This method only requires rank-ordered values, not replicates. This way it can be determined if when the exposure concentration goes up, the metal accumulation also goes up. For the water samples, additionally a Wilcoxon signed-rank test was performed to test whether filtered and non-filtered concentration differed significantly across treatments.

2.4 Ethical statement

D. rerio until the age of 120 hpf and *C. volutator* are not included in the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. The exposure experiments did therefore not require approval by the Ethical Committee for Animal Testing of the University of Antwerp.

3. Results

3.1 *Corophium volutator*

The water quality parameters remained within the following limits during the exposure period: temperature (14.47 ± 0.33 °C), salinity (33 ± 2 ppt), oxygen (8.22 ± 0.53 mg/L), pH (7.91 ± 0.10), and ammonium (<1.5 mg/L).

3.1.1 Survival and growth

Survival and mortality, for which the values can be found in appendix 1, could not be reliably assessed, since in many containers over twenty individuals were found after the experiment. No significant differences in survival between treatments were found (Kruskal-Wallis test; $\chi^2 = 5.614$, $df = 3$, $p = 0.132$). Survival is shown in figure 3.

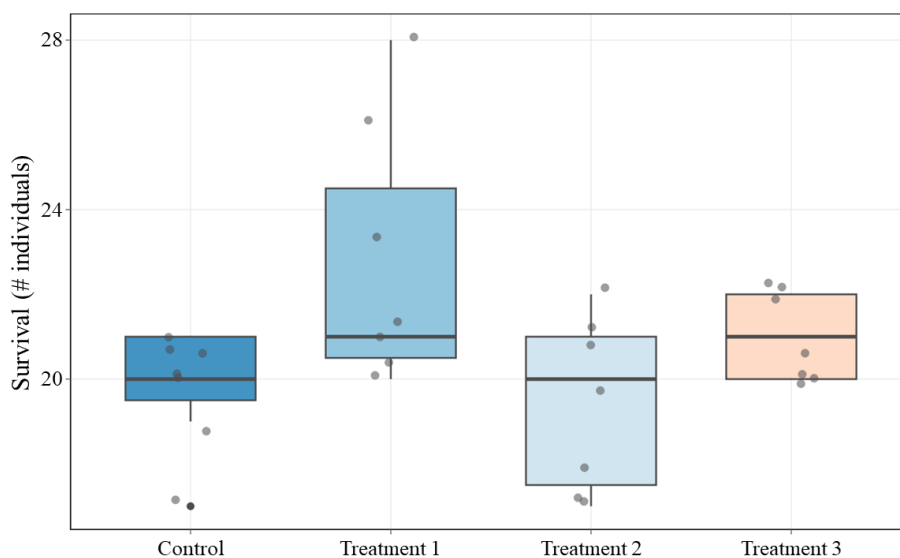


Figure 3: Amount of surviving individuals of *C. volutator* per treatment ($n = 7$). T1: $0.38 \mu\text{g In/L}$ and $2.34 \mu\text{g Ga/L}$; T2: $4.19 \mu\text{g In/L}$ and $25.8 \mu\text{g Ga/L}$; T3: $42.3 \mu\text{g In/L}$ and $260 \mu\text{g Ga/L}$.

Absolute growth showed a near-significant treatment effect, which suggests a possible influence on growth performance (Kruskal-Wallis test; $\chi^2 = 7.753$, $df = 3$, $p = 0.051$). This is shown in figure 4.

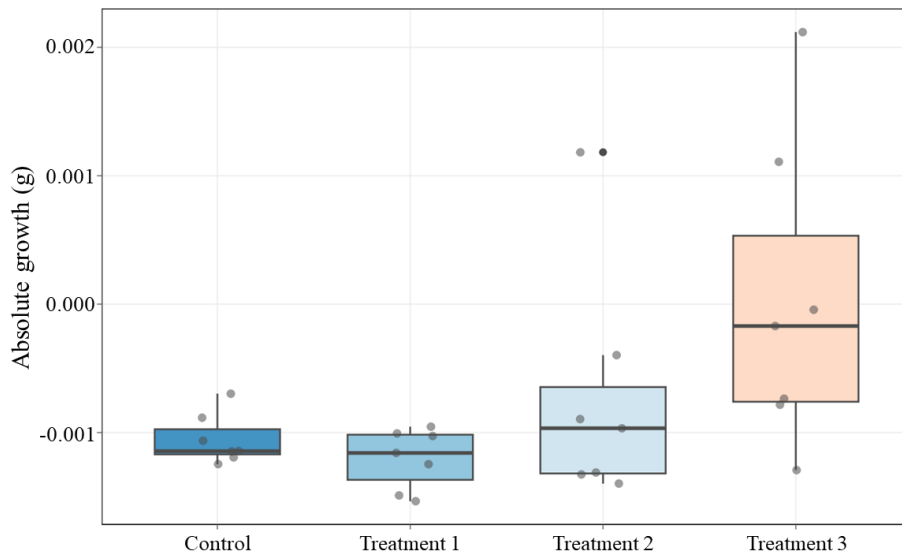


Figure 4: Absolute growth in grams per individual of C. volutator per treatment (n = 7). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

The specific growth rate (SGR) did differ significantly between the treatments (one-way ANOVA; $F(3,24) = 4.116$, $p = 0.017$). A post hoc Tukey's HSD test indicated that treatment 3 exhibited a significantly higher SGR than treatment 1 ($p = 0.013$). A Dunnett's test for comparing the different treatments with the control treatment, showed that treatment 3 also had a significantly higher SGR compared to the control treatment ($p = 0.041$). This is shown in figure 5.

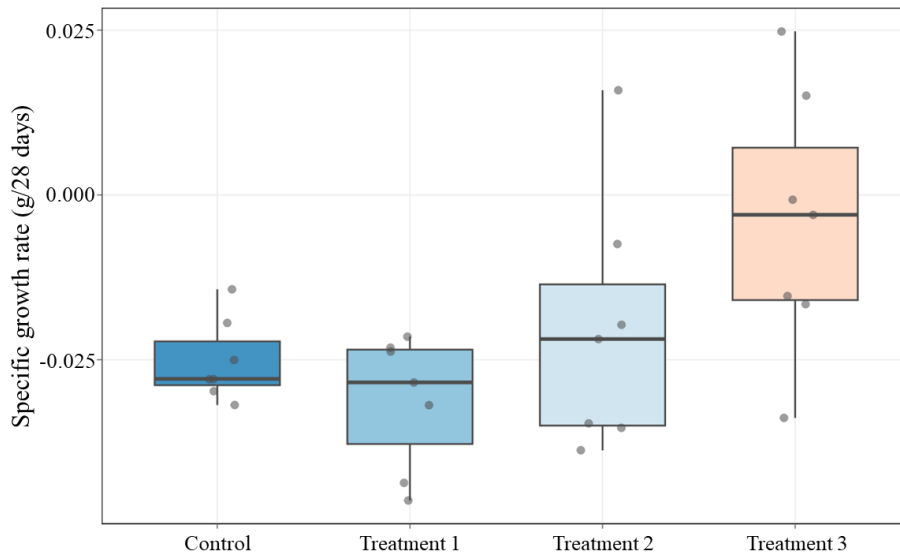


Figure 5: Specific growth rate in grams over 28 days per individual of *C. volutator* per treatment ($n = 7$). T1: $0.38 \mu\text{g In/L}$ and $2.34 \mu\text{g Ga/L}$; T2: $4.19 \mu\text{g In/L}$ and $25.8 \mu\text{g Ga/L}$; T3: $42.3 \mu\text{g In/L}$ and $260 \mu\text{g Ga/L}$.

For relative growth, after performing a Shapiro-Wilk test and a Levene's test, parametric assumptions were not met. There was no statistically significant difference in relative growth between treatments. (Kruskal-Wallis test; $\chi^2 = 7.002$, $\text{df} = 3$, $p = 0.072$). This is shown in figure 6.

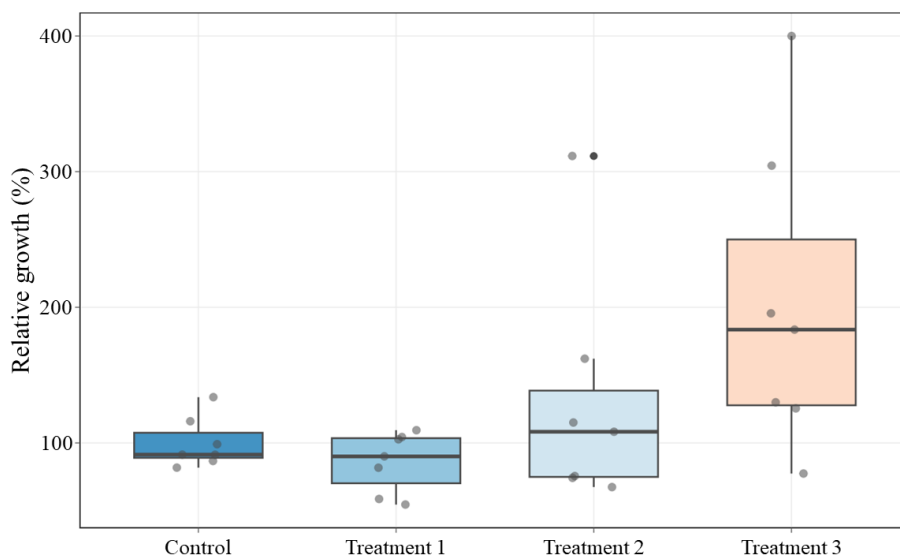


Figure 6: Relative growth in percentage, compared to the mean value of the control treatment, per individual of *C. volutator* per treatment ($n = 7$). T1: $0.38 \mu\text{g In/L}$ and $2.34 \mu\text{g Ga/L}$; T2: $4.19 \mu\text{g In/L}$ and $25.8 \mu\text{g Ga/L}$; T3: $42.3 \mu\text{g In/L}$ and $260 \mu\text{g Ga/L}$.

3.1.2 Metal accumulation

For the accumulation of In in the organic tissue of *C. volutator*, a significant difference between treatments was found (Kruskal-Wallis test; $\chi^2 = 20.861$, $df = 3$, $p < 0.001$). Assumption checks indicated non-normality and unequal variances (Levene's test; $p = 0.001$). Post hoc tests showed treatment 3 to differ significantly from the control treatment ($p = 0.001$) and treatment 1 ($p < 0.001$).

For the accumulation of In in the sediment, a significant difference between treatments was found (Kruskal-Wallis test; $\chi^2 = 22.972$, $df = 3$, $p < 0.001$). Assumption checks indicated non-normality and unequal variances (Levene's test; $p = 0.004$). Post hoc tests showed treatment 3 to differ significantly from the control treatment ($p = 0.011$) and treatment 1 ($p < 0.001$). Treatment 2 also differed significantly from treatment 1 ($p = 0.026$). Both accumulation in the organic tissue and in the sediment are shown in figure 7.

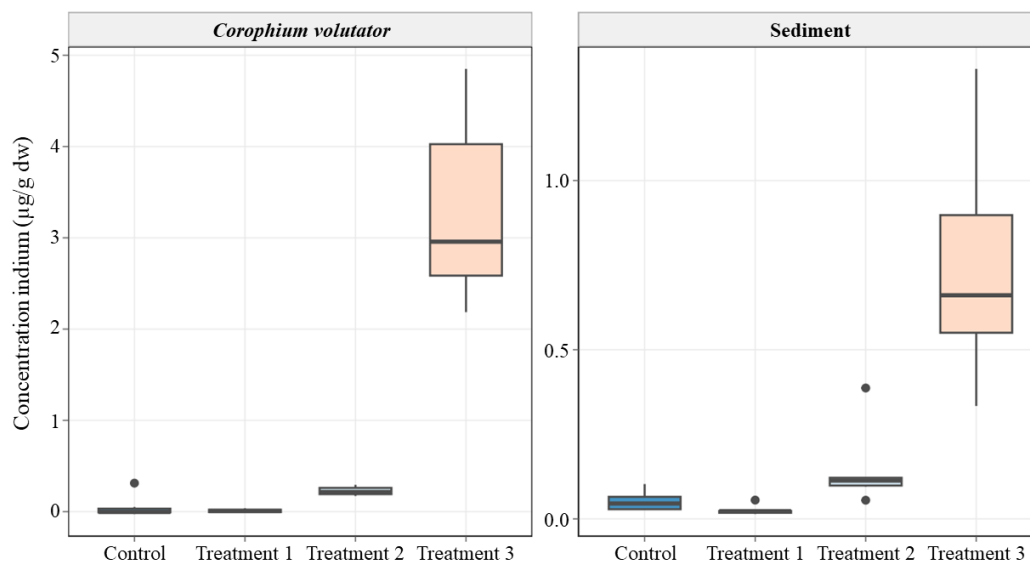


Figure 7: Indium concentrations in individuals of *C. volutator* and in the sediment in µg per g dry weight (dw), for each treatment ($n = 7$). T1: $0.38 \mu\text{g In/L}$ and $2.34 \mu\text{g Ga/L}$; T2: $4.19 \mu\text{g In/L}$ and $25.8 \mu\text{g Ga/L}$; T3: $42.3 \mu\text{g In/L}$ and $260 \mu\text{g Ga/L}$.

For the accumulation of Ga in the organic tissue of *C. volutator*, a significant difference between treatments was found (Kruskal-Wallis test; $\chi^2 = 22.864$, $df = 3$, $p < 0.001$). Assumption checks indicated non-normality and unequal variances (Levene's test; $p < 0.001$). Post hoc tests showed treatment 3 to differ significantly from the control treatment ($p < 0.001$) and treatment 1 ($p < 0.001$).

For the accumulation of Ga in the sediment, a significant difference between treatments was found (Kruskal-Wallis test; $\chi^2 = 22.938$, $df = 3$, $p < 0.001$). Assumption checks indicated non-normality and unequal variances (Levene's test; $p < 0.001$). Post hoc tests showed treatment 3 to differ significantly from the control treatment ($p < 0.001$) and treatment 1 ($p < 0.001$). Both accumulation in the organic tissue and in the sediment are shown in figure 8.

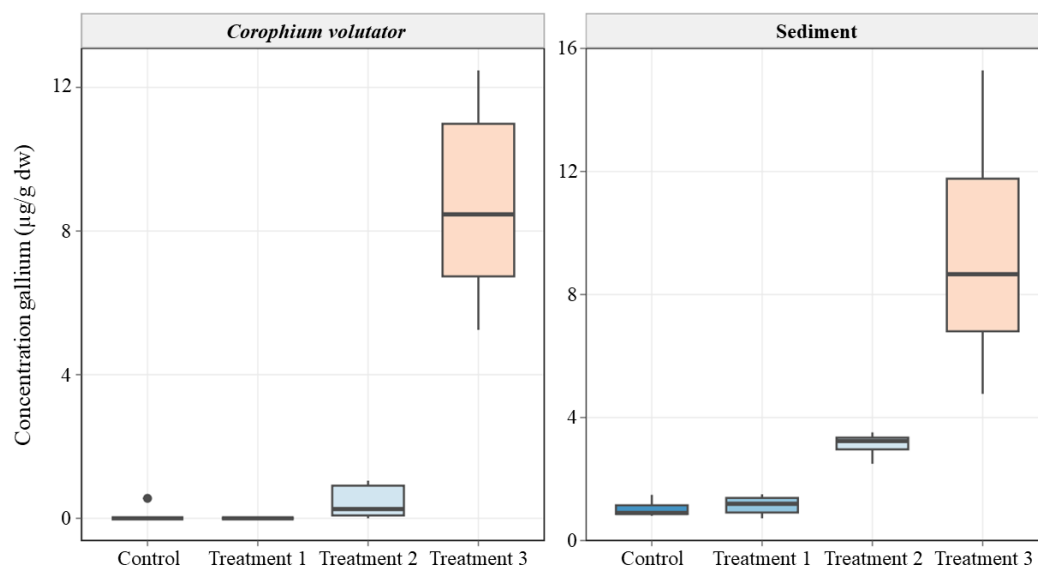


Figure 8: Gallium concentrations in individuals of *C. volutator* and in the sediment in µg per g dry weight (dw), for each treatment ($n = 7$). T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Biota-sediment accumulation factors (BSAFs) were calculated using the pooled *C. volutator* samples from each container relative to treatment-specific sediment concentrations. Mean BSAF values differed among treatments for both In and Ga, but with large standard deviations, as shown in table 1. Across both elements, treatment 3 showed the highest BSAF values, while treatment 1 showed the lowest.

Table 1: Mean BSAF values and standard deviations for indium and gallium per C. volutator treatment. The value for T2 Ga is 0, since all concentrations were below the LOD. T1: 0.38 µg In/L and 2.34 µg Ga/L; T2: 4.19 µg In/L and 25.8 µg Ga/L; T3: 42.3 µg In/L and 260 µg Ga/L.

Treatment	Mean BSAF In ± SD	Mean BSAF Ga ± SD
Control	0.916 ± 2.30	0.077 ± 0.204
T1	0.395 ± 0.719	0.000 ± 0.000
T2	1.59 ± 0.326	0.150 ± 0.151
T3	4.45 ± 1.41	0.936 ± 0.290

A significant treatment effect on In BSAF was found (Kruskal-Wallis test; $\chi^2 = 17.415$, $df = 3$, $p < 0.001$). A post hoc Dunn's test with Bonferroni correction showed that treatment 3 had significantly higher BSAF values for In than both the control treatment ($p = 0.003$) and treatment 1 ($p = 0.002$).

A significant treatment effect on Ga BSAF was found (Kruskal-Wallis test; $\chi^2 = 22.148$, $df = 3$, $p < 0.001$). A post hoc Dunn's test with Bonferroni correction showed that treatment 3 had significantly higher BSAF values for Ga than both the control treatment ($p < 0.001$) and treatment 1 ($p < 0.001$).

The higher BSAF values for In compared to Ga indicate a greater relative accumulation. Both are shown in figure 9.

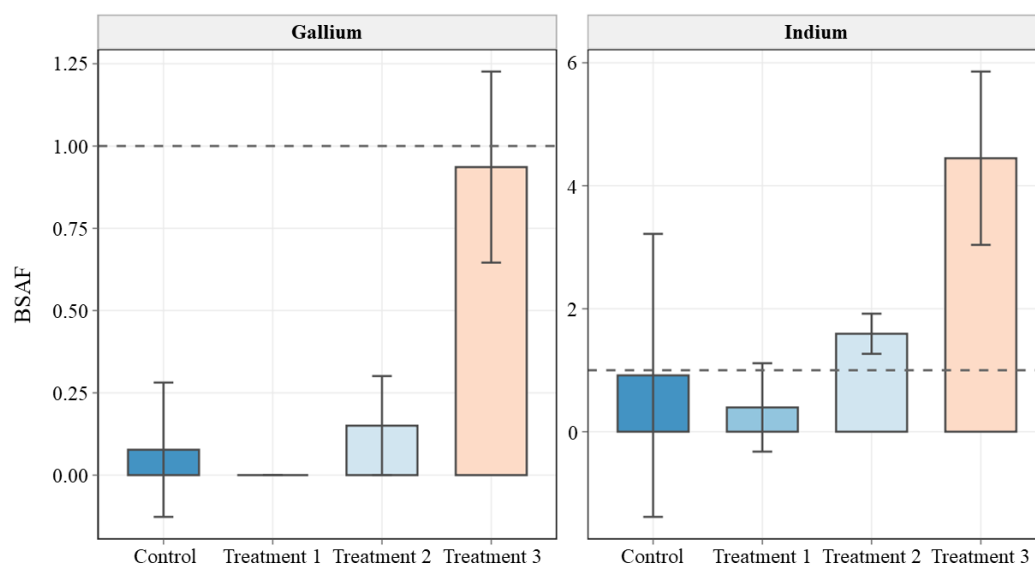


Figure 9: Mean BSAF values for gallium and indium per *C. volutator* treatment ($n = 7$). T1: $0.38 \mu\text{g In/L}$ and $2.34 \mu\text{g Ga/L}$; T2: $4.19 \mu\text{g In/L}$ and $25.8 \mu\text{g Ga/L}$; T3: $42.3 \mu\text{g In/L}$ and $260 \mu\text{g Ga/L}$.

3.1.2.1 Sediment metal analysis

For the metal analysis of the natural sediment collected at the sampling site of *C. volutator*, 17 metals including In and Ga were analysed to determine the current background concentrations, shown in appendix 2. As expected, these were relatively low for the metals of interest, namely $0.242 \pm 0.328 \mu\text{g In/g sediment}$ and $0.921 \pm 0.168 \mu\text{g Ga/g sediment}$. It should be noted that, even though there is no indication of contamination of the sediment, the presence of the other metals could have impacted the results.

3.1.2.2 Water metal analysis

The metal analysis of the water samples taken over the course of the experiment and from the different treatments were analysed for 14 metals including In and Ga, shown in appendix 3. Interestingly, the In concentrations stayed below the detection limit of $0.005 \mu\text{g/L}$ throughout the experiment. For Ga, a clear increase is visible between the treatments, with the highest concentrations in treatment 3. Given that the Ga concentrations for the control and treatment 1 at day 1 exceed the concentrations of the added water (and elevated manganese levels) possibly contamination occurred.

3.1.3 Reference toxicant test

The results of the reference toxicant test can be found in appendix 4. With these values, estimated LC_{50} , LC_{90} , and LC_{95} values were calculated, as shown in table 2, and a dose-response curve was made, as shown in figure 10. The estimated LC_{50} of 281.70 mg NH_4^+ /L was slightly above the 72h- LC_{50} range of 36-220 mg NH_4^+ /L mentioned by Schipper et al. (2006), which implies that the used *C. volutator* individuals were less sensitive to contaminants than normal.

Table 2: Estimated LC_{50} , LC_{90} , and LC_{95} values in mg NH_4^+ per litre, with their upper and lower limits, of the reference toxicant test for *C. volutator*.

	Estimate (mg NH_4^+ /L)	Lower (mg NH_4^+ /L)	Upper (mg NH_4^+ /L)
LC_{50}	282	245	324
LC_{90}	501	389	645
LC_{95}	609	448	827

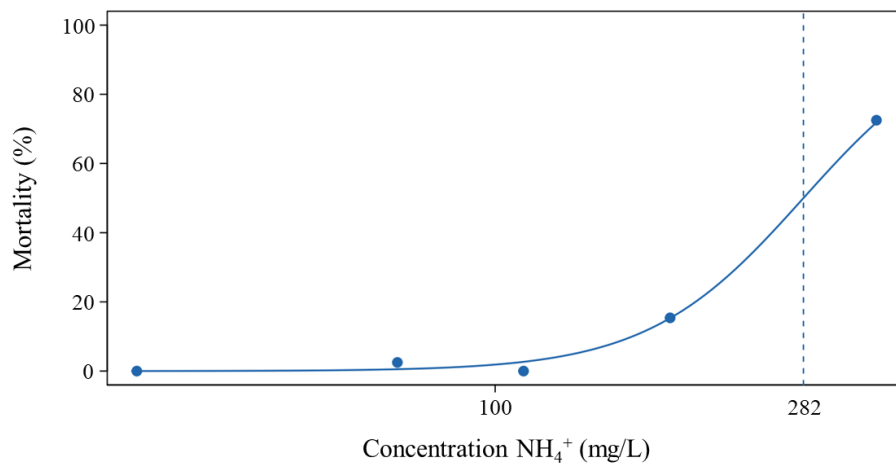


Figure 10: Dose-response curve showing the NH_4^+ -concentration in mg per litre and the percentual mortality of the reference toxicant test for *C. volutator*, including the LC_{50} ($n = 2$).

3.2 *Danio rerio*

3.2.1 Survival and hatching

Across all comparisons, no treatment showed a statistically significant difference in survival relative to the control treatment (all $p > 0.05$). The survival percentages are shown in figure 11.

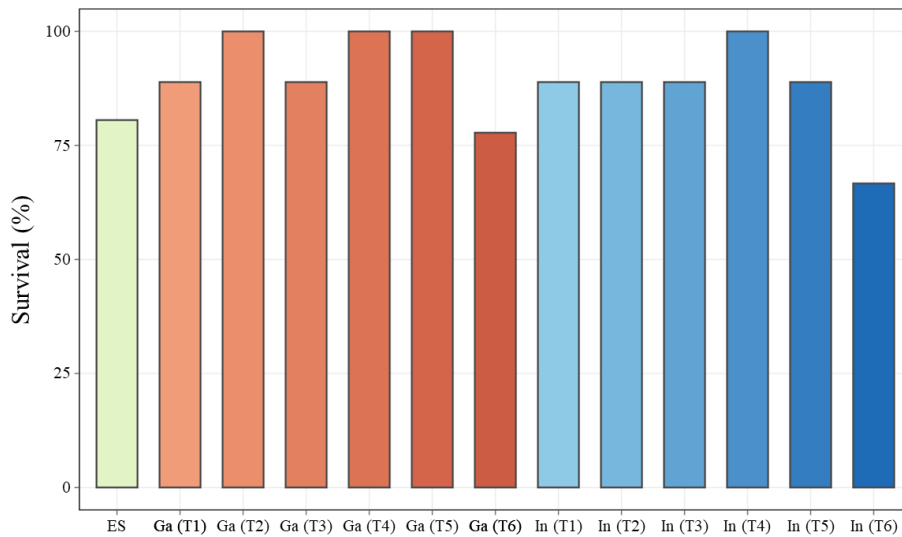


Figure 11: Percentage of surviving *D. rerio* individuals per treatment ($n = 9$, for ES $n = 36$). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

For comparison of the hatching between the experimental treatments and the control treatment, Kruskal-Wallis tests were performed. No significant differences in hatching were observed (all $p > 0.05$). This is shown in figure 12.

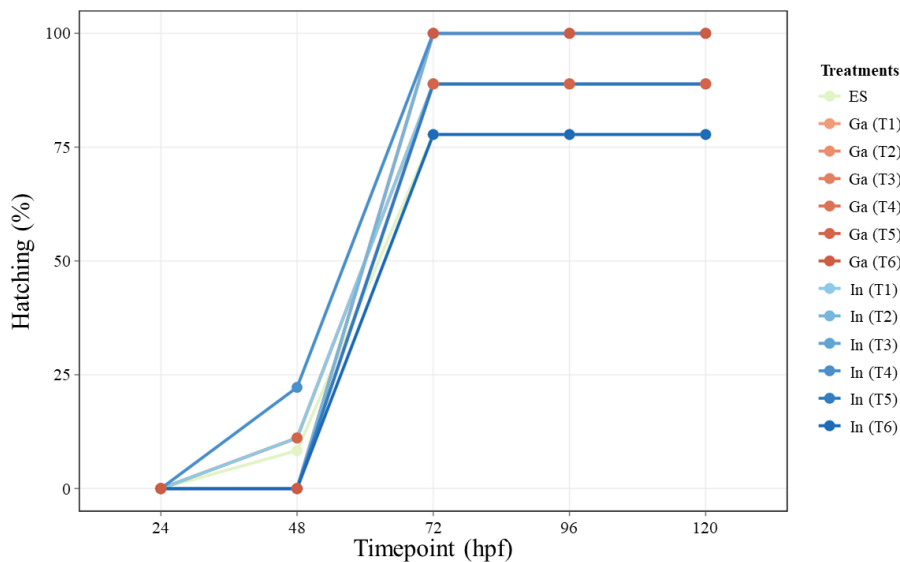


Figure 12: Hatching of *D. rerio* individuals over time in all treatments ($n = 9$, for ES $n = 36$). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

3.2.2 Metal accumulation

The low dry weight of the 120 hpf larvae made it impossible to convert the data to metal content per mass. Therefore, metal accumulation was expressed per individual larva, which is shown in appendix 5.

For the accumulation of In, a significant positive trend was shown (Spearman's rank correlation coefficient; $\rho = 0.943$, $p = 0.005$). With the In exposure going up from treatment 1 to treatment 6, the amount of In accumulated by the larvae also increased consistently. The reason why ρ is not 1, lies within the fact that treatment 4 had a lower amount of In as compared to treatment 3. This is shown in figure 13.

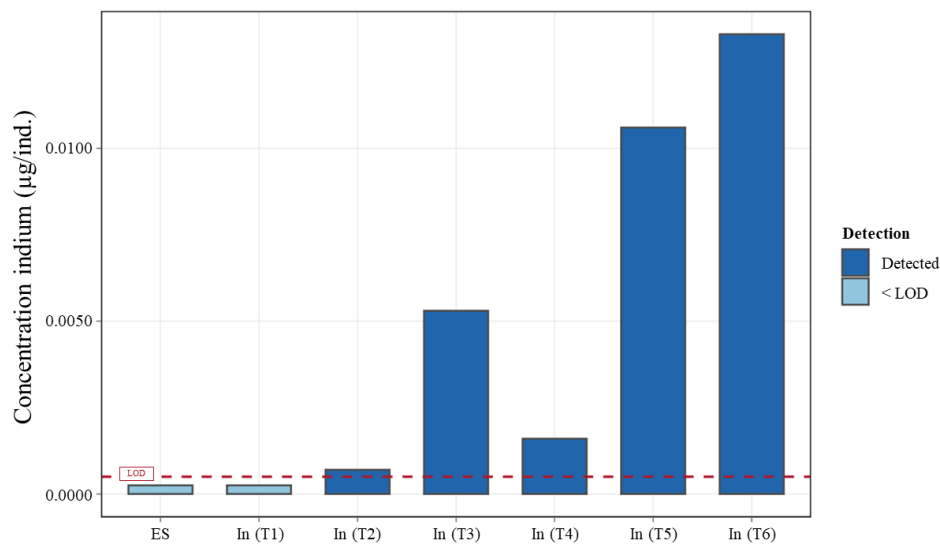


Figure 13: Indium accumulation in µg per individual of indium-exposed D. rerio larvae per treatment (n = 9, for ES n = 36). In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

For the accumulation of Ga, a significant positive trend was shown (Spearman's rank correlation coefficient; $\rho = 1$, $p < 0.005$). With the Ga exposure going up from treatment 1 to treatment 6, the amount of Ga accumulated by the larvae also increased consistently. This time, a perfect monotonic increase is observed. This is shown in figure 14.

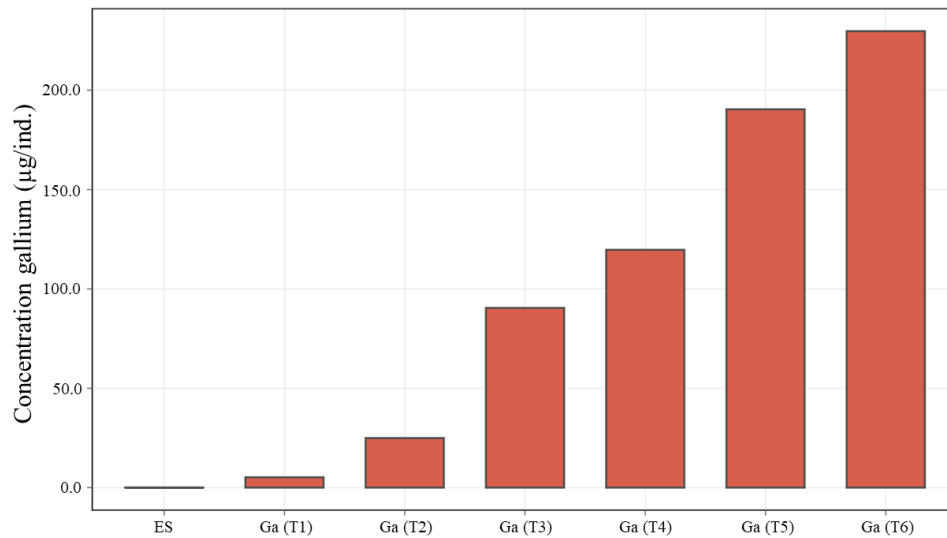


Figure 14: Gallium accumulation in μg per individual of gallium-exposed *D. rerio* larvae per treatment ($n = 9$, for ES $n = 36$). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

When putting the metal accumulation data for Ga on a log scale, a linear dose-response curve becomes visible (figure 15).

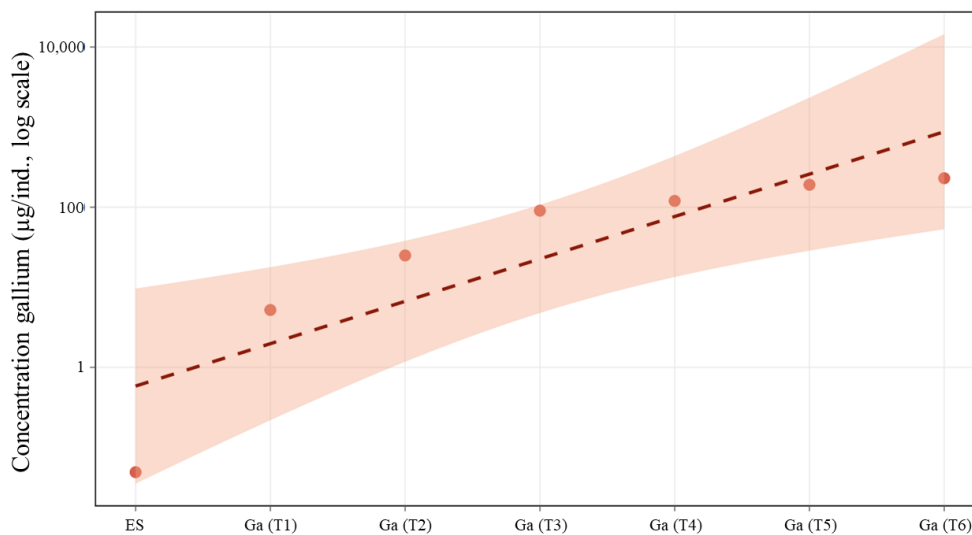


Figure 15: Dose-response curve for gallium accumulation in μg per individual of gallium-exposed *D. rerio* larvae for the different treatments ($n = 9$, for ES $n = 36$). Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

Interestingly, another perfect result was found, but in the opposite direction. As In exposure increased, the Ga measured in these larvae decreased consistently ($\rho = -1$, $p < 0.005$). No Ga

exposure was performed on these individuals, so Ga came purely from background uptake, through either water or contamination. This is shown in figure 16.

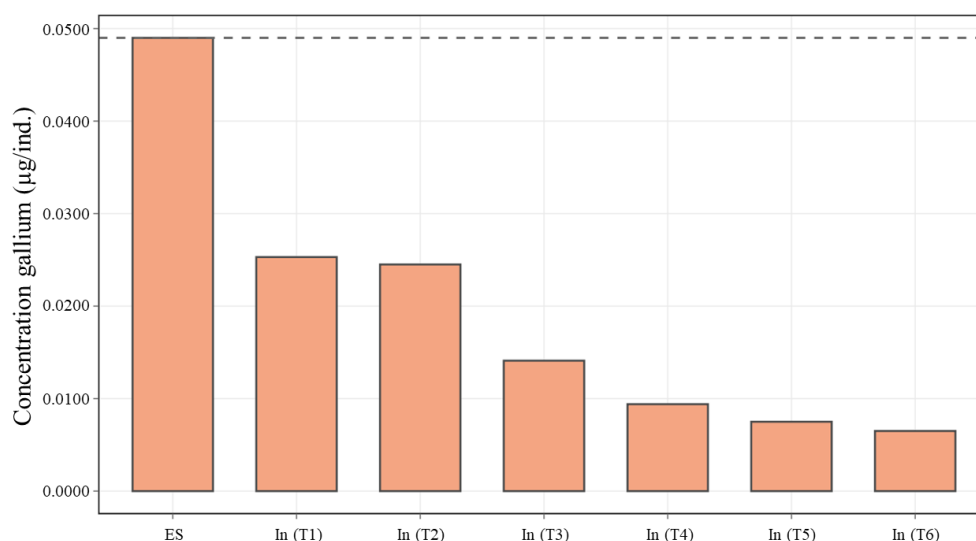
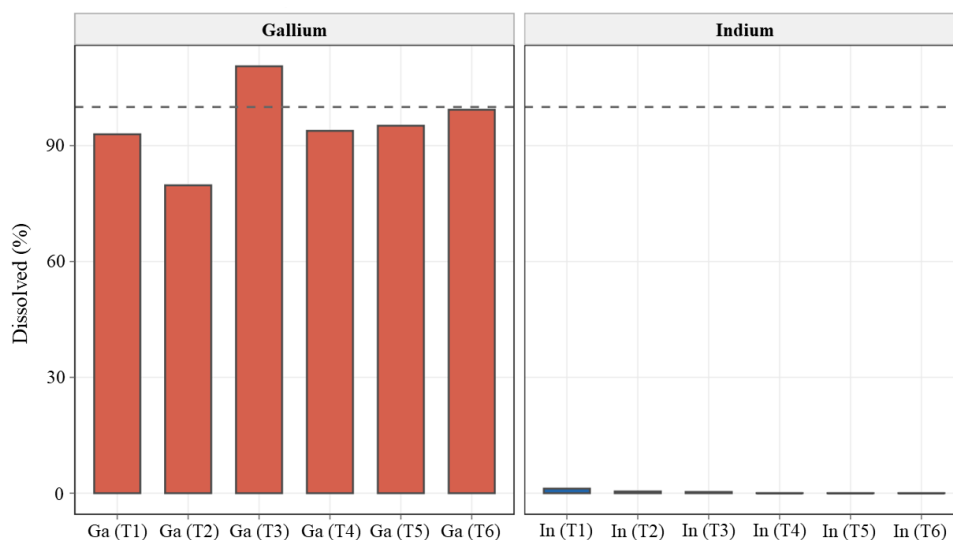


Figure 16: Gallium accumulation in µg per individual of indium-exposed *D. rerio* larvae per treatment ($n = 9$, for ES $n = 36$).

3.2.2.1 Water metal analysis

Both filtered and non-filtered water samples were analysed for their metal content, the results of which are shown in appendix 6. The filtered samples isolate the potentially bioavailable metal ions which can be taken up by *D. rerio*, which is the portion of the metals that is relevant for this study.

The dissolved fraction was calculated by dividing the filtered concentrations by the non-filtered concentrations and multiplied by 100. This value reflects the proportion of the total metal concentration that is truly dissolved and potentially bioavailable for the *D. rerio* larvae. For In, this was always below 2%, while for Ga this was always above 90% (apart from treatment 2, which was approximately 80%). These dissolved fractions are shown in figure 17.



*Figure 17: The dissolved fraction in percentage of the total fraction for all *D. rerio* treatments of both gallium and indium. Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L; In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.*

Spearman's rank correlation coefficients were calculated and showed perfect values for non-filtered In and Ga, and filtered Ga ($\rho = 1$, $p < 0.005$). For filtered In, the value was slightly lower due to treatment 3, but still significant ($\rho = 0.829$, $p = 0.042$). Also the percentages of dissolved In showed a perfect monotonic increase ($\rho = 1$, $p < 0.005$), but the ones for Ga did not ($\rho = 0.600$, $p = 0.208$). Following this, a Wilcoxon signed-rank test was performed to test whether filtered and non-filtered concentrations differed significantly across treatments. For In a significant difference was found, which means a large particulate fraction is present ($V = 21$, $p = 0.036$). For Ga no significant difference could be found, meaning that the metal is

predominantly dissolved ($V = 17$, $p = 0.208$). A comparison of the non-filtered and filtered metal concentrations for both In and Ga can be found in figures 18 and 19.

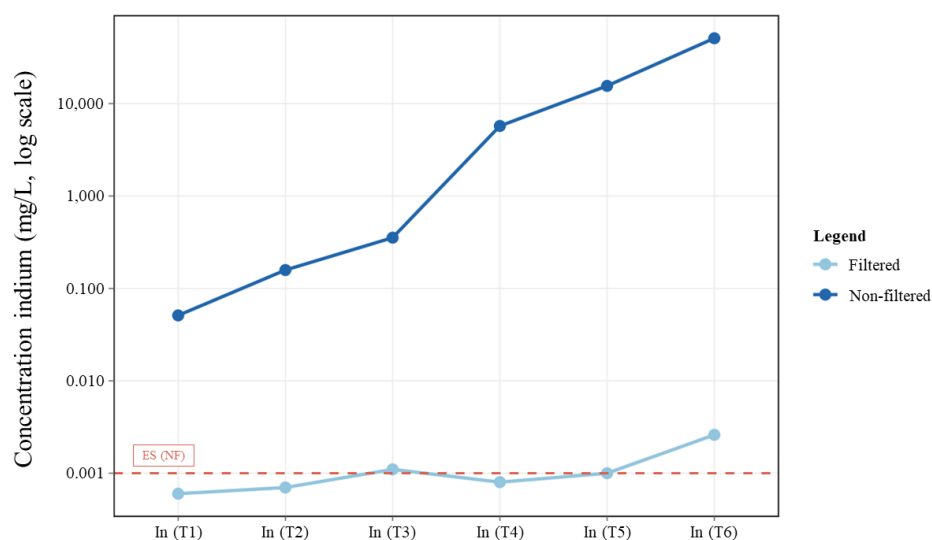


Figure 18: Water concentrations in mg per litre for all indium treatments, showing both the filtered and non-filtered samples, and the background values measured in the non-filtered ES (dashed line) for the *D. rerio* experiment. In (T1-T6): 0.06, 0.6, 1.2, 6.0, 30, and 90 mg In/L.

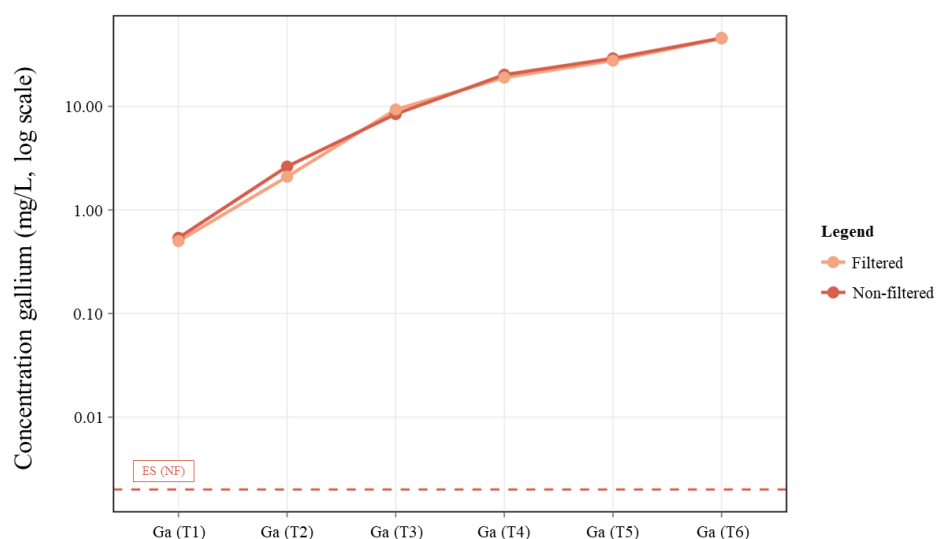


Figure 19: Water concentrations in mg per litre for all gallium treatments, showing both the filtered and non-filtered samples, and the background values measured in the non-filtered ES (dashed line) for the *D. rerio* experiment. Ga (T1-T6): 1.0, 5.0, 20, 40, 60, and 90 mg Ga/L.

3.2.3 Morphological abnormalities

Morphological abnormalities were only observed sporadically, and therefore they were not suitable to detect any differences between treatments. An example of a scoring sheet used at 120 hpf can be found in appendix 7. Pictures of the different stages at which scoring was performed can be found in figure 20.

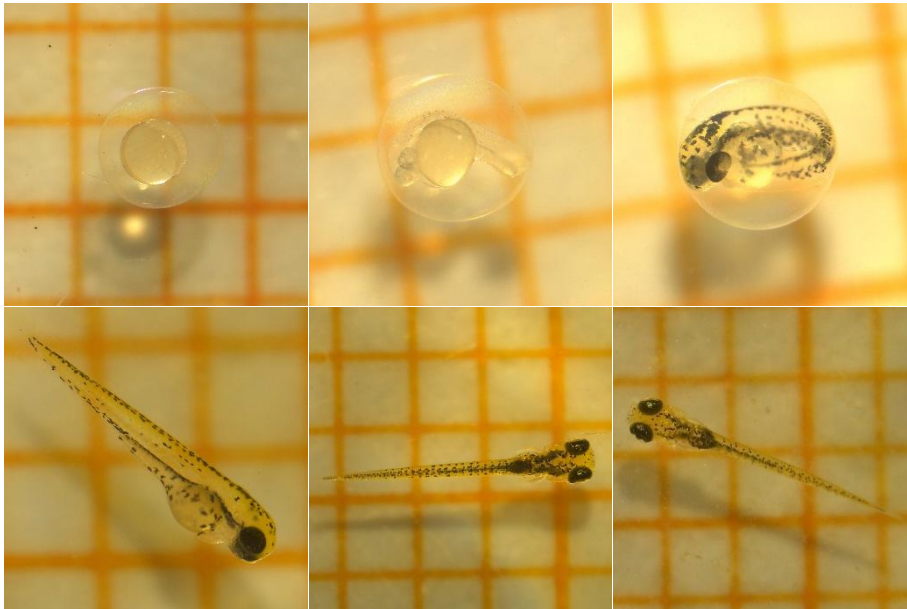


Figure 20: Different stages of D. rerio, photographed at approximately 0, 24, 48, 72, 96, and 120 hpf (left-to-right and top-to-bottom).

4. Discussion

This study investigated the ecotoxicological effects of indium (In) and gallium (Ga) on the sediment-dwelling amphipod *Corophium volutator* and embryo-larval stages of the zebrafish *Danio rerio*.

4.1 *Corophium volutator*

4.1.1 Survival and growth

The reference toxicant test revealed that the used *C. volutator* population was less sensitive to ammonium than reported in the literature, with an estimated LC_{50} of 282 mg NH_4^+ /L compared to the expected 36-220 mg NH_4^+ /L (Schipper et al., 2006). A less sensitive population would be expected to be less responsive to other stressors. This suggests that effects of In and Ga could be different in more sensitive populations.

No statistically significant effect on survival was found between the different treatments. This suggests that neither In nor Ga causes lethality in *C. volutator* under the experimental conditions. This is consistent with the low toxicity reported for both elements in aquatic invertebrates at environmentally relevant concentrations (e.g. Yang (2014) on *Macrobrachium nipponense*). Perhaps a 28-day period of exposure to In and Ga was not enough to assess mortality. Exposure in natural conditions happens over a longer, chronic period of time, and therefore this experiment cannot rule out the possibility of mortality over longer periods of exposure to In and Ga. Bat et al. (1998) observed decreased survival (but increased metal accumulation) of *C. volutator* to other metals (Cu, Zn, and Cd) in the absence of sediment in a 96-hour experiment, so repeating the present experiment without sediment could also provide valuable additional insights.

In absolute and relative growth, no significant differences were observed. On the contrary, the specific growth rate (SGR) showed significant differences, with treatment 3, the highest tested concentration, having a higher SGR compared to treatment 1 and the control treatment. This was an unexpected, counterintuitive result, which might be explained by unidentified indirect effects of In or Ga. They could have somehow induced growth of sediment microalgae or other similar species, which improved the (quality of) food available for *C. volutator* (Liang et al., 2020).

Another potential explanation is the process of hormesis (Calabrese & Baldwin, 2003). This is the biological phenomenon where mild, temporary exposure to a stressor triggers an adaptive response. This can make the organism stronger, more resilient, or enhance reproduction, or in this case promote growth. Higher doses of the stressor are still toxic. A similar response has been observed before in a wide variety of taxonomic groups, including in a similar species (*Gammarus locusta*) as a response to Cu exposure (Correia et al., 2001). While hormesis could not be proven with this study, it shows an interesting hypothesis for future research. The increase of organisms (above twenty) in some containers might also be a result of hormesis, which can stimulate reproduction. Since a period of fourteen days from copulation to the release of young, excluding five days in the brood pouch, was estimated for *C. volutator* by Fish & Mills (1979), it could also be possible that young individuals were unknowingly introduced in some containers at the beginning of the experiment. Lastly, imprecise counting is also a possible explanation. This uncertainty can be addressed in future experiments by only using clearly distinguishable juveniles, or by conducting the experiment in individual containers per organism.

4.1.2 Metal accumulation

Significant differences in accumulation of both In and Ga in both the sediment and the organic tissue of *C. volutator* were found. For each situation, treatment 3 showed significantly higher accumulation compared to the control treatment and treatment 1. This is in line with previous experiments on the same species with different metals (Bat et al., 1998). A concentration-dependent pattern of metal loading is demonstrated, which indicates that *C. volutator* can absorb In and Ga from the surrounding sediment and/or from the overlying water, and that both metals partition strongly to the sediment phase. Since *C. volutator* is a prey species for various predators like birds and fish, the trophic implications are worth considering for future research.

The strong sediment partitioning of In is further supported by the water chemistry data. The concentrations remained below the detection limit of 0.005 µg/L throughout the experiments in all treatments. This indicates that In, once introduced into the experimental system, rapidly and almost completely got adsorbed to sediment particles. This affinity for fine-grained sediment fractions has previously been shown (Ladenberger et al., 2015). Therefore, sediment-based exposure seems to have been the primary route of exposure for *C. volutator*.

A different situation was visible in the case of Ga. A clear concentration gradient across treatments was present in the water samples, with the highest concentrations in treatment 3. However, Ga concentrations in the water samples of the control treatment and treatment 1 at day 1 of the experiment exceeded those of the added water. Mn levels were also elevated compared to the following measurements, suggesting that contamination may have occurred.

Temperature and salinity were some of the water quality parameters that were kept constant over the course of this experiment. It has been shown previously that they can alter the toxicity of other metals like Zn and Ni to *C. volutator*, and therefore this would also be an interesting experiment to perform in the future (Bryant et al., 1985).

The biota-sediment accumulation factors (BSAFs) provided additional insight into the bioavailability of both metals. For both In and Ga, treatment 3 consistently showed the highest mean BSAF values, whereas treatment 1 showed the lowest. The significant treatment effects observed for the BSAFs of both metals indicate that metal accumulation in *C. volutator* is related to the metal concentrations in the sediment. This non-linear pattern may reflect saturation of sediment binding sites at higher exposure concentrations, resulting in an increased freely bioavailable fraction of the metals and consequently greater uptake into organism tissue. Alternatively, the observed pattern may be associated with physiological or behavioural changes in the organisms under elevated metal stress, such as altered feeding activity or metal regulation mechanisms.

Indium showed higher BSAF values compared to Ga across the treatments. This could mean that despite similar sediment partitioning patterns, In is more bioavailable to or less efficiently excreted by *C. volutator* than Ga. A BSAF value above 1, which is the case for treatment 2 and 3 of In, indicates the contaminant is accumulating in the organism at higher levels than in the sediment. This suggests an increased risk of bioaccumulation and toxicity. On the contrary, a value below 1, which is the case in all other treatments, implies the chemical is not meaningfully building up. This is an interesting finding, since sediment concentrations of In were lower in absolute terms, the organism's exposure may have been disproportionately high. The mechanisms underlying the bioaccumulation of these metals in invertebrate tissues are not well characterised in the literature, so this study provides novel data to this largely unexplored field.

4.2 *Danio rerio*

4.2.1 Survival and hatching

Neither survival nor hatching was significantly affected by any of the twelve experimental treatments in *D. rerio* embryos. The absence of significant effects on these fundamental developmental endpoints suggests that In and Ga, at the tested concentrations, do not elicit toxicity to the embryos or larvae, or interfere with the hatching process under the applied exposure conditions. Therefore, the 96-hour LC₅₀ values for *D. rerio* as described by Yang & Chen (2017), namely 12.84 mg/L to 18.35 mg/L for In, with a NOEC of 6 mg/L, and 7.25 mg/L to 11.42 mg/L for Ga, with a NOEC of 4 mg/L, could not be confirmed by the present study. The positive control DCA did perform as expected and caused a mortality above 30% as noted in the OECD guidelines (OECD, 2025). This implies that the used zebrafish embryos were sufficiently sensitive.

Given the sensitivity of *D. rerio* embryos and larvae to a wide range of chemical stressors, a main reason for its widespread use in research, it is notable that no significant effects were observed (Scholz et al., 2008). The absence of significant mortality and hatching delay across all twelve treatments indicates that the tested concentrations were likely below the threshold for disrupting these developmental processes. This cannot be completely confirmed, since the relatively small sample sizes may have affected the results.

Before hatching, the embryos are surrounded by an acellular envelope, the chorion. This chorion could serve as a barrier for some chemicals, which is why in some studies it gets removed prematurely (Henn & Braunbeck, 2010). This was not done in the present study but can be considered for future studies. Apart from that, a larger sample size of at least twenty embryos per treatment, as mentioned in the OECD guidelines, compared to the nine embryos in this study would likely improve the results (OECD, 2025).

4.2.2 Metal accumulation

A significant concentration-dependent accumulation of In in *D. rerio* larvae was demonstrated following the 120-hour waterborne exposure. This absorption most likely occurred through the skin and the yolk sac, rather than through the gills, which are not fully developed yet at this point (Rombough, 2002). The metal accumulation in the two treatments with the lowest In concentrations remained below or just above the analytical detection limit, which suggests that

the larvae may detoxify In to some extent at low concentrations. Sequestration by metallothionein-like proteins or storage in insoluble granules are possible explanations for this (Vijver et al., 2004). The non-monotonic step between treatment 3 and treatment 4 (respectively 0.0011 mg/L and 0.0008 mg/L) may reflect biological variability inherent to experiments with a low sample size. In the future, more replicates should be used to confirm or deny the findings from this study. Whether the accumulated In concentrations reported here are sufficient to cause subcellular injury at higher treatment levels should also be further researched, for example by using biomarkers of oxidative stress like superoxide dismutase activity, lipid peroxidation, or protein carbonylation (Zheng et al., 2016).

Gallium concentrations yielded a clear monotonic association, resulting in potential evidence of concentration-dependent bioaccumulation. This was previously already observed in another fish species, *Cyprinus carpio* (Betoulle et al., 2002). Compared to In, Ga accumulated to higher concentrations in *D. rerio* larvae following the 120-hour waterborne exposure. Across the tested concentration range an approximate 44-fold increase in Ga concentrations was found, and the highest concentration compared to the ES control treatment showed an approximate 4,687-fold increase. This difference with In could be explained by the ionic radius of Ga^{3+} (0.62 Å), which is very similar to that of Fe^{3+} (0.645 Å). This enables Ga to mimic Fe in biological systems and facilitates its uptake via Fe transport pathways (Chitambar, 2016). During embryogenesis of *D. rerio*, iron regulation is of high importance for survival of the embryo, so any disturbance caused by Ga could have large consequences (Lv & Liu, 2023).

A noteworthy and unexpected finding was the negative correlation between In exposure and the accumulation of Ga in In-exposed individuals. Since no additional Ga was added here, the detected Ga in these larvae represents solely background uptake from either the water or cross-contamination. The consistent, monotonic decrease of Ga concentrations with increasing In concentrations suggests a competitive inhibition mechanism. Potentially In^{3+} interferes with the cellular uptake of Ga^{3+} , for example by competing for the same membrane transporters. Since they have both been shown to interact with the Fe-carrier protein transferrin in humans, this could be a possible explanation (Harris & Messori, 2002). Another possible explanation is that In exposure induces a general downregulation of metal uptake transporters as a protective cellular response, which would also reduce Ga uptake. Which of these processes is happening, if any, requires transporter-specific gene expression analyses by qPCR, which falls beyond the scope of this study. Regardless of the precise mechanism, this finding highlights the need for

mixture toxicity studies, involving all the metals released by OWFs, to look for potential synergistic, antagonistic, or additive effects. This was recently done on a small scale by Majid et al. (2025) with Cd and Cu.

Apart from the larger sample size already mentioned in section 4.2.1, another recommendation for future research is to not pool all samples of one treatment for metal analysis. This way, statistical analyses like Kruskal-Wallis tests or one-way ANOVAs are not possible. With larger sample sizes, more (pooled) samples of the same treatment can provide more ICP-MS data to use in statistical analyses. Additionally, it should be noted that values below the analytical limit of detection (LOD; $< 0.0005 \mu\text{g}$) were substituted with LOD/2 ($0.00025 \mu\text{g}$) for statistical purposes, which is often done with left-censored data (e.g. Bergman et al., 2020; Ahmadi et al., 2021).

Alongside the measurements of metal concentrations in organic tissue, also the metal concentrations in water were measured. Both non-filtered (total recoverable metals) and filtered (bioavailable metals) exposure water samples were analysed from each treatment. Interestingly, In was found to be predominantly particulate or colloidal in solution. The dissolved fraction of In stayed below 2% throughout the different treatments, meaning the larvae were exposed to a nearly constant level of In (approximately 0.0006 to 0.0026 mg/L) regardless of the nominal concentration, which explains why the accumulation of In in the *D. rerio* larvae remained low across treatments. This difference between filtered and non-filtered concentrations was found to be significant. A similar pattern was found in previous research on a mining-influenced stream (White et al., 2016). This paper noted that the non-bioavailable InOH_3 , which gets removed in the filtered samples, tends to be the dominant chemical form of In. This is confirmed by decades of research on hydrolysis of In^{3+} (Moeller, 1941). Whether particulate In can contribute to accumulation in the organic tissue, for example by ingestion of these particles during feeding, cannot be determined with the present data but can be an interesting topic for future research.

Gallium on the other hand was shown to be predominantly dissolved. The dissolved Ga fraction stayed above 80%, and apart from one treatment even above 90%, throughout the different treatments. No significant differences between filtered and non-filtered concentrations were found. Therefore, the larvae were exposed to increasing, bioavailable levels of Ga, as intended. This explains the perfect dose-response in larval Ga accumulation. Although Ga^{3+} , just like In^{3+} , undergoes hydrolysis at the pH used in these experiments, it appears to remain more

bioavailable for the larvae. This may be related to the previously mentioned Fe mimicry in which Ga is involved (Chitambar, 2016).

4.2.3 Morphological abnormalities

Morphological abnormalities were observed only sporadically across the different treatments. They did not occur with sufficient frequency or consistency to allow statistical analyses. The observed abnormalities can therefore not be attributed to In or Ga exposure. It is assumed that they rather fall within the natural background incidence of developmental variation that is reported in unexposed populations of *D. rerio* (OECD, 2025).

4.3 Comparative evaluation and ecological relevance

The two model organisms used in this study represent ecologically contrasting exposure scenarios. *C. volutator* is a sediment-dwelling, deposit-feeding amphipod that is chronically exposed to contaminants associated with sediment particles. This makes this species an appropriate surrogate for benthic invertebrates in estuarine and coastal environments. *D. rerio* embryos and larvae are freshwater organisms, routinely used as an ecotoxicological model. The combined use of both organisms provides a multi-compartment perspective: benthic exposure via sediment for *C. volutator* and water-column exposure for *D. rerio*.

Ebeling et al. (2025) mentioned Ga concentrations between $1.37 \text{ ng/L} \pm 0.05 \text{ ng/L}$ and $4.9 \text{ ng/L} \pm 0.5 \text{ ng/L}$ in marine waters surrounding OWFs in the German Bight. Compared to natural Ga concentrations in North Atlantic surface ocean water (1.7 ng/L to 2.1 ng/L ; Kremling et al., 1999), only a slight increase is noted, probably due to natural variability. The In concentrations ranged between $0.011 \text{ ng/L} \pm 0.001 \text{ ng/L}$ and $0.27 \text{ ng/L} \pm 0.15 \text{ ng/L}$. Compared to natural In concentrations in North Atlantic surface ocean water (0.3 ng/L ; Kremling et al., 1999) a slight decrease is noted, probably due to natural variability. The values on which the concentrations used in this thesis were based are slightly higher, probably because they come from freshwater systems (below $0.17 \text{ } \mu\text{g Ga/L}$ and below $0.015 \text{ } \mu\text{g In/L}$; Salminen et al., 2005). Nonetheless, the filtered, and therefore assumed bioavailable, concentrations for the *D. rerio* experiment were well above these current environmental levels (between 0.5 and 45.2 mg Ga/L ; between 0.6 and $2.6 \text{ } \mu\text{g In/L}$). Given that no major effects were observed at these concentrations, In and Ga do not seem to pose an immediate risk in the water.

An earlier study by Ebeling et al. (2023) looked at the mass fractions of In and Ga in sediments in the same region. For Ga, these ranged between $8.6 \text{ mg/kg} \pm 0.5 \text{ mg/kg}$ and $27 \text{ mg/kg} \pm 14 \text{ mg/kg}$. This was close to the range of natural North Sea sediment mass fractions ($14 \text{ mg/kg} \pm 1 \text{ mg/kg}$ to $23 \text{ mg/kg} \pm 5 \text{ mg/kg}$; Klein et al., 2022). For the In mass fractions, values between $65 \text{ } \mu\text{g/kg} \pm 9 \text{ } \mu\text{g/kg}$ and $270 \text{ } \mu\text{g/kg} \pm 30 \text{ } \mu\text{g/kg}$ were noted. These values were also close to the natural North Sea sediment mass fractions ($73 \text{ } \mu\text{g/kg} \pm 4 \text{ } \mu\text{g/kg}$ to $237 \text{ } \mu\text{g/kg} \pm 13 \text{ } \mu\text{g/kg}$; Klein et al., 2022). The values in the spiked sediment of the present experiment ranged from $1.03 \text{ mg/kg} \pm 0.260 \text{ mg/kg}$ to $9.41 \text{ mg/kg} \pm 3.84 \text{ mg/kg}$ for Ga, and from $25 \text{ } \mu\text{g/kg} \pm 14 \text{ } \mu\text{g/kg}$ to $746 \text{ } \mu\text{g/kg} \pm 328 \text{ } \mu\text{g/kg}$ for In. These are roughly in the same order of magnitude as the current natural concentrations. No major negative effects were observed, but in future studies the effects of higher sediment concentrations should also be researched.

Based on the results from this study, the primary concern for OWF emissions of In and Ga lies in the sediment rather than in the water column, and therefore benthic organisms living in the vicinity of OWFs seem to be at greater risk of metal accumulation than pelagic species.

5. Conclusion

This study provides one of the first systematic ecotoxicological assessment of indium (In) and gallium (Ga) in two taxonomically and ecologically distinct aquatic organisms, addressing critical knowledge gaps in the environmental risk assessment of OWF emissions.

Neither *Corophium volutator* nor *Danio rerio* showed significant mortality or developmental impairment at the tested exposure concentrations, suggesting that In and Ga do not pose acute lethal risks under the conditions studied. However, the absence of mortality may not imply ecological safety. The most significant finding across both organisms was concentration-dependent bioaccumulation of both metals, in both *C. volutator* tissue and associated sediment, and *D. rerio* larvae.

The behaviour of the two metals differed between exposure media. In the *C. volutator* experiment, In partitioned almost completely to the sediment, rendering it undetectable in the overlying water across all treatments, while Ga maintained a measurable dissolved fraction. In the *D. rerio* experiment, this speciation contrast was even more pronounced: In was largely particulate in solution (less than 2% dissolved), whereas Ga remained predominantly bioavailable (more than 80% dissolved). These differences in aqueous speciation were reflected in the accumulation patterns observed in the larvae. This shows that total nominal concentrations are poor proxies for actual biological exposure.

The biota-sediment accumulation factors for In were consistently higher than those for Ga, indicating that In is disproportionately bioavailable to *C. volutator* relative to its sediment concentration. This indicates that benthic invertebrates in OWF-impacted areas may face greater risks of metal accumulation than pelagic species.

A noteworthy finding was the negative correlation between In exposure and Ga accumulation in *D. rerio* larvae. This could imply competitive inhibition of shared uptake pathways, possibly mediated by iron transport proteins such as transferrin. This finding showed the importance of mixture toxicity analyses when assessing OWF-related chemical emissions.

The elevated specific growth rate observed in *C. volutator* at the highest concentration tested, while counterintuitive, could be a sign of hormesis, previously documented in related crustaceans, and warrants further investigation.

Several methodological limitations constrain the strength of the conclusions. The reference toxicant test indicated that the *C. volutator* population used was less sensitive than literature benchmarks, which may have influenced the results. The small sample sizes in the *D. rerio* experiment (n = 9 per treatment) might have reduced the statistical power, and pooling of fish samples for metal analysis prohibited most statistical analyses. Future studies should increase replication, use dechorionated embryos to remove a potential barrier, and extend exposure durations to assess chronic, sublethal endpoints such as oxidative stress biomarkers and reproductive output.

Despite evidence of measurable accumulation in aquatic organisms, neither In nor Ga is currently regulated under the EU Water Framework Directive or the EU Marine Strategy Framework Directive. This study supports the future inclusion of sediment quality monitoring, specifically for In and Ga, in environmental management programmes for OWFs.

6. Bibliography

1. Ahmadi, H., Granger, D. A., Hamilton, K. R., Blair, C., & Riis, J. L. (2021). Censored data considerations and analytical approaches for salivary bioscience data. *Psychoneuroendocrinology*, 129, 105274. <https://doi.org/10.1016/j.psyneuen.2021.105274>
2. Barnes, R. S. K. (1994). The Brackish-Water Fauna of Northwestern Europe. *Cambridge University Press*, pp. 176-177. <https://doi.org/10.1002/aqc.3270050208>
3. Bat, L., & Raffaelli, D. (1998). Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. *Journal of Experimental Marine Biology and Ecology*, 226(2), 217–239. [https://doi.org/10.1016/s0022-0981\(97\)00249-9](https://doi.org/10.1016/s0022-0981(97)00249-9)
4. Batley, G. E., & Campbell, P. G. C. (2022). Metal contaminants of emerging concern in aquatic systems. *Environmental Chemistry*, 19(1), 23–40. <https://doi.org/10.1071/en22030>
5. Bell, A. M., Von Der Au, M., Regnery, J., Schmid, M., Meermann, B., Reifferscheid, G., Ternes, T., & Buchinger, S. (2020). Does galvanic cathodic protection by aluminum anodes impact marine organisms? *Environmental Sciences Europe*, 32(1). <https://doi.org/10.1186/s12302-020-00441-3>
6. Bergman, J., Svenningsson, A., Liv, P., Bergenheim, T., & Burman, J. (2020). Location matters: highly divergent protein levels in samples from different CNS compartments in a clinical trial of rituximab for progressive MS. *Fluids and Barriers of the CNS*, 17(1), 49. <https://doi.org/10.1186/s12987-020-00205-4>
7. Betoulle, S., Etienne, J., & Vernet, G. (2002). In vivo and in vitro modulation of carp (*Cyprinus carpio* L.) phagocyte oxidative burst activity by gallium. *Journal of Toxicology and Environmental Health*, 65(8), 603–615. <https://doi.org/10.1080/152873902317349754>
8. Bobba, S., Carrara, S., Huisman, J., Mathieux, F., & Pavel, C. (2020). Critical Raw Materials for Strategic Technologies and Sectors in the EU: A Foresight Study. *European Commission*. <https://doi.org/10.2873/58081>
9. Bower, C.E., & Holm-Hansen, T. (1980). A Salicylate–Hypochlorite method for determining ammonia in seawater. *Canadian Journal of Fisheries and Aquatic Sciences*, 37(5), 794–798. <https://doi.org/10.1139/f80-106>
10. Brun, N. R., Christen, V., Furrer, G., & Fent, K. (2014). Indium and Indium Tin Oxide Induce Endoplasmic Reticulum Stress and Oxidative Stress in Zebrafish (*Danio rerio*). *Environmental Science & Technology*, 48(19), 11679–11687. <https://doi.org/10.1021/es5034876>
11. Bryant, V., Newbery, D., McLusky, D., & Campbell, R. (1985). Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator*, *Macoma balthica*). *Marine Ecology Progress Series*, 24, 139–153. <https://doi.org/10.3354/meps024139>
12. Butcher, T., & Brown, T. (2014). Gallium. In *Critical Metals Handbook*, Gunn, G. (Ed.). *John Wiley & Sons*. <https://doi.org/10.1002/9781118755341.ch7>
13. Calabrese, E. J., & Baldwin, L. A. (2003). Hormesis: The Dose-Response Revolution. *The Annual Review of Pharmacology and Toxicology*, 43(1), 175–197. <https://doi.org/10.1146/annurev.pharmtox.43.100901.140223>
14. Ciarelli, S., Vonck, W. A., & Van Straalen, N. M. (1997). Reproducibility of spiked-sediment bioassays using the marine benthic amphipod, *Corophium volutator*. *Marine Environmental Research*, 43(4), 329–343. [https://doi.org/10.1016/s0141-1136\(96\)00095-5](https://doi.org/10.1016/s0141-1136(96)00095-5)
15. Chitambar, C. R. (2010). Medical applications and toxicities of gallium compounds. *International Journal of Environmental Research and Public Health*, 7(5), 2337–2361. <https://doi.org/10.3390/ijerph7052337>
16. Chitambar, C. R. (2016). Gallium and its competing roles with iron in biological systems. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*, 1863(8), 2044–2053. <https://doi.org/10.1016/j.bbamcr.2016.04.027>
17. Conradi, M., & Depledge, M. (1999). Effects of zinc on the life-cycle, growth and reproduction of the marine amphipod *Corophium volutator*. *Marine Ecology Progress Series*, 176, 131–138. <https://doi.org/10.3354/meps176131>

18. Correia, A. D., Costa, F. O., Neuparth, T., Diniz, M. E., & Costa, M. H. (2001). Sub-lethal effects of copper spiked sediments on the marine amphipod *Gammarus locusta*: evidence of hormesis. *Ecotoxicology and Environmental Restoration*, 4(2), 32–38. https://www.researchgate.net/publication/236620372_Sub-lethal_Effects_of_Copper-Spiked_Sediments_on_the_Marine_Amphipod_Gammarus_locusta_Evidence_of_Hormesis
19. De Backer, A., Van Ael, E., Vincx, M., & Degraer, S. (2009). Behaviour and time allocation of the mud shrimp, *Corophium volutator*, during the tidal cycle: a laboratory study. *Helgoland Marine Research*, 64(1), 63–67. <https://doi.org/10.1007/s10152-009-0167-6>
20. De Borger, E., Tiano, J., Braeckman, U., Ysebaert, T., & Soetaert, K. (2020). Biological and biogeochemical methods for estimating bioirrigation: a case study in the Oosterschelde estuary. *Biogeosciences*, 17(6), 1701–1715. <https://doi.org/10.5194/bg-17-1701-2020>
21. Degraer, S., Brabant, R., Rumes, B., & Vigin, L. (2022). Environmental Impacts of Offshore Wind Farms in the Belgian Part of the North Sea: Getting ready for offshore wind farm expansion in the North Sea. *Memoirs on the Marine Environment. Royal Belgian Institute of Natural Sciences*. https://odnature.naturalsciences.be/downloads/mumm/windfarms/winmon_report_2022_final.pdf
22. Ebeling, A., Wippermann, D., Zimmermann, T., Klein, O., Kirchgeorg, T., Weinberg, I., Hasenbein, S., Plaß, A., & Präfrock, D. (2023). Investigation of potential metal emissions from galvanic anodes in offshore wind farms into North Sea sediments. *Marine Pollution Bulletin*, 194, 115396. <https://doi.org/10.1016/j.marpolbul.2023.115396>
23. Ebeling, A., Wippermann, D., Zimmermann, T., Klein, O., Kirchgeorg, T., Weinberg, I., Plass, A., Hasenbein, S., & Präfrock, D. (2025). Coupling metal concentrations and drift simulations for tracing emissions from offshore wind farms. *Marine Pollution Bulletin*, 214, 117810. <https://doi.org/10.1016/j.marpolbul.2025.117810>
24. Elizalde-Velázquez, G. A., & Herrera-Vázquez, S. E. (2023). Zebrafish as Model Organism in Aquatic Ecotoxicology: Current trends and future perspectives. In *Zebrafish Research - An Ever-Expanding Experimental Model*, Disner, G. E. (Ed.). *IntechOpen*. <https://doi.org/10.5772/intechopen.1002731>
25. Eriksson, S. P., & Weeks, J. M. (1994). Effects of copper and hypoxia on two populations of the benthic amphipod *Corophium volutator* (Pallas). *Aquatic Toxicology*, 29(1–2), 73–81. [https://doi.org/10.1016/0166-445x\(94\)90049-3](https://doi.org/10.1016/0166-445x(94)90049-3)
26. Farrell, E. M., Beermann, J., Neumann, A., & Wrede, A. (2022). The interplay of temperature and algal enrichment intensifies bioturbation of the intertidal amphipod *Corophium volutator*. *Journal of Experimental Marine Biology and Ecology*, 559, 151837. <https://doi.org/10.1016/j.jembe.2022.151837>
27. Fasmina, M., Jayamanne, S. C., Liyanage, N. P., Perera, J. & De Silva, D. (2021). Determination of the Toxicity Cause by Trace Metals on Zebrafish (*Danio rerio*) Embryo. *Journal of Aquaculture and Fish Health*, 10(2), 176. <https://doi.org/10.20473/jafh.v10i2.21496>
28. Fish, J. D., & Mills, A. (1979). The reproductive biology of *Corophium volutator* and *C. arenarium* (Crustacea: Amphipoda). *Journal of the Marine Biological Association of the United Kingdom*, 59(2), 355–368. <https://doi.org/10.1017/s002531540004265x>
29. Fox, M., Ohlsson, C., Sharpe, A. D., & Brown, R. J. (2014). The use of a *Corophium volutator* chronic sediment study to support the risk assessment of medetomidine for marine environments. *Environmental Toxicology and Chemistry*, 33(4), 937–942. <https://doi.org/10.1002/etc.2515>
30. Harris, W. R., & Messori, L. (2002). A comparative study of aluminum(III), gallium(III), indium(III), and thallium(III) binding to human serum transferrin. *Coordination Chemistry Reviews*, 228(2), 237–262. [https://doi.org/10.1016/s0010-8545\(02\)00037-1](https://doi.org/10.1016/s0010-8545(02)00037-1)
31. Haynes, W. (2013). Holm's Method. In *Encyclopedia of Systems Biology*, Dubitzky, W., Wolkenhauer, O., Cho, K. H., & Yokota, H. (Ed.). *Springer Nature*. https://doi.org/10.1007/978-1-4419-9863-7_1214
32. Held, A., Kramer, G.N., Lamberty, A., Robouch, P., & Wätjen, U. (2006). The certification of the mass fractions of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Sb, Sc, Se, Sn, Th, Tl, U, V and Zn in three sediments: BCR-277R estuarine sediment,

BCR-280R lake sediment and BCR-320R channel sediment. *European Commission; Joint Research Centre; Institute for Reference Materials and Measurements*. <https://doi.org/10.2787/16519>

33. Hengstmann, E., Corella, P. Z., Alter, K., Belzunce-Segarra, M. J., Booth, A. M., Castro-Jiménez, J., Czermer, N., De Cauwer, K., Deviller, G., Gomiero, A., Goseberg, N., Hasenbein, S., Kirchgeorg, T., Mason, C., Pape, W., Parmentier, K., Plaß, A., Pröfrock, D., Sarhadi, A., Vanavermaete, D., van der Molen, J., Vinagre, P. A., Wood, D., Weinberg, I., Windt, C., Zonderman, A., Kenyon, J., & De Witte, B. (2025). Chemical emissions from offshore wind farms: From identification to challenges in impact assessment and regulation. *Marine Pollution Bulletin*, 215, 117915. <https://doi.org/10.1016/j.marpolbul.2025.117915>
34. Henn, K., & Braunbeck, T. (2010). Dechoriation as a tool to improve the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology*, 153(1), 91–98. <https://doi.org/10.1016/j.cbpc.2010.09.003>
35. Hirata, S., Toshimitsu, H., & Aihara, M. (2006). Determination of arsenic species in marine samples by HPLC-ICP-MS. *Analytical Sciences*, 22(1), 39–43. <https://doi.org/10.2116/analsci.22.39>
36. Ikhuazugbe, I. H., Ofoka, E. A., Odofin, O. L., Erumiseli, O., Edoka, O. E., Ezennubia, K. P., Ogbunike, P. M., Annan, E., Okonkwo, S. S., & Dike, J. C. (2025). Antibacterial activity and mechanistic insights of gallium-based nanoparticles: an emerging frontier in metal-based antimicrobials. *RSC Advances*, 15(38), 31122–31153. <https://doi.org/10.1039/d5ra04216j>
37. Jiang, W., Lin, S., Chang, C. H., Ji, Z., Sun, B., Wang, X., Li, R., Pon, N., Xia, T., & Nel, A. E. (2015). Implications of the Differential Toxicological Effects of III–V Ionic and Particulate Materials for Hazard Assessment of Semiconductor Slurries. *ACS Nano*, 9(12), 12011–12025. <https://doi.org/10.1021/acs.nano.5b04847>
38. Kaldellis, J., & Apostolou, D. (2017). Life cycle energy and carbon footprint of offshore wind energy. Comparison with onshore counterpart. *Renewable Energy*, 108, 72–84. <https://doi.org/10.1016/j.renene.2017.02.039>
39. Kater, B. J., Hannewijk, A., Postma, J. F., & Dubbeldam, M. (2000). Seasonal changes in acute toxicity of cadmium to amphipod *Corophium volutator*. *Environmental Toxicology and Chemistry*, 19(12), 3032–3035. <https://doi.org/10.1002/etc.5620191224>
40. Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Developmental Dynamics*, 203(3), 253–310. <https://doi.org/10.1002/aja.1002030302>
41. Kirchgeorg, T., Weinberg, I., Hörnig, M., Baier, R., Schmid, M., & Brockmeyer, B. (2018). Emissions from corrosion protection systems of offshore wind farms: Evaluation of the potential impact on the marine environment. *Marine Pollution Bulletin*, 136, 257–268. <https://doi.org/10.1016/j.marpolbul.2018.08.058>
42. Klein, O., Zimmermann, T., Ebeling, A., Kruse, M., Kirchgeorg, T., & Pröfrock, D. (2022). Occurrence and Temporal Variation of Technology-Critical Elements in North Sea Sediments—A determination of preliminary reference values. *Archives of Environmental Contamination and Toxicology*, 82(4), 481–492. <https://doi.org/10.1007/s00244-022-00929-4>
43. Kremling, K., Andreae, M.O., Brüggemann, L., van den Berg, C.M.G., Prange, A., Schirmacher, M., Koroleff, E., Kremling, K., & Kus, J. (1999). Determination of trace elements. In *Methods of Seawater Analysis*, Grasshoff, K., Kremling, K., & Ehrhardt, M. (Ed.). Wiley. <https://doi.org/10.1002/9783527613984.ch12>
44. Ladenberger, A., Demetriades, A., Reimann, C., Birke, M., Sadeghi, M., Uhlbäck, J., Andersson, M., & Jonsson, E. (2015). GEMAS: Indium in agricultural and grazing land soil of Europe — Its source and geochemical distribution patterns. *Journal of Geochemical Exploration*, 154, 61–80. <https://doi.org/10.1016/j.gexplo.2014.11.020>
45. Le, P. T. T., & Boyd, C. E. (2012). Comparison of phenate and salicylate methods for determination of total ammonia nitrogen in freshwater and saline water. *Journal of the World Aquaculture Society*, 43(6), 885–889. <https://doi.org/10.1111/j.1749-7345.2012.00616.x>
46. Lee, H., Morrison, C., Doriean, N. J., Welsh, D. T., & Bennett, W. W. (2024). Trace metal distribution in seagrass-vegetated sediments of an urbanized estuary in Queensland, Australia. *Marine Pollution Bulletin*, 208, 116981. <https://doi.org/10.1016/j.marpolbul.2024.116981>

47. Lewis, E., & Perkin, R. (1981). The practical salinity scale 1978: conversion of existing data. *Deep Sea Research Part A. Oceanographic Research Papers*, 28(4), 307–328. [https://doi.org/10.1016/0198-0149\(81\)90002-9](https://doi.org/10.1016/0198-0149(81)90002-9)
48. Liang, S. X. T., Wong, L. S., Dhanapal, A. C. T. A., & Djearamane, S. (2020). Toxicity of metals and metallic nanoparticles on nutritional properties of microalgae. *Water Air & Soil Pollution*, 231(2). <https://doi.org/10.1007/s11270-020-4413-5>
49. Lv, P., & Liu, F. (2023). Heme-deficient primitive red blood cells induce HSPC ferroptosis by altering iron homeostasis during zebrafish embryogenesis. *Development*, 150(20). <https://doi.org/10.1242/dev.201690>
50. Majid, S., Smeets, K., Vergauwen, L., Pilehvar, A., Knapen, D., & Blust, R. (2025). Insights into the combined toxicity of copper and cadmium in zebrafish (*Danio rerio*) embryos and adults. *Ecotoxicology and Environmental Safety*, 299, 118368. <https://doi.org/10.1016/j.ecoenv.2025.118368>
51. Manna, S., & Firdous, S. M. (2025). Unravelling the developmental toxicity of heavy metals using zebrafish as a model: a narrative review. *BioMetals*, 38(2), 419–463. <https://doi.org/10.1007/s10534-025-00671-z>
52. Meadows, P. S., & Reid, A. (1966). The behaviour of *Corophium volutator* (Crustacea: Amphipoda). *Journal of Zoology*, 150(4), 387–399. <https://doi.org/10.1111/j.1469-7998.1966.tb03013.x>
53. Mejías, O., Poulet, T., Behnoudfar, P., Jackson, L., Freeman, J., Jones, T. R., Howard, D. L., & Parbhakar-Fox, A. (2025). Fate, distribution, and transport dynamics of indium in a polymetallic mine waste environment: An integrated mineralogical characterisation and geochemical modelling study. *The Science of the Total Environment*, 998, 180268. <https://doi.org/10.1016/j.scitotenv.2025.180268>
54. Moeller, T. (1941). Contributions to the Chemistry of Indium. II. The hydrolysis of indium trichloride solutions. *Journal of the American Chemical Society*, 63(5), 1206–1207. <https://doi.org/10.1021/ja01850a013>
55. Nguyen, C. H., Field, J. A., & Sierra-Alvarez, R. (2019). Microbial toxicity of gallium- and indium-based oxide and arsenide nanoparticles. *Journal of Environmental Science and Health Part A*, 55(2), 168–178. <https://doi.org/10.1080/10934529.2019.1676065>
56. Nielsen, M., & Kofoed, L. (1982). Selective Feeding and Epipsammic Browsing by the Deposit-Feeding Amphipod *Corophium volutator*. *Marine Ecology Progress Series*, 10, 81–88. <https://doi.org/10.3354/meps010081>
57. OECD (2025). Test No. 236: Fish Embryo Acute Toxicity (FET) Test. In *OECD guidelines for the testing of chemicals. Section 2: Effects on biotic systems*. <https://doi.org/10.1787/9789264203709-en>
58. Olivares, C. I., Field, J. A., Simonich, M., Tanguay, R. L., & Sierra-Alvarez, R. (2016). Arsenic (III, V), indium (III), and gallium (III) toxicity to zebrafish embryos using a high-throughput multi-endpoint in vivo developmental and behavioral assay. *Chemosphere*, 148, 361–368. <https://doi.org/10.1016/j.chemosphere.2016.01.050>
59. Peters, C., & Ahlf, W. (2005). Reproduction of the estuarine and marine amphipod *Corophium volutator* (Pallas) in laboratory for toxicity testing. *Chemosphere*, 59(4), 525–536. <https://doi.org/10.1016/j.chemosphere.2005.01.053>
60. Porteus, C. S., Waples, E., Dempsey, A., Paull, G., & Wilson, R. W. (2024). A survey of water chemistry used in zebrafish facilities and their effects on early zebrafish development. *F1000Research*, 13, 168. <https://doi.org/10.12688/f1000research.134520.2>
61. Posit Team (2026). RStudio: Integrated Development Environment for R. *Posit Software*. <http://www.posit.co>
62. R Core Team (2024). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*. <https://www.R-project.org>
63. Radowitz, B. (2020). Dipping into lakes: why offshore wind has moved into inland waters. *Recharge*. <https://www.rechargenews.com/wind/dipping-into-lakes-why-offshore-wind-has-moved-into-inland-waters/2-1-907526>
64. Reese, A., Voigt, N., Zimmermann, T., Irrgeher, J., & Pröfrock, D. (2020). Characterization of alloying components in galvanic anodes as potential environmental tracers for heavy metal emissions from offshore wind structures. *Chemosphere*, 257, 127182. <https://doi.org/10.1016/j.chemosphere.2020.127182>
65. Ringering, K., Kouhail, Y., Yecheskel, Y., Dror, I., & Berkowitz, B. (2018). Mobility and retention of indium and gallium in saturated porous media. *Journal of Hazardous Materials*, 363, 394–400. <https://doi.org/10.1016/j.jhazmat.2018.09.079>

66. Rombough, P. (2002). Gills are needed for ionoregulation before they are needed for O₂ uptake in developing zebrafish, *Danio rerio*. *Journal of Experimental Biology*, 205(12), 1787–1794. <https://doi.org/10.1242/jeb.205.12.1787>
67. Rueda-Bayona, J. G., Eras, J. J. C., & Chaparro, T. R. (2022). Impacts generated by the materials used in offshore wind technology on Human Health, Natural Environment and Resources. *Energy*, 261, 125223. <https://doi.org/10.1016/j.energy.2022.125223>
68. Salminen, R., Batista, M. J., Bidovec, M., Demetriades, A., De Vivo, B., De Vos, W., Duris, M., Gilucis, A., Gregorauskiene, V., Halamic, J., Heitzmann, P., Lima, A., Jordan, G., Klaver, G., Klein, P., Lis, J., Locutura, J., Marsina, K., Mazreku, A., O'Connor, P. J., Olsson, S. Å., Ottesen, R.-T., Petersell, V., Plant, J. A., Reeder, S., Salpeteur, I., Sandström, H., Siewers, U., Steenfelt, A. & Tarvainen, T. (2005). Geochemical Atlas of Europe. Part 1: Background Information, Methodology and maps. *Geological Survey of Finland*. <https://edepot.wur.nl/486313>
69. Satymov, R., Bogdanov, D., & Breyer, C. (2025). Techno-economics of offshore wind power in global resolution. *Applied Energy*, 393, 125980. <https://doi.org/10.1016/j.apenergy.2025.125980>
70. Savenko, A. V., & Savenko, V. S. (2010). Gallium (III) speciation in seawater. *Geochemistry International*, 48(9), 950–952. <https://doi.org/10.1134/s0016702910090090>
71. Scarlett, A., Rowland, S., Canty, M., Smith, E., & Galloway, T. (2007). Method for assessing the chronic toxicity of marine and estuarine sediment-associated contaminants using the amphipod *Corophium volutator*. *Marine Environmental Research*, 63(5), 457–470. <https://doi.org/10.1016/j.marenvres.2006.12.006>
72. Schipper, C. A., Burgess, R. M., & van den Dikkenberg, L. C. (2006). Sediment toxiciteitstest met de slijkgarnaal *Corophium volutator*. *Rijksinstituut voor Kust en Zee*, 6, 18 pp. <https://doi.org/10.13140/RG.2.2.12730.59847>
73. Schiwy, S., Herber, A., Hollert, H., & Brinkmann, M. (2020). New Insights into the Toxicokinetics of 3,4-Dichloroaniline in Early Life Stages of Zebrafish (*Danio rerio*). *Toxics*, 8(1), 16. <https://doi.org/10.3390/toxics8010016>
74. Scholz, S., Fischer, S., Gündel, U., Küster, E., Luckenbach, T., & Voelker, D. (2008). The zebrafish embryo model in environmental risk assessment—applications beyond acute toxicity testing. *Environmental Science and Pollution Research*, 15(5), 394–404. <https://doi.org/10.1007/s11356-008-0018-z>
75. Schulze, Y., Ghiaci, P., Zhao, L., Biver, M., Warringer, J., Filella, M., & Tamás, M. J. (2023). Chemical-genomic profiling identifies genes that protect yeast from aluminium, gallium, and indium toxicity. *Metallomics*, 15(6). <https://doi.org/10.1093/mtomcs/mfad032>
76. Schwarz-Schampera, U. (2014). Indium. In *Critical Metals Handbook*, Gunn, G. (Ed.). John Wiley & Sons. <https://doi.org/10.1002/9781118755341.ch9>
77. Snell, J., Emteborg, H., & Schimmel, H. (2012a). The Certification of the Mass Fractions of Elements in Fish Muscle: Certified Reference Material ERM® - BB422. *European Commission; Joint Research Centre; Institute for Reference Materials and Measurements*. <https://doi.org/10.2787/69685>
78. Snell, J., De Vos, E., Emteborg, H., & Schimmel, H. (2012b). The Certification of the Mass Fractions of Elements in Mussel Tissue: Certified Reference Material ERM® - CE278k. *European Commission; Joint Research Centre; Institute for Reference Materials and Measurements*. <https://doi.org/10.2787/6829>
79. Spitsbergen, J. M., & Kent, M. L. (2003). The State of the Art of the Zebrafish Model for Toxicology and Toxicologic Pathology Research—Advantages and Current Limitations. *Toxicologic Pathology*, 31, 62–87. <https://doi.org/10.1080/01926230390174959>
80. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Proceedings of the Fourth International Symposium on Polarization Phenomena in Nuclear Reactions*, 101, 133–164. https://doi.org/10.1007/978-3-7643-8340-4_6
81. Thain, J., & Roddie, B. (2001). Biological effects of contaminants: *Corophium* sp. sediment bioassay and toxicity test. *ICES Techniques in Marine Environmental Sciences*, 28, 21 pp. <https://doi.org/10.17895/ices.pub.5079>
82. Tsai, P., Wu, S., Chiang, C., Lee, M., Chen, H., Chen, W., Chen, C., Yang, S., Yeh, C., & Kuan, Y. (2020). Evaluation of cytotoxicity, apoptosis, and genotoxicity induced by indium chloride in macrophages through mitochondrial dysfunction

- and reactive oxygen species generation. *Ecotoxicology and Environmental Safety*, 193, 110348. <https://doi.org/10.1016/j.ecoenv.2020.110348>
83. USGS (2026). Mineral commodity summaries 2026. *U.S. Geological Survey*. <https://doi.org/10.3133/mcs2026>
 84. Vijver, M. G., Van Gestel, C. a. M., Lanno, R. P., Van Straalen, N. M., & Peijnenburg, W. J. G. M. (2004). Internal metal sequestration and its ecotoxicological relevance: A review. *Environmental Science & Technology*, 38(18), 4705–4712. <https://doi.org/10.1021/es040354g>
 85. Watson, G. J., Banfield, G., Watson, S. C. L., Beaumont, N. J., & Hodkin, A. (2025). Offshore wind energy: assessing trace element inputs and the risks for co-location of aquaculture. *Npj Ocean Sustainability*, 4(1). <https://doi.org/10.1038/s44183-024-00101-6>
 86. Westerfield, M. (2000). The Zebrafish Book: A guide for the laboratory use of zebrafish (*Danio rerio*). *University of Oregon Press*. https://zfin.org/zf_info/zfbook/zfbk.html
 87. White, S. J. O., Hussain, F. A., Hemond, H. F., Sacco, S. A., Shine, J. P., Runkel, R. L., Walton-Day, K., & Kimball, B. A. (2016). The precipitation of indium at elevated pH in a stream influenced by acid mine drainage. *The Science of the Total Environment*, 574, 1484–1491. <https://doi.org/10.1016/j.scitotenv.2016.08.136>
 88. Yandem, G., & Jabłońska-Czapla, M. (2024). Review of indium, gallium, and germanium as emerging contaminants: occurrence, speciation and evaluation of the potential environmental impact. *Archives of Environmental Protection*, 50(3), 84–99. <https://doi.org/10.24425/aep.2024.151688>
 89. Yang, J. (2014). Comparative acute toxicity of gallium(III), antimony(III), indium(III), cadmium(II), and copper(II) on freshwater swamp shrimp (*Macrobrachium nipponense*). *Biological Research*, 47(1), 13. <https://doi.org/10.1186/0717-6287-47-13>
 90. Yang, J. L., & Chen, L. H. (2017). Toxicity of antimony, gallium, and indium toward a teleost model and a native fish species of semiconductor manufacturing districts of Taiwan. *Journal of Elementology*, 23(1), 191–199. <https://doi.org/10.5601/jelem.2017.22.3.1470>
 91. Zheng, J., Yuan, S., Wu, C., & Li, W. (2016). Chronic waterborne zinc and cadmium exposures induced different responses towards oxidative stress in the liver of zebrafish. *Aquatic Toxicology*, 177, 261–268. <https://doi.org/10.1016/j.aquatox.2016.06.001>

Appendix

Appendix 1: Surviving and dead organisms of C. volutator per experimental unit, including their total weight in grams at the end of the experiment.

Container	Treatment	Surviving	Dead	Total weight (g)
1	C	21	0	0.0297
2	C	21	0	0.0203
3	C	20	0	0.0173
4	C	20	0	0.0193
5	C	17	3	0.0156
6	C	19	1	0.0233
7	C	21	0	0.0220
8	1	26	0	0.0150
9	1	20	2	0.0173
10	1	23	0	0.0254
11	1	28	1	0.0174
12	1	20	0	0.0217
13	1	21	0	0.0200
14	1	21	0	0.0243
15	2	22	0	0.0157
16	2	17	2	0.0136
17	2	17	3	0.0560
18	2	21	0	0.0165
19	2	20	0	0.0229
20	2	21	0	0.0360
21	2	18	2	0.0219
22	3	21	0	0.0172
23	3	22	0	0.0292
24	3	22	0	0.0455
25	3	22	0	0.0427
26	3	20	0	0.0644
27	3	20	0	0.0275
28	3	20	0	0.0846

Appendix 2: The mean natural concentrations and standard deviations of fifteen metals (excluding indium and gallium, since they are already mentioned in the main text) in µg per gram of sediment at the C. volutator sampling site.

Element	Li	Sb	V	Cr	Mn
Mean (µg/g)	5.12 ± 0.437	0.449 ± 0.405	15.0 ± 1.04	17.3 ± 1.49	79.6 ± 5.51
Element	Fe	Co	Ni	Sn	Ag
Mean (µg/g)	8,130 ± 982	2.35 ± 0.348	5.38 ± 0.428	0.613 ± 0.587	0.433 ± 0.278
Element	Cd	Pb	Al	Cu	Zn
Mean (µg/g)	0.0006 ± 0.0000	5.50 ± 0.531	2900 ± 19.3	2.26 ± 0.155	19.5 ± 0.427

Appendix 3: The concentrations of twelve metals (excluding indium and gallium, since they are already mentioned in the main text) in µg per litre in the water samples of the C. volutator experiment, over time in the different treatments.

	Concentration (µg/L)											
	Cd	Pb	Cr	Co	Ni	Zn	As	Ag	Al	Mn	Fe	Cu
Day 1 (C)	0.005	0.600	0.430	0.220	0.510	0.450	95.5	0.005	76.1	129	124	10.3
Day 1 (T1)	0.005	0.360	0.280	0.240	0.460	0.560	48.4	0.005	74.2	135	117	4.95
Day 1 (T2)	0.005	0.400	0.380	0.170	0.490	0.450	97.0	0.005	83.6	136	180	4.19
Day 1 (T3)	0.005	0.650	7.02	0.230	3.420	6.34	76.1	0.005	80.9	137	211	6.08
Day 14 (C)	0.005	3.96	1.34	0.240	0.770	3.34	56.6	0.005	293	5.07	413	2.37
Day 14 (T1)	0.005	1.21	1.73	0.250	0.880	4.31	43.7	0.005	370	5.39	494	2.70
Day 14 (T2)	0.005	1.46	1.71	0.350	1.04	4.51	65.2	0.005	315	5.83	478	5.48
Day 14 (T3)	0.005	1.45	1.64	0.260	1.06	4.33	72.4	0.005	340	6.06	518	3.55
Day 28 (C)	0.005	1.06	1.81	0.160	0.820	28.5	69.3	0.005	216	3.37	341	2.47
Day 28 (T1)	0.005	0.990	1.22	0.140	0.670	4.15	58.8	0.005	250	3.43	359	3.42
Day 28 (T2)	0.005	1.20	1.61	0.060	0.870	4.11	55.7	0.005	253	3.93	378	4.49
Day 28 (T3)	0.005	1.29	1.53	0.070	0.780	4.35	69.0	0.005	174	3.66	353	1.79

Appendix 4: Reference toxicant test results per treatment for C. volutator, showing the surviving and dead organisms per concentration in mg NH₄⁺ per litre.

Treatment	Concentration (mg NH ₄ ⁺ /L)	Surviving	Dead
1	0	39	0
2	30	37	0
3	72	39	1
4	110	40	0
5	180	33	6
6	360	11	29

*Appendix 5: Metal content in μg per individual of *D. rerio*, shown per treatment.*

	In per individual (μg)	Ga per individual (μg)
T1 (In)	< 0.0005	0.025
T2 (In)	0.001	0.025
T3 (In)	0.005	0.014
T4 (In)	0.002	0.009
T5 (In)	0.011	0.008
T6 (In)	0.013	0.007
T1 (Ga)	< 0,0005	5.20
T2 (Ga)	< 0,0005	24.9
T3 (Ga)	< 0,0005	90.4
T4 (Ga)	< 0,0005	120
T5 (Ga)	< 0,0005	190
T6 (Ga)	< 0,0005	230
ES	< 0,0005	0.049

*Appendix 6: Concentrations of indium (left) and gallium (right) in mg per litre, for both non-filtered and filtered water samples per treatment of *D. rerio* larvae.*

	Not filtered (mg/L)	Filtered (mg/L)		Not filtered (mg/L)	Filtered (mg/L)
In (T1)	0.051	0.0006	Ga (T1)	0.538	0.500
In (T2)	0.158	0.0007	Ga (T2)	2.62	2.09
In (T3)	0.354	0.0011	Ga (T3)	8.42	9.31
In (T4)	5.73	0.0008	Ga (T4)	20.2	18.9
In (T5)	15.6	0.0010	Ga (T5)	29.0	27.6
In (T6)	51.0	0.0026	Ga (T6)	45.6	45.2

*Appendix 7: Example of a scoring sheet for *D. rerio* larvae at 120 hpf (indium T6).*

