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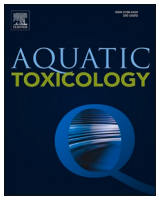

Genotoxicity in Artemia spp.: An old model with new sensitive endpoints

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ABSTRACT

Artemia spp. represent models species widely used in ecotoxicological studies due to its simple and fast manipulation in laboratory conditions that makes this crustacean well adaptable to several methodological approaches. Although cysts hatching, swimming behavior, reproductive success and mortality are the main endpoints used for the determination of toxicity, the detection of slight alterations induced by certain substances found at low concentrations in the environment may require more sensitive biomarkers. For this reason, the identification of DNA or chromosomal damages has been proposed as an additional and appreciable endpoint for the ecotoxicological assessment of environmental chemicals. Concerning *Artemia* models, only few studies indicated that the exposure to organic and inorganic compounds (*i.e.* pesticides, nanoparticles, bacterial products or heavy metals) can reduce the survival and fitness through the onset of DNA breaks or the dysregulation of key genes. In contrast, literature research revealed a lot of works primarily focusing on the mortality and hatching rates of *Artemia* nauplii and cysts despite the well-known low sensitivity of these species.

The present review reports the current state of knowledge concerning the effects induced by various chemicals, including organic and inorganic compounds, on the common parameters and genotoxicity in both *Artemia franciscana* and *Artemia salina*. Advantages and limitations of *Artemia* spp. models in eco-toxicological investigations together with the most used classes of compounds are briefly discussed. Moreover, a mention is also addressed to scarce availability of literature data focusing on genotoxic effects and the great reliability of molecular approaches observed in this poorly sensitive model organism. Thus, the opportunity to take advantage of genotoxic analyses has also been highlighted, by suggesting this approach as a novel endpoint to be used for the eco-toxicological assessment of several stressors.

1. Introduction

The genus *Artemia* shows a wide geographical distribution and groups together six species characterized by sexual reproduction, such as *Artemia franciscana, Artemia persimilis, Artemia salina, Artemia sinica, Artemia tibetiana, Artemia urmiana* plus diverse strains belonging to the same species that reproduce asexually (Abatzopoulos et al., 2002; Asem et al., 2010; Dhont et al., 2013; Maniatsi et al., 2011). Brine shrimps are well adapted to extreme habitats, including hypersaline lakes and ponds that are often subjected to water evaporation and, in turn, characterized by a broad range of temperatures (6-35°C) and high salt concentrations (Gajardo and Beardmore, 2012). Due to its high nutritional value, comprising a huge abundance of highly unsaturated fatty acids (HUFAs), *Artemia* spp. have been often used for aqua- and larvae-culture purposes, particularly with enriched formulations that balance the whole content by adding several micro-nutrients, such as vitamins, sterols, pigments and antioxidants (Bengtson et al., 2018; Cavrois-Rogacki et al., 2020; K. V. et al., 2021; Nafisi Bahabadi et al., 2018; Van Stappen et al., 2020).

Artemia species possess a quite short life-cycle (the development of nauplii into sub-adults occurs within 1–3 weeks) with a great fecundity rate which makes them suitable models for laboratory experiments (Hollergschwandtner et al., 2017; Yu, 2018). Interestingly, depending on the environmental conditions, the reproductive process can follow two completely different paths: i) under favorable environmental conditions the embryos develop directly inside the egg pouch and are released from them directly as nauplii; ii) in contrast, under harsh environmental conditions the oviparous sac produces diapausing eggs which dry up after their release (cysts) (Abatzopoulos et al., 2002). For

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instance, manipulating the surrounding temperature, *Artemia* cysts can be easily induced to hatch in laboratory conditions (Kumar and Babu, 2015). Due to several attractive features such as, high market availability, abundance of cysts, simple manipulation and maintenance in laboratory conditions, the *Artemia* bioassay is one of the most used approaches in (eco-)toxicological studies to study the toxicity of various compounds and media including nanoparticles (Bergami et al., 2016; Bhuvaneshwari et al., 2018; Rajabi et al., 2015), pesticides (Xu et al., 2015), pharmaceuticals (Albendín et al., 2021), heavy metals (Danabas et al., 2020; Frías-Espericueta et al., 2022; Khoshnood et al., 2017; Ñañez Pacheco et al., 2021; Palácio et al., 2021; Venkateswara Rao et al., 2007; Zhu et al., 2017a), toxic algae (Mutalipassi et al., 2022; Neves et al., 2017), and sewages (Borba et al., 2019; Libralato et al., 2007; Svensson et al., 2005), with increasing applications addressed to the toxicity screening of plant or marine natural compounds for drug discovery purposes (Hamrun et al., 2020; Longo et al., 2021; Ntungwe N et al., 2020; Hamidi et al., 2014). *Artemia* acute toxicity tests are performed according to standard methods (CNR, 2003).

Within the *Artemia* genus, a great variety of sensitivity to toxicants has been observed so that a correct taxonomic identification is extremely important.. To this purpose, genetic analyses according to the geographic origin, morphological, reproductive and physiological features, have been applied to investigate the phylogenetic relationships. On the whole, the *Artemia* evolutionary trajectory probably started from some strains of *A. salina* that underwent a reproductive isolation generating the bisexual and parthenogenetic species, including *A. franciscana* (Kappas et al., 2004). Due to several ecological barriers, *A. franciscana* diversified into separated populations that, in turn, lead to development of new species that tolerated different habitats (Triantaphyllidis et al., 1998). In some cases, the geographic distribution of *Artemia* species and habitat-induced isolation of monospecific populations has been investigated by using molecular approaches evaluating the pattern of hypervariable regions (*e. g.* RFLP) (Baxevanis et al., 2014; Beristain et al., 2010).

Concerning ecotoxicological studies, *A. franciscana* was the most employed species due to the presence of clear distinctive features and a good phenotypic plasticity that makes this model well adapted to several ecological contexts (Kappas et al., 2004; Nunes et al., 2006). The classical endpoints commonly include hatching, swimming, mortality, growth and reproduction rates together with several biomarkers reflecting the health state such as, acetylcholinesterase (AChE), heat shock proteins (HSPs), glutathione-peroxidase (GPx), glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH) (Libralato et al., 2016).

Despite the cost-effectiveness and simplicity of using both species for acute and chronic tests, the scientific community is starting to employ alternative models since a low sensitivity and a lack of optimized protocols were evinced (Libralato, 2014). The crucial step is the choice of the specific endpoint used for investigating the effects of certain toxicants, particularly in the case of short-time exposures (Nunes et al., 2006). In fact, an extreme low sensitivity of *Artemia* spp. have been found in acute toxicity tests while, despite a higher sensitivity of long-term exposures in comparison to short-term ones, there is still no standardized methods like International Standard Organization (ISO), American Society for Testing and Materials (ASTM) or Organization for Economic Cooperation and Development (OECD) (Libralato et al., 2016). A good balance has been found in the hatching assay that was proposed as a reliable tool to evaluate toxicity in *Artemia* spp. allowing to achieve consistent results in a very short time and revealing a high sensitivity in the case of short-term tests (Rotini et al., 2015).

In addition to the most common approaches measuring the larval survivalsurvival or hatching success, molecular analyses evaluating the expression levels of genes involved in larval growth, molting, stress and detoxification have been recently applied on *A. franciscana* nauplii (Bergami et al., 2017; Comeche et al., 2017; Yu, 2018; Varó et al., 2019). Interestingly, the stress response of *A. salina* and *A. franciscana* has been also studied through Next Generation Sequences (NGS) approaches (De Vos et al., 2019; Huylmans et al., 2019; Lee et al., 2022; Yi et al., 2020), by making available a huge amount of sequences to be used for novel molecular studies in the future.

Here we review the literature on *A. salina* and *A. franciscana* evaluating the toxicity of several classes of chemical compounds (inorganic and organic) by grouping them on the basis of the endpoint observed. Then, we report a few works investigating the genotoxicity on both species and highlight this endpoint as suitable tool that scientists still not extensively employed, so far. A recent work demonstrated that, although the classical endpoints were not able to reveal a clear evidence of toxicity induced by benzo(k)fluoranthene treatments, gene expression analyses revealed a significant alteration of heat shock proteins (Albarano et al., 2022). These findings have opened the hypothesis that molecular approaches evaluating DNA damage or mRNA levels of key genes involved in stress response or detoxification processes could represent a helpful method to perform ecotoxicological assessments in low sensitive models as *Artemia* spp. Moreover, taking into consideration the need of developing novel suitable endpoints, we aim at revitalizing the role in eco-toxicological investigations of *Artemia* spp. models that in the last years have been almost abandoned. In fact, *A. franciscana* has been little used during the past twenty-two years, particularly when compared to other well-known model organisms as *Paracentrotus lividus* and *Daphnia magna* that greatly contributed to scientific publications in aquatic ecotoxicology (Fig. 1).

1.1. Data collection and information management

The present review examined 115 papers including original ecotoxicological researches embracing data on both *A. franciscana* and *A. salina* from 2000 up to the end of May 2022. Bibliographic search engines were Google Scholar, PubMed, Scopus National Center for Biotechnology Information (NCBI) and Web of Science (WOS). Review papers were not considered. Only studies written in English were considered. The search was performed from 1th of January 2000 to 1th of June 2022. The selected keywords in the search include "organic compounds", "inorganic compounds", "heavy metals", "mortality", "hatching success", "reproduction", "bioaccumulation", "ecotoxicity", "genotoxicity"etc.

Among the investigated papers, the endpoints used to evaluate the ecotoxicity of organic and inorganic compounds included mortality (49 %, n = 86), hatching and swimming rate(16 %, n = 29), reproduction (11 %, n = 19), bioaccumulation (9 %, n = 16), enzymatic activity (12

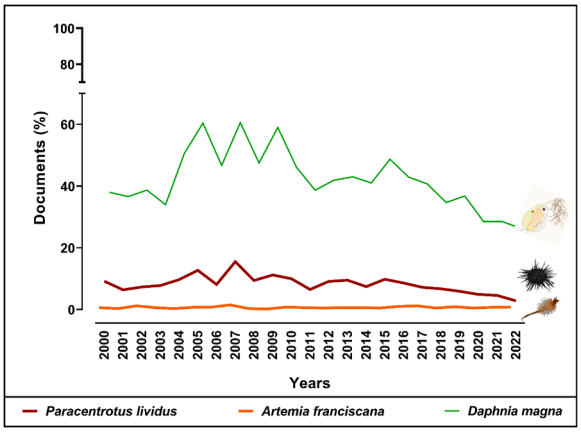

Fig. 1. The trend chart reports the relative abundance of published papers from 1th of January 2000 to 1th of June 2022 using *D. magna* (green line), *A. franciscana* (orange line) and *P. lividus* (red line) as ecotoxicological models. Abundance (%) of papers for each biological model has been calculated on the number of selected papers in the field of aquatic ecotoxicology using the following keywords "organic compounds, inorganic compounds, heavy metals, mortality, reproduction, bioaccumulation, ecotoxicity, genotoxicity etc.".

%, n = 21) and gene expression (3 %, n= 6), (Fig. 2).

The whole literature discussed in the present review paper is listed in Table 1.

2. Endpoints

2.1. Mortality

Mortality rate represents the most used endpoint (Fig. 2) since it provides extremely simple and quick results for evaluating the acute toxicity of the compounds under analysis. In fact, *Artemia* can be easily exposed to a certain stressor and the toxicity data are then achieved in 24 h and 48 h by applying some toxicity parameters such as 50 % Lethal Concentration (LC_{50}), no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC). Among toxicants, several biocides (e.g. pesticides, disinfectants) have been studied since the occurrence in the aquatic environment has been reported worldwide due to their extensive use in agriculture, medicine and industry fields (de Campos et al., 2021). For instance, fungal biocontrol agents, alamethicin (ALA), destruxin A (DA) and elsinochrome A (EA), and the biocides potassium dichromate (PDC) and p-coumaric acid (p-CA) showed a considerable toxicity, while oosporein (OOS) displayed no effects on the crustacean survival at the tested concentrations after both 24 and 36 h of exposure (Favilla et al., 2006; Alyürük and Çavaş, 2013). Comparative studies evaluating theimpact of tributylin (TBT), diuron and irgarol on the survival of *A. salina* nauplii revealed that TBT was the most toxic, with a survival rate that decreased over time (about 73, 48 and 26 % after 12, 24 and 48 h, respectively) (Lee et al., 2017). Other studies reported that antifouling biocides, plus Zinc (ZnPT) and copper pyrithione (CuPT), induced a higher mortality in ZnPT supplied exposures in comparison to CuPT after 24h of treatment (Gutner-Hoch et al., 2019). Mixtures of these latter compounds together with diuron and chlorothalonil at different concentration ratios showed i) synergistic effects in ZnPT-CuPT blends, ii) antagonist interaction in binary mixtures with chlorothalonil, and iii) synergistic, antagonist and additive effects in binary combinations of diuron with ZnPT and CuPT depending on concentration ratios. Additional exposures with ZuPT-CuPT-chlorothalonil and ZuPT-CuPT-diuron mixtures resulted in synergistic and additive effects, respectively; whereas Chlorothalonil-Diuron-CPT and Chlorothalonil-Diuron-ZPT exhibited antagonistic interactions. Interestingly, when quaternary combinations were applied, additive effects on *Artemia* survival were observed (Koutsaftis and Aoyama, 2007; Lavtizar et al., 2018). Moreover, the organophosphorus insecticides diazinon, clorpyrifos, dichlorvos and profenofos, and ammonium sulfate, which is a pesticide ingredient, also showed a considerable toxicity causing high mortality rate of *Artemia* nauplii (Bustos-Obregon and Vargas, 2010); Varó et al., 2002; Venkateswara Rao et al., 2007; Benmeddah et al., 2020).

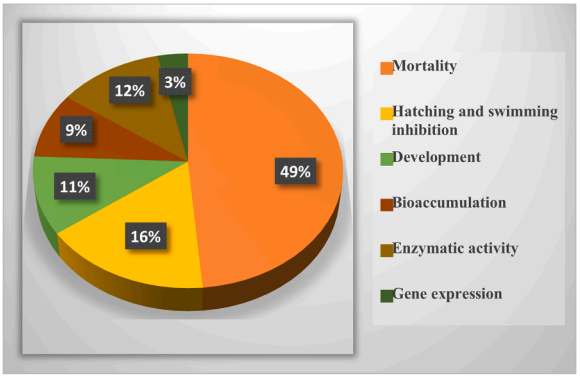

Fig. 2. Pie chart reporting the relative abundance (%) of papers separated on the basis of the following endpoint: mortality (49 %, n = 86), hatching and swimming (16 %, n = 29), development (11 %, n = 19), enzymatic activity (12 %, n = 21), bioaccumulation (9 %, n = 16), and gene expression (3 %, n = 6). The percentage of each endpoint has been calculated on the total number (n = 115) of papers examined in the present review.

The *Artemia* bioassay has been also found extremely useful to evaluate the ecological impact and possible toxicity of natural compounds (Chan et al., 2021; Ntungwe N et al., 2020). For instance, literature works demonstrated that palytoxin (PLTX), a compound isolated from *Trichodesmium* cyanobacteria, and polyunsatured aldehydes (2E, 4E-decadienal, decanal, undecanal) and eicosapentaenoic acid (EPA) extracted from the diatoms *Skeletonema costatum* and *Nitzschia commutate* induced a significant reduction of nauplii survival after 24 h and 72 h of exposure (Caldwell et al., 2003). The *Artemia* mortality assay was also useful to detect the toxicity of secondary metabolites extracted from several plants. For instance, compounds and oils extracted from the leaves and roots of several species such as, *Chaetomium globosum, Cochlospermum regium Amaranthus spinosus L., Ricinus communis* (Castor), *Capsicum frutescence L.* (Chili), *Azadirachta indica L.* (Neem), *Cymbopogon nardus L.* (Lemon grass), *Zingiber officinale L.* (Ginger), *Clerodendrum paniculatum L.* (Pagoda flower) and *Lippia javanica* displayed a considerable cytotoxic activity on *Artemia* nauplii (Qin et al., 2009; Atchou et al., 2021; Magalhães et al., 2021; Hafiz et al., 2019; Khaleel, 2019; Adeogun et al., 2018). In addition, among the extracts of roots, bark, leaves, and fruit/hypocotyl obtained from five species of Rhizophoraceae (*Bruguieria cylindrica, B. gymnorrhiza, Ceriops decandra, Rhizophora apiculata*, and *R. mucronata*), the bark was the most toxic by reducing nauplii survival (Indriaty et al., 2022). In contrast, *Artemia* nauplii did not display any significant sensitivity to the extract of *Lomandra hysteric,* a perennial rhizomatus herb belonging to the family Asparagaceae (Rumbudzai Chikowe, 2021). Concerning natural compounds from fungi, phyllostictines A and B, extracted from the species *Phyllosticta cirsii*, caused extremely high mortality rate in *Artemia* larvae (Evidente et al., 2008), while three fungal toxins, diplobifuranylones A and B, 5′-Monosubstituted Tetrahydro-2H-bifuranyl-5-ones, did not show any toxicity on *Artemia* nauplii after 48 h of exposure (Evidente et al., 2007). Moreover, atranorin, thamnolic, usnic, gyrophoric, barbatic, fumarprotocetraric, perlatolic, norstictic and protolichesterinic isolated from nine lichens (*Lobaria erosa, Cladia aggregata, Cladonia confusa, C. crispatula, C. furcata, Stereocaulon microcarpum, S. ramulosum, Punctelia canaliculata* and *Cladonia dimorphoclada*) have also been tested for toxicity against *Artemia*. In particular, atranorin and perlatotic, usnic and anziaic acids had a high toxicity with LC_{50} values (Honda et al., 2016).

The negative effects on nauplii survival of emerging pollutants such as, nanoparticles, pharmaceuticals, additives, personal care products and so on, were also investigated in *Artemia* models. For instance, PS-NH2, silver (AgNPs) and zinc nanoparticles (ZnNPs, 40-60 nm), and synthetic microfibers (polypropylene and polyethylene terephthalate) increased mortality rate (Bergami et al., 2017; Varó et al., 2019; Kim et al., 2021;Arulvasu et al., 2014; Danabas et al., 2020). A compound extensively used in the production of polycarbonate plastics, named bisphenol A, was also found to affect the survival of *Artemia* (Castritsi-Catharios et al., 2013) and when its negative impact was compared to sodium dodecyl sulfate (SDS), the toxicity was particularly strong, with LC50 concentration five times lower (Ekonomou et al., 2019). Interestingly, to verify the potential role of humic acid (HA) in surfactants inactivation, the compounds SDS, Triton X-100 (Tx-100) and cetylpyridinium chloride (CPC) have been evaluated both in the presence and absence of HA. As a result, both terrestrial and aquatic HAs were able to mitigate the toxicity of CPC and SDS, while the mortality rate in the presence of Tx-100 was limited to the sole terrestrial HAs (Deese et al., 2016). In other works, *Artemia* nauplii (6-24 h old) were exposed to graphene oxide (GO), a compound commonly applied in various areas including adsorption, catalysis, biosensor, and drug delivery, revealing that these nanomaterials were able to cause immobilization and mortality at long exposure times (48 h and 72 h) (Lu et al., 2018; Shokry et al., 2021). Produced formation water (PFW), naturally

Table 1
Type, concentrations (mg/L) and toxic effects of organic and inorganic compounds on *A. franciscana* from 1th of January 2000 to 1th of June 2022.

Compound type	Concentrations (mg/L)	Morphological effects	Molecular and metabolomic effects	Reference
TMTC, DMTC and DBTA	0.08, 0.1, 0.15, 0.2, 0.25, 0.3, and 0.35 for TMTC; 70, 75, 79, 81, 83, 85, 90, and 95 for DMTC; and 55, 60, 70, 80, 90, 95, 105, and 110 for DBTA	Mortality and bioaccumulation	Not detected	(Hadjispyrou et al., 2001)
Cr, Cd and mixture of Cr and Cd	100 and 200 for Cd; 50 for Cr	Mortality; antagonist effect of mixture	Not detected	(Beňová et al., 2007; Hadjispyrou et al., 2001)
clorpyrifos and dichlorvos	0.1 to 18 for chlorpyrifos; 0.56 to 100 for dichlorvos.	LD50 = 9.3 at 24h for dichlorvos; LD50 = 3.2 at 24h for chlorpyrifos	inhibition of ChE activity	(Varó et al., 2002)
Cd	0, 100, 200, 250, 300, 350, 400, and 500	LC50 = 142	Not detected	(Sarabia et al., 2006, 2002)
microcystin-LR, Dhb-microcystin-HtyR and nodularin	0.0005	Not detected	Elevation of sGST and mGST activity in adults	(Beattie et al., 2003)
aldehydes and fatty acid eicosapentaenoic acid	0.1	Hatching cysts inhibition; Mortality	Not detected	(Caldwell et al., 2003)
As	4, 8, 15, 31 and 56	Mortality; LC50 = 15.78 at 24 h	Not detected	(Brix et al., 2003; Sánchez et al., 2016)
$CuSO_4$	2, 4, 8, 16 and 32	Swimming speed alteration, mortality, hatching rate inhibition	Not detected	(Manfra et al., 2016; Pati and Belmonte, 2003)
Se	42, 56, 75, 100, 133	Not detected	Not detected	(Brix et al., 2004)
compounds present in landfill leachate	45, 68, 79, 86 and 91	Hatching cysts inhibition	Not detected	(Svensson et al., 2005)
Alamethicin, paracelsin, antiamoebin, gliotoxin, destruxin A, oosporein and elsinochrome A	0.0002–0.0612	LC50 = 0.005 for ALA; LC50 = 0.021 for PCS; LC50 = 0.019 for AAM; LC50 = 0.039 for GTX; LC50 = 0.017 for DA; LC50 = 0.020 for EA	Not detected	(Favilla et al., 2006)
Cu, Cd and Zn	0.005 to 0.07	Hatching cysts inhibition by Cu	Not detected	(Brix et al., 2006)
Mixture of ZnPT, CuPT, diuron and chlorothalonil	20 to 80	synergistic, antagonist and additive effect	Not detected	(Koutsaftis and Aoyama, 2007, 2008; Lavtizar et al., 2018)
acephate, chlorpyrifos, monocrotophos, and profenofos	0.075 to 1.0 for clorpyrifos; 0.08 to 75 for profenos; 125 to 325 for monocrotophos; and 750 to 3000 for achephate	LD50 = 0.385 at 48h for chlorpyrifos; LD50 = 2350 at 48h for achephate; LD50 = 7.7 at 48h for profenos; LD50 = 262.7 at 48h for monocrotophos; Hatching cysts inhibition by chlorpyrifos and profenofos	inhibition of AChE activity by chlorpyrifos and profenofos	(Venkateswara Rao et al., 2007)
untreated wood leachates	0.006, 0.012, 0.018, 0.024 and 0.036	Not effect	Not detected	(Libralato et al., 2007)
Mixture of $CdCl_2$ and $ZnSO_4$	31 of $FeCl_3$, 5 of $CdCl_2$	Synergistic effect	Not detected	(Nováková et al., 2007)
Diplobifuranylones A and B, 5′-Monosubstituted Tetrahydro-2H-bifuranyl-5-ones	0.03 to 0.3	Mortality	Not detected	(Evidente et al., 2007)
phyllostictines A and B	297	Mortality	Not detected	(Evidente et al., 2008)
Chaetomugilin D, chaetomugilin A, chaetoglobosins A and C	0.01	Mortality	Not detected	(Qin et al., 2009)
organophosphate diazinon	1.0 to 29.4	LD50 = 6 after 24h	Not detected	(Bustos-Obregon and Vargas, 2010)
juice of *Aloe barbadensis*	0.0019	Not detected	thioredoxin reductase, glutathione reductase and glutathione peroxidase inhibition	(Sindaarta and Cock, 2010)
produced formation waters	0.048 to 0.128 for volatile aromatic compounds; 2.4 to 13 for semivolatile aromatic compounds;	Not effect	Not detected	(Manfra et al., 2011, 2010)
monoethanolamine, diethanolamine and triethanolamine	98.34 for MEA, at 498.54 for DEA and at 907.97 for TEA	EC50 = 43.0, LOEC = 2.85, NOEC = <2.85 for MEA; EC50 = 378, LOEC = 124.9, NOEC = 62.5 for DEA; EC50 = 577, LOEC = 150, NOEC = 100 for TEA	Not detected	(Libralato et al., 2010)
Cr, Hg, Pb and Zn	0, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24 and 0.28	Inhibition of growth by Pb and Cr; Bioaccumulation of Pb after 4 days	Not detected	(Shaojie and Wenli, 2012; Soto-Jiménez et al., 2011)
$ZnSO_4$, $FeSO_4$, $CuSO_4$, $CdCl_2$, $Cu(NO_3)_2$	0.001, 0.01, 0.1, 1, 10, 100 and 1000	LC50 = 710.7 for $CdCl_2$, LC50 = 19.5 for Cu and LC50 = 1000 for $ZnSO_4$	Not detected	(Cortés et al., 2018; Kokkali et al., 2011)
Ni and V	0.001, 0.002 and 0.003	LC50 = 0.0107 for Ni; LC50 = 0.011 for V; Bioaccumulation both in nauplii and adults; Growth inhibition	Not detected	(Asadpour et al., 2013; Manavi and Baniamam, 2011; Sujatha Devi et al., 2016)
compounds present in landfill leachate	from 0.3 to 1.3 for Hg; from 77.1 to 131 for Cr; from 21 to 143 for Pb; from 29 to 349 for Cu; from 38 to 61 for Ni; from	Mortality	Not detected	(Lu et al., 2012)

(*continued on next page*)

Table 1 (*continued*)

Compound type	Concentrations (mg/L)	Morphological effects	Molecular and metabolomic effects	Reference
	0.7 to 4 for Cd; from 399 to 1768 for Zn; and from 6 to 565 for Mn			
Mixture of nine PAHs	370	Not effect	Not detected	(Rojo-Nieto et al., 2012)
Cr, Hg and Cd	0.052	Inhibition of growth and mortality by Cr	Increase of protein, lipid and carbohydrate concentration	(Umarani et al., 2012)
diuron and irgarol	1 and 5 for irgarol, 1.5 and 25 for diuron	Hatching cysts inhibition by diuron	inhibition of serine proteases activity	(Alyürük and Çavaş, 2013; Jung et al., 2017)
potassium dichromate and p-coumaric acid	7 to 100	Mortality; swimming velocity decrease	Not detected	(Alyuruk et al., 2013)
bisphenol A	20 to 50	LC50 = 44.8 at 24h, LC50 = 34.7	Not detected	(Castritsi-Catharios et al., 2013)
Zn and La	10, 50 and 100	LC50 = 100 for Zn; LC50 = 78.1 for La	Not detected	(Ates et al., 2013; Bergsten-Torralba et al., 2020)
$HgCl_2$, KCN, $K_2Cr_2O_7$, C_6H_6 and $C_6H_6Cl_6$	not reported	LC50 = 0.12 for $HgCl_2$; LC50= 0.06 for KCN; LC50 = 0.72 for $K_2Cr_2O_7$; LC50 = 1.57 for C_6H_6 and LC50 = 0.44 for $C_6H_6Cl_6$	Not detected	(Lu et al., 2013)
antifouling paints	0.2 and 1	Body development inhibition	Not detected	(Castritsi-Catharios et al., 2014)
carbon black nanoparticles	50 to 1000	Not effect	Increase of *hsp70* activity	(Rodd et al., 2014)
Ag	0.0002 to 0.001	Hatching cysts inhibition; LC50 = 1078000 at 24h and 1293600 at 48h; bioaccumulation in gut	DNA demage	(Arulvasu et al., 2014)
SnO_2, CeO_2 and Fe_3O_4	0.01, 0.1, 1.0	Swimming speed inhibition by CeO_2; Hatching cysts inhibition, mortality and swimming speed alteration byFe_3O_4	Increase of ChE activity by CeO_2 and Fe_3O_4; Inhibition of GST and ChE activity by SnO_2; Increase of CAT activity by Fe_3O_4	(Gambardella et al., 2014; Selvinsimpson et al., 2021; Zhu et al., 2017b)
$FeCl_3$, $CdCl_2$ and mixure	30 of $FeCl_3$, 5 of $CdCl_2$	Not detected	Increase of CAT, GPX and SOD activity, metallothioneins concentration by $CdCl_2$;	(Mohamed et al., 2014)
diethylene glycol	From 365 to 25000	NOEC = 25000	Not detected	(Manfra et al., 2015)
triclosan and triclocarban	0.1 to 0.5 for triclosan and 0.001 to 0.1	LC50 = 0.171 for triclosan; LC50 = 0.018 for triclocarban	Not detected	(Xu et al., 2015)
Ag	0, 1, 5, 10 and 50	Mortality and swimming speed inhibition dose dependent	Not detected	(Gambardella et al., 2015; Palácio et al., 2021)
atranorin, thamnolic, usnic, gyrophoric, barbatic, fumarprotocetraric, perlatolic, norstictic and protolichesterinic acid	0.01 to 0.5	LC50 = 45000 for atranorin and perlatotic, anziaic acids; LC50 = 10000 for usnic acid	tyrosinase inhibition by barbatic, usnic and anziaic acids	(Honda et al., 2016)
anionic carboxylated and cationic amino polystyrene nanoparticles	0.005 to 0.1	PN-COOH bioaccumulation in gut; PS-NH_2 bioaccumulation in antennules and appendages	Not detected	(Bergami et al., 2016)
Triton X-100, cetylpyridinium chloride and sodium dodecyl sulphate	100 for Tx-100, 3.5 for CPC and 25 sor SDS	Mortality by Tx-100; hatching cysts inhibition by CPC and SDS	Not detected	(Deese et al., 2016)
TRO	1 to 10	Mortality	Not detected	(Duan et al., 2016)
TiO_2 and Ag-TiO_2	0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 10, 50 and 100 for TiO_2; 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 5, 10, 20 and 40 for Ag-TiO_2	LC50 = 23.03 at 24h, 3.74 at 48h, 1.06 at 72h and 0.79 for Ag-TiO_2; LC50 = 381.6 at 24h, 70.12 at 48h, 41.26 at 72h and 18.77 for TiO_2	Not detected	(Ozkan et al., 2016)
TiO_2, ZnO and CuO	100, 120, 140, 160, 180 and 200 for ZnO; 1, 3, 5, 7, 9 and 10 for CuO; and 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 for TiO_2	LC50 = 11.42 at 24h, 8.51 at 48h, 6.21 at 72h and 4.32 at 96 h for CuO; LC50 = 293.1 at 24h, 247.13 at 48h, 201.21 at 72h and 173.20 at 96 h for ZnO; LC50 = 115.55 at 24h, 86.11 at 48h, 57.31 at 72h and 30.54 at 96 h for TiO_2	Not detected	(Khoshnood et al., 2017)
tributylin, diuron and irgarol	0.000005-0.001 for tributylin, 0.5-15 for diuron, 0.01-2 for irgarol	LC50 = 0.0002 for tributylin, LC50 = 0.982 and LC50 = 10.3 for diuron	inhibition of acetylcholinesterase activity	(Lee et al., 2017)
anionic carboxylated and cationic amino polystyrene nanoparticles	0.0005 to 0.01	Mortality by PS-NH_2 exposure	Up-regulation of *clap* and *cstb* in 48 h larvae by PS-NH_2	(Bergami et al., 2017)
graphene oxide	0, 25, 50, 100, 200, 400 and 600	Hatching cysts inhibition dose-dependent, mortality and immobilization	Not detected	(Zhu et al., 2017b)
methylparaben	0.0085 and 0.017	LC50 = 36.7 at 24h	Down-regulation of *CAT* gene	(Comeche et al., 2017)
Zn and Ni; mixture	50 to 1200 for Zn and 25 to 600 for Ni; seven dilution ratios (4:1, 3:2, 2:1,1:1, 1:2, 2:3, and 1:4) for mixture	LC50 = 44.0 for Zn; LC50 = 271 for Ni; additive effect at salinity of 35 ppm and antagonist effect at salanity of 10 ppm	Not detected	(Damasceno et al., 2017)
TiO_2 and mixture of TiO_2 - As	0, 1, 10, 100 and 1000	Synergistic effect		

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Table 1 (*continued*)

Compound type	Concentrations (mg/L)	Morphological effects	Molecular and metabolomic effects	Reference
			Increase of protein concentration by mixture	(Thiagarajan et al., 2022; Yan et al., 2017)
CuO	0.2, 1, 5, 10, 25 e 50	Bioaccumulation at 72h; LC50 = 61.4 for nauplii and 175.2 for adults	Increase of GSH and TBARS activity	(Cimen et al., 2020; Madhav et al., 2017)
Fe_3O_3	600	Hatching cysts inhibition; LC50 = 177.4 for nauplii II; inhibition of growth	Increase of MDA content, T-AOC, ROS and antioxidant enzymes activities	(Zhu et al., 2017b)
CeO_2	100	Hatching cysts inhibition; mortality	Increase of ROS activities	(Sugantharaj David et al., 2017)
monoterpene hydrocarbons, oxygenated monoterpenes, ketones, aldehydes, sesquiterpene hydrocarbon, esters extracted from *Lippia javanica*	0.031 to 1	Hatching cysts inhibition; Mortality	Not detected	(Adeogun et al., 2018)
graphene oxide	1, 10, 50, 100 and 500	Mortality, immobilization and growth inhibition	Not detected	(Lu et al., 2018; Shokry et al., 2021)
chemical discharges	1 to 100	LC50 = 1 for glutaraldehyde; LC50 = 5.92 for sodium hypochlorite	Not detected	(Cortés et al., 2018)
zinc and copper pyrithione	0.001 to 100	LC50 = 1.37 for ZnPT; LC50 = 4.58 for CuPT	Not detected	(Gutner-Hoch et al., 2019)
2-phenyl-1H-benzo[d]imidazole, phenyl (2-phenyl-1Hbenzo[d]imidazol-6-yl) methanone, (2-(2,3-dichlorophenyl)-1Hbenzo[d]imidazol-6-yl)(phenyl) methanone	10, 100, 500 and 1000	LC50 = 138.7 for (2-(2,3-dichlorophenyl)-1Hbenzo[d]imidazol-6-yl)(phenyl) methanone after 6 h; LC50 = < 10 for 2-phenyl-1H-benzo[d]imidazole and phenyl (2-phenyl-1Hbenzo[d]imidazol-6-yl) methanone after 24 h	Not detected	(Lapetaje and Creencia, 2019)
Polyphenol, flavonoid, saponins and alkaloids	0.05, 0.1, 0.25, 0.5	Hatching cysts inhibition	Not detected	(Braguini et al., 2019)
alkaloids, flavonoids, steroids/ triterpenoids, tannins, saponins and glycosides	20 to 100	LC50 = 49.4	Not detected	(Hafiz et al., 2019)
plants extract	5 to 320	Mortality	Not detected	(Khaleel, 2019)
cationic amino polystyrene nanoparticles	0.0001, 0.001, 0.003 and 0.01	Mortality after 14d	inhibition of ChE and CbE activity	(Varó et al., 2019)
microplastics	0.4, 0.8 and 1.6	Reproduction inhibition	Not detected	(Peixoto et al., 2019)
bisphenol A and sodium dodecyl sulfate	7, 8, 9, 10, and 12 for SDS; 40, 42, 44, 46, and 48 for bisphenol A	LC50 = 45.5 at 24h, LC50 = 34.4; LC50 =17.2 at 72h for bisphenol A; 8.4 at 24h, LC50 = 7.8 at 48h; LC50 = 7.1 at 72h for SDS	Not detected	(Ekonomou et al., 2019)
compounds present in untreatted and treatted landfill leachate	20-80	LC50 = 50 for treatted LL	Not detected	(Borba et al., 2019)
eugenol and consequently crude oil	10000, 25000, 50000, 75000, 100000, 125000 and 150000	LC50 = 27000 for eugenol; LC50 = 16000 for crude oil	Not detected	(Rahman and Pratama, 2019; Viega et al., 2020)
TiO_2 and Mixture of TiO_2, ZnO, MgO	25, 50, 100 and 200	Mortality; LC50 = 140.4 for TiO_2; LC50 = 238.1 for MONs	Not detected	(Anaya-Esparza et al., 2019)
$K_2Cr_2O_7$	0.005, 0.01, 0.02, 0.025, 0.05, 0.1 and 0.25	LC50 = 0.021 for nauplii I; LC50 = 0.009 for nauplii II; LC50 = 0.015 for metanauplii; LC50 = 0.022 for juvenile; LC50 = 0.0186 for adults	Not detected	(Ocaranza-Joya et al., 2019)
ammonium sulfate	50, 75, 100, 125, 150 and 200	LC50 = 75	Not detected	(Benmeddah et al., 2020)
polystyrene nanoparticles	0, 0.006 and 0.6	Bioaccumulation	Not detected	(Kim et al., 2022; Marta et al., 2020)
Nanoparticles of Zn	0, 0.2, 1, 5, 10, 25 and 50	Bioaccumulation; mortality at dose and time-dependent	Not detected	(Danabas et al., 2020)
Cu	0.2, 1, 5, 10, 25 e 50	Bioaccumulation at 72h	Increase of GSH and TBARS activity	(Cimen et al., 2020)
TiO_2 and Cr-TiO_2	0.25, 0.5, 1, 2, and 4 for TiO_2; 0.5, 1, 2, and 4 for Cr-TiO_2	Antagonist effect	Increase of ROS activity by mixture	(Thiagarajan et al., 2020)
gel with testosterone and gel with estradiol	0.001 to 0.025 for gel with testosterone; 0.00003 to 0.015 for gel with estradiol	Mortality	Not detected	(Viega et al., 2020)
Br	79, 97, 190, 380, 780, 1900, 3900, 7700, and 11000	Mortality	Not detected	(Pillard and Tapp, 2021)
tributyltin chloride	0.025, 0.050. 0.1, 0.2 and 0.3	Lesions in the testis and ovary section	Immunoreactivity to Caspase 3 and HSP70 antibody	(Abushaala et al., 2021)
Amaranthus spinosus root extract	25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, 0.10 and 0.05	LC50 = 1.18	Not detected	(Atchou et al., 2021)
polystyrene nanoparticles	0, 1, 10, 100, 1000 and 10000	Bioaccumulation	Not detected	(Albano et al., 2021)
microplastics	500	Mortality	Not detected	(Kim et al., 2021)
microplastic+simvastatin, microplastic+carbamazepine,	0.26 for microplastic; 12.03, 10.03, 8.35, 6.96, 5.80 and 52.08 for simvastatin; 43.40,	LC50 = 10.29 for microplastic+simvastatin, LC50 = 46.50 for microplastic+carbamazepine, LC50 =	inhibition of ChE activity	(Albendín et al., 2021)

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Table 1 (*continued*)

Compound type	Concentrations (mg/L)	Morphological effects	Molecular and metabolomic effects	Reference
microplastic+chlorpyrifos, microplastic+triclosan	36.17, 30.14and 25.16 for carbamazepine; 12, 6, 3, 1.5, 0.75 and 0.0312 for triclosan; 0.0156, 0.0078, 0.0039 and 0.00195 for chlorpyrifos	0.012 for microplastic+chlorpyrifos, LC50 = 4.96 for microplastic+triclosan		
N-2-methoxybenzyl-phenethylamines	0.00075, 0.001, 0.0015, 0.002, 0.003, 0.006, 0.009, 0.012, 0.015, 0.018, 0.024 and 0.030	Mortality and immobilization	Not detected	(Álvarez-Alarcón et al., 2021)
carbon black nanoparticles	100 to 5000	Not effect	Not effect	(Tretyakova et al., 2021)
$ZnCl_3$, $CdCl_2$ and $HgCl_2$	0.81 of $FeCl_3$, 0.97 of $CdCl_2$, 0.08 of $HgCl_2$	Hatching rate inhibition; mortality;	Not detected	(Ñañez Pacheco et al., 2021)
essential oil of *Cochlospermum regium*	0.001, 0.0025, 0.005, 0.007, 0.010, 0.020, 0.050, 0.070, 0.1 and 1	LC50 = 0.09	Not detected	(Magalhães et al., 2021)
Lomandra hystric extract	2	Mortality	Not detected	(Rumbudzai Chikowe, 2021)
Palytoxin	0.2, 2.68 and 26.8	Hatching cysts inhibition and mortality	Increase of ROS levels, catalase and peroxidase activity	(Cavion et al., 2022)
plants extract	0.001 to 1	LC50 = 0.032 for bark	Not detected	(Indriaty et al., 2022)
microplastics	0.001, 0.025, 0.05, 0.075 and 0.1	LC50 = 0.041 for nauplii, LC50 = 0.052 for metanauplii; swimming behaviour alteration in juvenile	inhibition of SOD, CAT, GSH, GST and AChE activity	(Jeyavani et al., 2022)
benzofenone, 2,5-diaminotoluen solfate, p-phenyl enediamine e tetrabromobisphenol A	0 to 300	LC50 = 0.017 for tetrabromobisphenol A; LC50 = 0.014 for benzofenone	Not detected	(Tapia-Salazar et al., 2022)
naphthalene, phenanthrene, benzo-k-fluoranthene, fluoranthene	0.015, 0.032, 0.078, 0.11, 0.26, 0.41, 0.76, 1.45, 4.23 and 10.1 for NAP; 0.21, 0.71, 1.15, 2.26, 3.45, 4.23, 7.48, 48.7, 98.6 and 223.4 for PHE; 0.29, 0.81, 2.14, 4.41, 9.91, 20.4, 45.6, 91.6, 179 and 325 for FLT; 0.016, 0.41, 0.78, 0.98, 2.4, 5.3, 10.4, 19.5, 41.7 and 84.6 for BkF	LC50 = 0.60 (nauplii) and 44.3 (adults) for NAP; LC50 = 3.07 (nauplii) and 1.68 (adults) for PHE; LC50 = 0.09 (nauplii) and 0.77 (adults) for FLT; LC50 = 6.12 (adults) for BkF	Increase of *hsp60* and *UCP2* activity by all PAHs	(Albarano et al., 2022)

present in sedimentary formations from which metals, aromatic and aliphatic hydrocarbons, phenols and additives are mined, was also tested on *Artemia* models. PFW showed toxicity on different marine organisms, but did not display high impact on *A. franciscana* after both 24 h and 48 h of exposure (Manfra et al., 2011, 2010). Moreover, the diethylene glycol (DEG), commonly used in the dehydration of natural gas streams (e.g. PFW), displayed long-term effects on the crustacean, reported as NOEC values (Manfra et al., 2015). A recent study also showed that N-2-methoxybenzyl-phenethylamines, psychoactive substances with poorly defined pharmacological properties, had a great impact on the survival of the brine shrimp after 24 h of exposure (Álvarez-Alarcón et al., 2021). Toxicity tests of compounds found within the untreated and treated landfill leachate (LL) demonstrated the high efficiency of the mediated photo-Fenton process in landfill leachate treatment. In fact, untreated LL induced mortality in all tested dilutions (20–80%), while treated LL was able to reduce of 56 % the LC_{50} value (Borba et al., 2019).

A great risk for the marine environment was also represented by chemical discharges (Libralato et al., 2007), with glutaraldehyde, sodium hypochlorite, copper sulfate, potassium permanganate and iron (III) chloride) displaying a considerable toxicity with antagonistic or additive effects depending on the mixture tested (Cortés et al., 2018). Concerning personal care products, the toxic effect of monoethanolamine (MEA), diethanolamine (DEA) and triethanolamine (TEA), benzofenone, 2,5-diaminotoluen solfate, p-phenyl enediamine, tetrabromobisphenol A, benzofenone, tetrabromobisphenol A, and gels commonly formulated with hormones and other ingredients also demonstrated a great impact on *Artemia* survival (Libralato et al., 2010; Tapia-Salazar et al., 2022; Rahman and Pratama, 2019; Viega et al., 2020). Comparative analyses with the organic wastewater compounds, triclosan and triclocarban, and trimethyltin chloride (TMTC), dimethyltin dichloride (DMTC) and dibutyltin diacetate (DBTA), revealed different effects on exposed nauplii. In particular, triclocarban was more toxic than triclosan at 24h of exposure, and TMTC induced significant increase of mortality rate (Hadjispyrou et al., 2001; Xu et al., 2015). Mixtures of nine Polycyclic Aromatic Hydrocarbons (PAHs; naphthalene, phenanthrene, acenaphthene, fluorene, pyrene, fluoranthene, anthracene, benzo(a)anthracene and benzo(a)pyrene) that have been widely found in water sewages, did not show any toxic effects in *Artemia* individuals (Rojo-Nieto et al., 2012). In contrast, a recent paper demonstrated that, among four different PAHs, fluoranthene was the most toxic by inducing a dose-dependent reduction of *Artemia* survival in both nauplii and adults after 24 h and 48 h of exposure, whereas benzo(k)fluoranthene did not report any negative effects on *Artemia* survival (Albarano et al., 2022).

Artemia is also a valuable model species to evaluate the efficiency and safety of remediation techniques commonly applied on water effluents to remove contaminants. As an example, the electrolyzed water used in the treatment of ballast water is rich of active constituents, which are measured as Total Residual Oxidant (TRO). TRO decay experiments revealed that electrolyzed water was capable to induce mortality in a dose-dependent manner, registering a considerable mortality rate (>75%) (Duan et al., 2016). In contrast, other works demonstrated that carbon black (CB) nanoparticles, normally used to remove oil spills in wastewater, were non-toxic on *A. franciscana* and *A. salina*, suggesting the overall safety of this application (Rodd et al., 2014; Tretyakova et al., 2021).

Concerning the effects of heavy metals on *Artemia* survival, several

works have been published in the last twenty-two years. For instance, vanadium (V) and nickel (Ni) were found to increase the mortality of nauplii (Asadpour et al., 2013; Manavi and Baniamam, 2011), even at low concentrations (Fichet and Miramand, 1998). Similarly, the metals Zinc (Zn), lanthanum (La) and Cd also induced an increase of nauplii mortality after 96 h of exposure (Ates et al., 2013; Bergsten-Torralba et al., 2020; Sarabia et al., 2006, 2002). Similarly, arsenic (As) was able to reduce the survival of nauplii, without triggering negative consequences on cysts hatching and adults survival (Brix et al., 2003; Sánchez et al., 2016), while selenium (Se) and bromide (Br) did not reveal toxic effects at all concentration tested (Brix et al., 2004; Pillard and Tapp, 2021). Comparative analyses of several metals used in both single and mixture experiments have been widely reported. For instance, toxicity studies with Cd and chromium (Cr), showed that Cr was more toxic than Cd, and metals combination exerted antagonistic interactions (Beňová et al., 2007; Hadjispyrou et al., 2001). Comparisons among Cr, Hg and Cd metals, revealed a much stronger toxic effect of Cr on the survival of nauplii (Umarani et al., 2012). Other experiments also reported that Zn was more toxic than Ni, but both metals displayed higher toxicity at low salinities (10 ppm). When mixture effects have been analyzed, the transition from additive to antagonistic interactions depending on salinity rate, significantly decreased (Damasceno et al., 2017). Experiments with Titanium dioxide (TiO_2) and a mixture of oxide nanoparticles made of TiO_2-Zinc oxide (ZnO)-Magnesium oxide (MgO) revealed that TiO_2 was more toxic than the tertiary mixture (Anaya-Esparza et al., 2019). Conversely, by comparing the toxicity of TiO_2 and TiO_2 associated to silver nanoparticles (Ag-TiO_2), Ag-TiO_2 was found up to 17-fold more toxic than pure TiO_2 (Ozkan et al., 2016). Moreover, a comparative study reported that CuO was up to 26-fold more toxic than pure ZnO in *Artemia* nauplii (Khoshnood et al., 2017).

Mortality rates, together with other endpoints, were found relatively altered in nauplii exposed to several metals such as stannic oxide (SnO_2), cerium (IV) oxide (CeO_2) and iron (II, III) oxide (Fe_3O_4) (Gambardella et al., 2014; Selvinsimpson et al., 2021; Zhu et al., 2017a). A significant decrease of nauplii survival was also detected when exposures were applied with CeO_2, TiO_2-As and Fe_2O_3-nanoparticle combinations with synergistic effects clearly evident in the case of mixtures (Wang et al., 2017). Moreover, copper sulphate ($CuSO_4$) was able to impair the survival after 24 h and 48 h of exposure (Manfra et al., 2016; Pati and Belmonte, 2003). Interestingly, other works revealed that Cu salts were up to 35- and 50-fold more toxic than cadmium (II) chloride ($CdCl_2$) and zinc sulphate ($ZnSO_4$), respectively (Browne, 1980; Cortés et al., 2018; Kokkali et al., 2011; Saliba and Ahsanullah, 1973; Saliba and Krzyz, 1976). Testing $CdCl_2$ in combination with $ZnSO_4$, a higher toxic profile has been shown, underlining synergistic effects of both substances (Nováková et al., 2007). Recent works also reported that the nauplii and metanauplii stages of the crustacean life cycle were the most sensitive to potassium dichromate ($K_2Cr_2O_7$) exposures (Ocaranza-Joya et al., 2019), and survival of nauplii after 48 h was inhibited by $CdCl_2$, zinc chloride ($ZnCl_2$) and mercury chloride ($HgCl_2$) (Lu et al., 2013; Ñañez Pacheco et al., 2021).

2.2. Hatching and swimming behavior

Hatching is a crucial step that allows the transition from dormant cysts to swimming nauplii when the environmental conditions are favorable. In *Artemia* models, the ecotoxicological assessment of several compounds has been also evaluated on the hatching and swimming behavior. For instance, exposures of *Artemia* hydrated cysts to low and high concentrations of two biocides, diuron and irgarol revealed that irgarol did not show any effect on egg hatching at both low and high concentrations (1 and 5 mg/L, respectively), whereas diuron decreased the hatching rate of about 50 % (Alyürük and Çavaş, 2013; Jung et al., 2017). Other biocides, PDC and p-CA, already mentioned for their effects on *Artemia* survival, reduced the swimming velocity of nauplii of 50% and 70%, respectively (Alyuruk et al., 2013). Similarly, the natural compounds PLTX, polyundatured aldehydes and EPA, that impairs the survival rate of *Artemia*, were also found to induce a dose-dependent reduction of the hatching success (Caldwell et al., 2003; Cavion et al., 2022).

The extract of leaves and flowers obtained from *Lavandula officinalis* was also found to reduce the hatching success of *Artemia* cysts. In particular, at high concentrations (0.5 and 1 mg/L), the aqueous extract of leaves, significantly decreased hatching of about 60-90 % at 36 h and 72 h, whereas at the same concentrations and timing, the exposure of the flower extract was able to reduce hatching within a range of 18-60 % (Braguini et al., 2019). Similarly, the ecotoxicological assessment of essential oils extracted from fresh and dried leaves of *L. javanica* showed that the number of hatched eggs dose-dependently reduced, by reaching only the 10 % of hatching success after 12 h at 0.5 mg/L (Adeogun et al., 2018).

Several pollutants already mentioned for their capabilities to affect *Artemia* survival such as water leachates, heavy metals, microplastics, rare metals, AgNPs, salts and combinations with nanoparticles were all found to strongly inhibit the hatching success and swimming speed (Brix et al., 2006; Lu et al., 2012; Svensson et al., 2005).

Moreover, other studies reported that the salts $CuSO_4$, $K_2Cr_2O_7$, $CdCl_2$, $ZnCl_2$ and $HgCl_2$ were able to impair the hatching rate and swimming speed, after 48 h of exposure (Manfra et al., 2016; Pati and Belmonte, 2003), with nauplii and metanauplii stages being the most sensitive to potassium dichromate exposures (Ocaranza-Joya et al., 2019). Recent studies also reported that molecules and delivery systems used in biomedical applications strongly influenced the hatchability and swimming behavior in juveniles after 24 h of exposure (Álvarez-Alarcón et al., 2021).

2.3. Development

Artemia embryo development and reproduction, as for others model organism, represents a key endpoint to assess the toxicity of several contaminants. For instance, a recent study reported a significant decrease of the reproduction success in *Artemia* adults after 44 days of exposure to microplastics (Peixoto et al., 2019).

Concerning biocide compounds, such as (2-(2,3-dichlorophenyl)-1Hbenzo[d]imidazol-6-yl)(phenyl)methanone, 2-phenyl-1H-benzo[d]imidazole, phenyl(2-phenyl-1Hbenzo[d]imidazol-6-yl)methanone, and the aforementioned diuron and irgarol, a considerable delay or arrest of *Artemia* development was observed, except in the case of irgarol whose toxicity was not detected at both low and high concentrations (Lapetaje and Creencia, 2019). Moreover, the exposure of four antifouling paints (ANTI F, SHARKSKIN, OCEAN T/F, MICRON), additives (SDS, bisphenol A, GO) also reported a significant decrease of *A. franciscana* body length after 24h and 48h (Ekonomou et al., 2019), with GO impairing the *Artemia* development only at long exposure time (48 h and 72 h) (Lu et al., 2018; Shokry et al., 2021).

In addition, several works resulted in that the heavy metals V, Ni, Cr and Pb were able to reduce the growth success of nauplii after 24 h, 48 h, 72 h and 96 h (Shaojie and Wenli, 2012). Interestingly, comparisons among Cr, Hg and Cd metals, revealed a much stronger toxic effect of Cr on growth of nauplii (Umarani et al., 2012). Concerning metal oxides mixtures, a synergistic effect of TiO_2 and As on the development of *Artemia* nauplii was observed (Yan et al., 2017; Thiagarajan et al., 2022).

2.4. Bioaccumulation

Toxicants may also accumulate through the feeding process and impair the physiology and behavior of *Artemia* larvae and adults. The effects of two polystyrene nanoparticles (PS NPs), 40 nm anionic carboxylated (PS-COOH) and 50 nm cationic amino (PS-NH_2), were evaluated in *A. franciscana* larvae. After 48h of exposure, PS-COOH bioaccumulation has been shown in gut limiting food intake, while PS-NH_2 was detected at the surface of sensorial antennules and

appendages, probably preventing larvae motility (Bergami et al., 2016). Successively, PS-NPs were observed in the mandible, stomach, gut, tail gut and appendages after 24 h of exposure (Albano et al., 2021; Kim et al., 2022; Marta et al., 2020). ZnO nanoparticles (80-100 nm) were also found to highly bioaccumulate by triggering severe and acute effects on the survival of the brine shrimp (Danabas et al., 2020). The same authors evaluated the toxicity of Zn NPs (40-60 nm) on *Artemia* nauplii. Specifically, they highlighted that these NPs were able to bioaccumulate through the filtration system, and influence the survival at higher concentrations (50 mg/L) and lower concentration ($\leq$ 5 mg/L) after 24 h and 72 h, respectively (Danabas et al., 2020). Bioaccumulation of the organic wastewater compound TMTC was measured as 25 and 50 times greater than DMTC and DBTA, respectively (Hadjispyrou et al., 2001).

Concerning heavy metals, a significant bioaccumulation was observed. After seven days of exposure, Cd was able to bioaccumulate in a dose-dependent manner and increase the intake depending on feeding activities(Cimen et al., 2020; Jayasekara et al., 1986; Jennings and Rainbow, 1979), and V, Ni and Pb were found to bioaccumulate in both nauplii and adults (Asadpour et al., 2013; Sujatha Devi et al., 2016). Moreover, a significant bioaccumulation at 72 h was reported in *Artemia* nauplii exposed to copper oxide (CuO) (Cimen et al., 2020; Madhav et al., 2017).

2.5. Enzymatic biomarker

Enzymatic biomarkers represent a useful tool to assess and understand how aquatic organisms respond to several stressors, by looking at the activity of proteins particularly involved in detoxification processes. The biocide diuron was found to inhibit the activity of the hatching enzyme serine protease (Alyürük and Çavaş, 2013) and, together with TBT, irgarol, clorpyrifos, dichlorvos and profenofos showed a considerable decrease of AChE activity (Lee et al., 2017; Varó et al., 2002; Venkateswara Rao et al., 2007). Moreover, TBT was able to also cause histological lesions and immunoreactivity to apoptosis markers (Caspase 3 and HSP70) in adults ovary (Abushaala et al., 2021). After 24 h of exposure to the cyanobacterial toxins CDNB, DCNB and EPNP, CDNB was able to increase mGST activity, while MCLR and NODLN enhanced sGST levels in adults exposed to all toxins (Beattie et al., 2003). A recent paper, also demonstrated that PLTX, another common compound extracted from cyanobacteria, caused oxidative stress in adults by increasing Reactive Oxygen Species (ROS) levels, Catalase (CAT) and Peroxidase activity (Cavion et al., 2022). Instead, the exposure to juice of *Aloe barbadensis,* was demonstrated to induce oxidative stress, by decreasing of thioredoxin reductase, glutathione reductase and glutathione peroxidase activity (Sindaarta and Cock, 2010). Moreover, atranorin, thamnolic, usnic, gyrophoric, barbatic, fumarprotocetraric, perlatolic, norstictic and protolichesterinic isolated from lichens have been also tested showing that only barbatic, usnic and anziaic acids inhibited the tyrosinase activity (Honda et al., 2016). Emerging pollutants like microplastics were also able to decrease Superoxide Dismutase (SOD), Glutathione (GSH), CAT, GST and AChE activities in all tested life stages (nauplii, metanauplii and juvenile) (Jeyavani et al., 2022). The cationic amino polystyrene nanoparticles inhibiting ChE and Carboxylesterase (CbE) activity in 48 h aged larvae (Bergami et al., 2017; Varó et al., 2019). However, when microplastics were tested together with pesticides, they were still able to cause a decline in ChE activity, confirming their neurotoxic effect (Albendín et al., 2021). In addition to pollution due to organic compounds, the likely effects of inorganic compounds have been extensively studied. Cu significantly caused high oxidative stress, through the activation of GSH and Thiobarbituric acid reactive substances (TBARS) activity at all times of exposure (24 h, 48 h and 72 h) (Cimen et al., 2020). The same authors reported that Cu treatment caused a significant increase of lipid, protein and carbohydrate contents (Umarani et al., 2012). A significant increase of GSH and TBARS activity after 24 h, 48 h and 72 h, was reported in *Artemia* nauplii exposed to copper oxide (CuO) (Cimen et al., 2020; Madhav et al., 2017). Furthermore, CeO_2 increased ChE activity, SnO_2 inhibited ChE and GST levels, and Fe_3O_4 increased ChE and CAT activities (Zhu et al., 2017a). Combinations of Fe_2O_3 - nanoparticles were also able to increase the malondialdehyde (MDA) content, Total Antioxidant Capacity (T-AOC), ROS and antioxidant enzymes activities (Wang et al., 2017). Similarly, the cerium oxide (CeO_2) and Cu-TiO_2 combinations showed high impact on ROS activity (Sugantharaj David et al., 2017). Moreover, adults of *Artemia* were also exposed to $CdCl_2$, iron chloride ($FeCl_3$) and their mixtures for 1, 3 and 7 days. In particular, the exposure to $CdCl_2$ was able to increase CAT, GPX and SOD activities and metallothionein concentration in tissues more than $FeCl_3$ and their mixtures (Mohamed et al., 2014).

2.6. Gene expression

Very few studies have evaluated the expression of genes involved in stress response or development in *Artemia* models exposed to nanoparticles, Polycyclic Aromatic hydrocarbons (PAHs) and food/cosmetic preservatives. For instance, anionic carboxylated and cationic amino polystyrene nanoparticles up-regulate the expression levels of two target genes, *clap* and *cstb*, involved in brine shrimp larval growth and molting (Bergami et al., 2017), while carbon black (CB) nanoparticles, normally used to remove oil spills in wastewater, were able to increase the expression levels of *hsp70* gene (Rodd et al., 2014; Tretyakova et al., 2021). Similarly, methylparaben (MeP), mainly used as preservative of personal care products, food and pharmaceuticals, induced down-regulation of *CAT* expression levels only with acute exposures (24 h) of *Artemia* (Comeche et al., 2017). A recent paper, also demonstrated that benzo-k-fluornthene was able to up-regulated the expression level of nine genes involved in stress response (*hsp26, hsp60, hsp70, COXI* and *COXIII*) and in developmental process (*HAD-like, CDC48, UCP2* and *tcp*), despite its exposure has not caused toxic effect on nauplii and adult survival (Albarano et al., 2022).

3. *A. salina* and *A. franciscana* as models for genotoxicity detection

Genotoxicity assay have been historically applied to perform the ecotoxicity assessment of several mutagens in both freshwater and marine model species by evaluating the amount of DNA damage, including double-strand breaks, gene mutation and aneuploidy (Brendler-Schwaab et al., 2005; Depledge, 1998; Frenzilli et al., 2009). As mentioned in the introduction section, only a few studies have reported the genotoxicity in *Artemia* individuals, so far, probably due to the scarce availability of standardized protocols. In 2013, single-cell electrophoresis techniques for employing Comet Assay and Polymerase Chain Reaction (PCR) of genetic markers have been set-up in *A. franciscana* larvae to evaluate the amount of DNA damage (Chandra and Sukumaran, 2019; Del Carmen Guzmán-Martínez et al., 2013; Sukumaran and Grant, 2013a, 2013b, 2013c). For instance, Inter-Simple Sequence Repeat (ISSR) and population growth rate approaches were performed to compare the transgenerational effects of genotoxicity between sexual (*A. franciscana*) and asexual (*Artemia parthenogenetica*) species of brine shrimp exposed to the mutagen ethylmethane sulfonate (EMS) (Sukumaran and Grant, 2013a, 2013b). Genomic template stability (GTS) quantified in parental individuals and F1/F2 generations revealed that GST was particularly divergent between the species that reproduce sexually and asexually. In fact, GTS reduced at all concentrations tested (85, 110, 135 and 160 ppm) in parental *A. franciscana* and in both parental and F1 generations of *A. parthenogenetica*, associated to a lesser fecundity, growth, survival and population growth-rate of asexual F1/F2 populations. In contrast, GTS did not decrease in the F1 of *A. franciscana* with a significant restore in F1 and F2 life-cycle parameters that suggests a possible recovery over generations in species that reproduce sexually (Sukumaran and Grant, 2013a). These results were deeper explored by Comet Assay revealing that the chronic exposure to EMS induced a

dose-dependent increase (concentration range= 0.78-1.48 mM) of Tail DNA percentage (%TDNA) in nauplii of both *Artemia* species (Sukumaran and Grant, 2013c). Comet Assay were also performed to evaluate the genotoxicity of two personal care products, triclosan (TCS) and triclocarban (TCC) on *A. salina* larvae measured as Olive Tail Moment (OTM) and %TDNA (Xu et al., 2015). Results showed that TCS (171 μg/L) induced significant DNA damage in *Artemia* coelomocytes at 24 h of exposure while TCC (18 μg/L) was particularly effective at both 12 h and 24 h. Moreover, single-cell gel electrophoresis and apoptotic frequency assays (Annexin V-FITC/PI assay) also demonstrated that TCS and TCC triggered early apoptosis (22.3 % and 45.8 %) at 24 h. These evidences suggested two possible scenarios inducing DNA damage that involve the release of ROS or TCS/TCC mutagens that directly act as DNA intercalants (Xu et al., 2015).

4. Concluding remarks

The *Artemia* bioassay is one of the most used approaches both in toxicological and eco-toxicological studies because of several attractive features such as, high market availability, abundance of cysts, simple manipulation and maintenance in laboratory conditions. As reported above, the hatching rate of cysts, reproductive success, swimming velocity, survival and gene expression of key genes involved in stress response and molting, was found to be quite interfered in *Artemia* by several contaminants widely found in natural environments. Among ecotoxicological studies reviewed in the present work, mortality and rates were the most employed, representing about the 50 % of the whole investigations analyzed (Fig. 1). This result might be probably due to the extremely easy and fast ecotoxicological approaches that allows the evaluation of *Artemia* survival in experimental conditions. In contrast, quantitative analyses revealed that hatching (16%), enzymatic biomarkers (12%), development (11%), bioaccumulation (9%) and gene expression (3%) were poorly used, which suggests the need of employing more diversified tools for studying ecotoxicity in *Artemia* models. Moreover, it should be considered that genotoxic approaches have also been partly used, with existing data being quite old and scattered. Nevertheless, molecular data clearly showed that the negative effects might be significantly detected at the DNA level by evaluating the presence of double strand breaks and abnormal mRNA expression of key genes, even at low doses of toxicants as corroborated by literature analysis. The impairment of suchendpoints was also correlated to several physiological responses contemporarily occurring in the organism. Interestingly, NGS approaches recently added a great amount of sequences to be used as novel tools for studying the molecular response of *Artemia* spp. to environmental stressors. Combining the great simplicity of manipulating *Artemia* spp. cysts and the easy-to-use and fast methods for detecting DNA damage or gene expression (*e.g.* Comet Assay, RT-qPCR), new possible scenarios might be opened in the future for this poorly sensitive crustacean. Thus, the present review highlights that besides the usefulness of *Artemia* models in ecotoxicology through the classical endpoints (*e.g.* mortality, hatching), genotoxicity could represent an additional tool due to its high sensitivity and reliability.

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CRediT authorship contribution statement

Luisa Albarano: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Nadia Ruocco:** Writing – original draft, Writing – review & editing. **Giusy Lofrano:** Writing – review & editing. **Marco Guida:** Writing – review & editing. **Giovanni Libralato:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Abatzopoulos, T.J., Kappas, I., Bossier, P., Sorgeloos, P., Beardmore, J.A., 2002. (Crustacea : Anostraca) 333–344.

Abushaala, N.M., Elfituri, A.M., Zulkifli, S.Z., 2021. Histological study of gonadal tissues of adult *Artemia salina* (Linnaeus 1758) and immunohistochemistry by caspase 3 and HSP70 to detect specific apoptosis markers on gonadal tissues after exposure to TBTCL. Open Vet. J. 11, 112–120. https://doi.org/10.4314/ovj.v11i1.17.

Adeogun, O.O., Maroyi, A., Afolayan, A.J., 2018. Comparative evaluation of essential oils from lippia javanica L leaf obtained by two methods and their effect on *artemia salina L.* Trop. J. Pharm. Res. 17, 105–115. https://doi.org/10.4314/tjpr.v17i1.16.

Albano, M., Panarello, G., Di Paola, D., Capparucci, F., Crupi, R., Gugliandolo, E., Spanò, N., Capillo, G., Savoca, S., 2021. The influence of polystyrene microspheres abundance on development and feeding behavior of *Artemia salina* (Linnaeus, 1758). Appl. Sci. 11 https://doi.org/10.3390/app11083352.

Albarano, L., Serafini, S., Toscanesi, M., Trifuoggi, M., Zupo, V., Costantini, M., Vignati, D.A.L., Guida, M., Libralato, G., 2022. Genotoxicity Set Up in *Artemia franciscana* Nauplii and Adults. Water 14.

Albendín, M.G., Aranda, V., Coello, M.D., González-Gómez, C., Rodríguez-Barroso, R., Quiroga, J.M., Arellano, J.M., 2021. Pharmaceutical products and pesticides toxicity associated with microplastics (Polyvinyl chloride) in *artemia salina*. Int. J. Environ. Res. Public Health 18. https://doi.org/10.3390/ijerph182010773.

Álvarez-Alarcón, N., Osorio-Méndez, J.J., Ayala-Fajardo, A., Garzón-Méndez, W.F., Garavito-Aguilar, Z.V., 2021. Zebrafish and *Artemia salina* in vivo evaluation of the recreational 25C-NBOMe drug demonstrates its high toxicity. Toxicol. Reports 8, 315–323. https://doi.org/10.1016/j.toxrep.2021.01.010.

Alyürük, H., Çavaş, L., 2013. Toxicities of diuron and irgarol on the hatchability and early stage development of *Artemia salina*. Turkish J. Biol. 37, 151–157. https://doi.org/10.3906/biy-1205-39.

Alyuruk, H., Demir, G.K., Cavas, L., 2013. A video tracking based improvement of acute toxicity test on *Artemia salina*. Mar. Freshw. Behav. Physiol. 46, 251–266. https://doi.org/10.1080/10236244.2013.814224.

Anaya-Esparza, L.M., González-Silva, N., Yahia, E.M., González-Vargas, O.A., Montalvo-González, E., Pérez-Larios, A., 2019. Effect of TiO2-ZnO-MgO mixed oxide on microbial growth and toxicity against *artemia salina*. Nanomaterials 9. https://doi.org/10.3390/nano9070992.

Arulvasu, C., Jennifer, S.M., Prabhu, D., Chandhirasekar, D., 2014. Toxicity effect of silver nanoparticles in brine shrimp *artemia*. Sci. World J. 2014 https://doi.org/10.1155/2014/256919.

Asadpour, Y.A., Nejatkhah Manavi, P., Baniamam, M., 2013. Evaluating the Bioaccumulation of Nickel and Vanadium and their effects on the Growth of *Artemia urmiana* and *A. franciscana*. Iran. J. Fish. Sci. 12, 183–192.

Asem, A., Rastegar-Pouyani, N., de Los Ríos-Escalante, P., 2010. El género *Artemia* leach, 1819 (Crustacea: Branchiopoda). I. Descripciones taxonómicas verdaderas y falsas. Lat. Am. J. Aquat. Res. 38, 501–506. https://doi.org/10.3856/vol38-issue3-fulltext-14.

Atchou, K., Lawson-Evi, P., Diallo, A., Eklu-Gadegbeku, K., 2021. Toxicological evaluation of the dried hydroethanolic extract of *Amaranthus spinosus L.* roots in *Artemia salina* larvae and *Sprague Dawley* rats. Clin. Phytoscience 7, 1–9. https://doi.org/10.1186/s40816-021-00304-1.

Ates, M., Daniels, J., Arslan, Z., Farah, I.O., Rivera, H.F., 2013. Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: Effects of particle size and solubility on toxicity. Environ. Sci. Process. Impacts 15, 225–233. https://doi.org/10.1039/c2em30540b.

Baxevanis, A.D., Maniatsi, S., Kouroupis, D., Marathiotis, K., Kappas, I., Kaiser, H., Abatzopoulos, T.J., 2014. Genetic identification of South African *Artemia* species: Invasion, replacement and co-occurrence. J. Mar. Biol. Assoc. United Kingdom 94, 775–785. https://doi.org/10.1017/S0025315414000083.

Beattie, K.A., Ressler, J., Wiegand, C., Krause, E., Codd, G.A., Steinberg, C.E.W., Pflugmacher, S., 2003. Comparative effects and metabolism of two microcystins and nodularin in the brine shrimp *Artemia salina*. Aquat. Toxicol. 62, 219–226. https://doi.org/10.1016/S0166-445X(02)00091-7.

Bengtson, D.A., Léger, P., Sorgeloos, P., 2018. Use of *artemia* as a food source for aquaculture. Artemia Biol 255–286. https://doi.org/10.1201/9781351069892.

Benmeddah, M.M., Bouzidi, M.A., Toumi-Benali, F., Amar, Y., Bouazza, S., Dellal, A., Dif, M.M., 2020. Ecobiological study of *Artemia salina L.* And first determination of the toxicity of ammonium sulphate. Limnol. Rev. 18, 109–113. https://doi.org/10.2478/limre-2018-0012.

Beňová, K., Dvořák, P., Falis, M., Sklenář, Z., 2007. Interaction of low doses of ionizing radiation, potassium dichromate and cadmium chloride in *Artemia franciscana* biotest. Acta Vet. Brno 76, 35–40. https://doi.org/10.2754/avb200776010035.

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp *Artemia franciscana* larvae. Ecotoxicol. Environ. Saf. 123, 18–25. https://doi.org/10.1016/j.ecoenv.2015.09.021.

Bergami, E., Pugnalini, S., Vannuccini, M.L., Manfra, L., Faleri, C., Savorelli, F., Dawson, K.A., Corsi, I., 2017. Long-term toxicity of surface-charged polystyrene nanoplastics to marine planktonic species *Dunaliella tertiolecta* and *Artemia franciscana*. Aquat. Toxicol. 189, 159–169. https://doi.org/10.1016/j.aquatox.2017.06.008.

Bergsten-Torralba, L.R., Magalhães, D.P., Giese, E.C., Nascimento, C.R.S., Pinho, J.V.A., Buss, D.F., 2020. Toxicity of three rare earth elements, and their combinations to algae, microcrustaceans, and fungi. Ecotoxicol. Environ. Saf. 201, 110795 https://doi.org/10.1016/j.ecoenv.2020.110795.

Beristain, P., Gajardo, G., Bossier, P., 2010. Species-specific RFLP pattern in the Heat Shock Protein26 gene (Hsp26): A single-locus tool for species identification and experimental testing of habitat-induced isolation in the New World *Artemia* species. Mol. Ecol. Resour. 10, 229–231. https://doi.org/10.1111/j.1755-0998.2009.02744.x.

Bhuvaneshwari, M., Thiagarajan, V., Nemade, P., Chandrasekaran, N., Mukherjee, A., 2018. Toxicity and trophic transfer of P25 TiO2 NPs from *Dunaliella salina* to *Artemia salina*: Effect of dietary and waterborne exposure. Environ. Res. 160, 39–46. https://doi.org/10.1016/j.envres.2017.09.022.

Borba, F.H., Leichtweis, J., Bueno, F., Pellenz, L., Inticher, J.J., Seibert, D., 2019. Pollutant removal and acute toxicity assessment (*Artemia salina*) of landfill leachate treated by photo-Fenton process mediated by oxalic acid. J. Water Process Eng. 28, 159–168. https://doi.org/10.1016/j.jwpe.2019.01.017.

Braguini, W.L., Alves, B.B., Pires, N.V., 2019. Toxicity assessment of Lavandula officinalis extracts in Brine Shrimp (*Artemia salina*). Toxicol. Mech. Methods 29, 411–420. https://doi.org/10.1080/15376516.2019.1567892.

Brendler-Schwaab, S., Hartmann, A., Pfuhler, S., Speit, G., 2005. The in vivo comet assay: Use and status in genotoxicity testing. Mutagenesis 20, 245–254. https://doi.org/10.1093/mutage/gei033.

Brix, K.V., Cardwell, R.D., Adams, W.J., 2003. Chronic toxicity of arsenic to the Great Salt Lake brine shrimp, *Artemia franciscana*. Ecotoxicol. Environ. Saf. 54, 169–175. https://doi.org/10.1016/S0147-6513(02)00054-4.

Brix, K.V., DeForest, D.K., Cardwell, R.D., Adams, W.J., 2004. Derivation of a chronic site-specific water quality standard for selenium in the Great Salt Lake, Utah, USA. Environ. Toxicol. Chem. 23, 606–612. https://doi.org/10.1897/02-623.

Brix, K.V., Gerdes, R.M., Adams, W.J., Grosell, M., 2006. Effects of copper, cadmium, and zinc on the hatching success of brine shrimp (*Artemia franciscana)*. Arch. Environ. Contam. Toxicol. 51, 580–583. https://doi.org/10.1007/s00244-005-0244-z.

Browne, R.A., 1980. Acute response versus reproductive performance in five strains of brine shrimp exposed to copper sulphate. Mar. Environ. Res. 3, 185–193. https://doi.org/10.1016/0141-1136(80)90026-4.

Bustos-Obregon, E., Vargas, Á., 2010. Chronic toxicity bioassay with populations of the crustacean *Artemia salina* exposed to the organophosphate diazinon. Biol. Res. 43, 357–362. https://doi.org/10.4067/S0716-97602010000300013.

Caldwell, G.S., Bentley, M.G., Olive, P.J.W., 2003. The use of a brine shrimp (*Artemia salina*) bioassay to assess the toxicity of diatom extracts and short chain aldehydes. Toxicon 42, 301–306. https://doi.org/10.1016/S0041-0101(03)00147-8.

Castritsi-Catharios, J., Alambritis, G., Miliou, H., Cotou, E., Zouganelis, G.D., 2014. Comparative toxicity of "tin free" self-polishing copolymer antifouling paints and their inhibitory effects on larval development of a non-target organism. Mater. Sci. Appl. 05, 158–169. https://doi.org/10.4236/msa.2014.53020.

Castritsi-Catharios, J., Syriou, V., Miliou, H., Zouganelis, G.D., 2013. Toxicity effects of bisphenol A to the nauplii of the brine shrimp *Artemia franciscana*. J. Biol. Res. 19, 38–45.

Cavion, F., Pelin, M., Ponti, C., Della Loggia, R., Tubaro, A., Sosa, S., 2022. Ecotoxicological Impact of the Marine Toxin Palytoxin on the Micro-Crustacean *Artemia franciscana*. Mar. Drugs 20, 1–14. https://doi.org/10.3390/md20020081.

Cavrois-Rogacki, T., Rolland, A., Migaud, H., Davie, A., Monroig, O., 2020. Enriching *Artemia* nauplii with selenium from different sources and interactions with essential fatty acid incorporation. Aquaculture 520, 734677. https://doi.org/10.1016/j.aquaculture.2019.734677.

Chan, W., Shaughnessy, A.E.P., van den Berg, C.P., Garson, M.J., Cheney, K.L., 2021. The validity of brine shrimp (*Artemia* Sp.) toxicity assays to assess the ecological function of marine natural products. J. Chem. Ecol. 47, 834–846. https://doi.org/10.1007/s10886-021-01264-z.

Chandra, S., Sukumaran, S., 2019. Physiological, biochemical and neurochemical responses of *Cirrhinus mrigala* upon short term exposure to cerium oxide. Int. J. Aquat. Biol. 7, 368–373. https://doi.org/10.22034/ijab.v7i6.657.

Cimen, I.C.C., Danabas, D., Ates, M., 2020. Comparative effects of Cu (60–80 nm) and CuO (40 nm) nanoparticles in *Artemia salina*: Accumulation, elimination and oxidative stress. Sci. Total Environ. 717, 137230 https://doi.org/10.1016/j.scitotenv.2020.137230.

CNR, 2003. Metodo 8060 di valutazione della tossicità acuta con *Artemia* sp. Manuali e Linee Guida-Metodi analitici per le acque. APAT IRSA CNR, pp. 1043–1049. Vol. 3.

Comeche, A., Martín-Villamil, M., Picó, Y., Varó, I., 2017. Effect of methylparaben in *Artemia franciscana*. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 199, 98–105. https://doi.org/10.1016/j.cbpc.2017.04.004.

Cortés, A.A., Sánchez-Fortún, S., García, M., Martínez, H., Bartolomé, M.C., 2018. Toxicological assessment of binary mixtures and individually of chemical compounds used in reverse osmosis desalination on *artemia franciscana* nauplii. Lat. Am. J. Aquat. Res. 46, 673–682. https://doi.org/10.3856/vol46-issue4-fulltext-4.

Damasceno, É.P., de Figuerêdo, L.P., Pimentel, M.F., Loureiro, S., Costa-Lotufo, L.V., 2017. Prediction of toxicity of zinc and nickel mixtures to *Artemia* sp. at various salinities: From additivity to antagonism. Ecotoxicol. Environ. Saf. 142, 322–329. https://doi.org/10.1016/j.ecoenv.2017.04.020.

Danabas, D., Ates, M., Ertit Tastan, B., Cicek Cimen, I.C., Unal, I., Aksu, O., Kutlu, B., 2020. Effects of Zn and ZnO Nanoparticles on *Artemia salina* and *Daphnia magna* Organisms: Toxicity, Accumulation and Elimination. Sci. Total Environ. 711, 134869 https://doi.org/10.1016/j.scitotenv.2019.134869.

de Campos, B.G., Figueiredo, J., Perina, F., Abessa, D.M., de, S., Loureiro, S., Martins, R., 2021. Occurrence, effects and environmental risk of antifouling biocides (EU PT21): Are marine ecosystems threatened? Crit. Rev. Environ. Sci. Technol. 0, 1–32. https://doi.org/10.1080/10643389.2021.1910003.

De Vos, S., Van Stappen, G., Sorgeloos, P., Vuylsteke, M., Rombauts, S., Bossier, P., 2019. Identification of salt stress response genes using the *Artemia* transcriptome. Aquaculture 500, 305–314. https://doi.org/10.1016/j.aquaculture.2018.09.067.

Deese, R.D., Leblanc, M.R., Cook, R.L., 2016. Surfactant toxicity to *Artemia franciscana* and the influence of humic acid and chemical composition. Environ. Chem. 13, 507–516. https://doi.org/10.1071/EN15108.

Del Carmen Guzmán-Martínez, M., Ramírez-Romero, P., La Cruz, K.D., De los Ángeles Aguilar Santamaría, M., 2013. Protocolo para obtener células en suspensión de *Artemia franciscana* para realizar electroforesis unicelular alcalina. Hidrobiologica 23, 133–137.

Depledge, M.H., 1998. The ecotoxicological significance of genotoxicity in marine invertebrates. Mutat. Res. - Fundam. Mol. Mech. Mutagen. 399, 109–122. https://doi.org/10.1016/S0027-5107(97)00270-4.

Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., Sorgeloos, P., 2013. Rotifers, Artemia and copepods as live feeds for fish larvae in aquaculture, Advances in Aquaculture Hatchery Technology. Woodhead Publishing Limited. https://doi.org/10.1533/9780857097460.1.157.

Duan, D., Liu, G., Yao, P., Wang, H., 2016. The effects of organic compounds on inactivation efficacy of *Artemia salina* by neutral electrolyzed water. Ocean Eng 125, 31–37. https://doi.org/10.1016/j.oceaneng.2016.08.003.

Ekonomou, G., Lolas, A., Castritsi-Catharios, J., Neofitou, C., Zouganelis, G.D., Tsiropoulos, N., Exadactylos, A., 2019. Mortality and effect on growth of *Artemia franciscana* exposed to two common organic pollutants. Water (Switzerland) 11. https://doi.org/10.3390/w11081614.

Evidente, A., Andolfi, A., Fiore, M., Spanu, E., Franceschini, A., Maddau, L., 2007. Diplofuranones A and B, two further new 4-monosubstituted 2(3H)-dihydrofuranones produced by *Diplodia corticola*, a fungus pathogen of cork oak. Arkivoc 2007, 318–328. https://doi.org/10.3998/ark.5550190.0008.728.

Evidente, A., Cimmino, A., Andolfi, A., Vurro, M., Zonno, M.C., Cantrell, C.L., Motta, A., 2008. Phyllostictines A-D, oxazatricycloalkenones produced by Phyllosticta cirsii, a potential mycoherbicide for Cirsium arvense biocontrol. Tetrahedron 64, 1612–1619. https://doi.org/10.1016/j.tet.2007.12.010.

Favilla, M., Macchia, L., Gallo, A., Altomare, C., 2006. Toxicity assessment of metabolites of fungal biocontrol agents using two different (*Artemia salina* and *Daphnia magna*) invertebrate bioassays. Food Chem. Toxicol. 44, 1922–1931. https://doi.org/10.1016/j.fct.2006.06.024.

Fichet, D., Miramand, P., 1998. Vanadium toxicity to three marine invertebrates larvae: Crassostrea gigas, P*aracentrotus lividus* and *Artemia salina*. Chemosphere 37, 1363–1368. https://doi.org/10.1016/S0045-6535(98)00118-0.

Frenzilli, G., Nigro, M., Lyons, B.P., 2009. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat. Res. - Rev. Mutat. Res. 681, 80–92. https://doi.org/10.1016/j.mrrev.2008.03.001.

Frías-Espericueta, M.G., Soto-Jiménez, M.F., Abad-Rosales, S.M., López-Morales, M.L., Trujillo-Alvarez, S.Y., Arellano-Sarabia, J.A., Quintero-Alvarez, J.M., Osuna-López, J.I., Bojórquez, C., Aguilar-Juárez, M., 2022. Physiological and histological effects of cadmium, lead, and combined on *Artemia franciscana*. Environ. Sci. Pollut. Res. 29, 7344–7351. https://doi.org/10.1007/s11356-021-16147-9.

Gajardo, G.M., Beardmore, J.A., 2012. The brine shrimp *Artemia*: Adapted to critical life conditions. Front. Physiol. (3 JUN), 1–8. https://doi.org/10.3389/fphys.2012.00185.

Gambardella, C., Costa, E., Piazza, V., Fabbrocini, A., Magi, E., Faimali, M., Garaventa, F., 2015. Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar. Environ. Res. 111, 41–49. https://doi.org/10.1016/j.marenvres.2015.06.001.

Gambardella, C., Mesarič, T., Milivojević, T., Sepčić, K., Gallus, L., Carbone, S., Ferrando, S., Faimali, M., 2014. Effects of selected metal oxide nanoparticles on *Artemia salina* larvae: Evaluation of mortality and behavioural and biochemical responses. Environ. Monit. Assess. 186, 4249–4259. https://doi.org/10.1007/s10661-014-3695-8.

Triantaphyllidis, GeorgeV., Abatzopoulos, TheodoreJ., Sorgeloos, Patrick, 1998. Review of the biogeography of the genus *Artemia* (Crustacea, Anostraca). J. Biogeogr. 25, 213–226.

Gutner-Hoch, E., Martins, R., Maia, F., Oliveira, T., Shpigel, M., Weis, M., Tedim, J., Benayahu, Y., 2019. Toxicity of engineered micro- and nanomaterials with antifouling properties to the brine shrimp *Artemia salina* and embryonic stages of the sea urchin Paracentrotus lividus. Environ. Pollut. 251, 530–537. https://doi.org/10.1016/j.envpol.2019.05.031.

Hadjispyrou, S., Kungolos, A., Anagnostopoulos, A., 2001. Toxicity, bioaccumulation, and interactive effects of organotin, cadmium, and chromium on *Artemia franciscana*. Ecotoxicol. Environ. Saf. 49, 179–186. https://doi.org/10.1006/eesa.2001.2059.

Hafiz, I., Fitri, K., Helvin, S.J., 2019. The Phytochemical Screning and Assesment of Bioactivity of Pagoda Flower (Clerodendrum paniculatum L.) Using Brine Shrimp (*Artemia salina*) Lethality Assay. Asian J. Pharm. Res. Dev. 7, 10–13. https://doi.org/10.22270/ajprd.v7i3.527.

Hamrun, N., Nabilah, T., Hasyim, R., Ruslin, M., Dammar, I., Anugrah, M., 2020. Toxicity test of bioactive red alga extract eucheuma spinosum on shrimp *artemia*

salina leach. Syst. Rev. Pharm. 11, 672–676. https://doi.org/10.31838/srp.2020.5.94.
Hollergschwandtner, E., Schwaha, T., Neumüller, J., Kaindl, U., Gruber, D., Eckhard, M., Stöger-Pollach, M., Reipert, S., 2017. Novel mesostructured inclusions in the epidermal lining of *Artemia franciscana* ovisacs show optical activity. PeerJ 2017, 1–25. https://doi.org/10.7717/peerj.3923.
Honda, N.K., Gonçalves, K., Brandão, L.F.G., Coelho, R.G., Micheletti, A.C., Spielmann, A.A., Canêz, L.S., 2016. Screening of Lichen Extracts Using Tyrosinase Inhibition and Toxicity Against *Artemia salina*. Orbital - Electron. J. Chem. 8 https://doi.org/10.17807/orbital.v8i3.842.
Huylmans, A.K., Toups, M.A., MacOn, A., Gammerdinger, W.J., Vicoso, B., 2019. Sex-biased gene expression and dosage compensation on the *artemia franciscana* Z-chromosome. Genome Biol. Evol. 11, 1033–1044. https://doi.org/10.1093/gbe/evz053.
Indriaty, I., Ginting, B., Hasballah, K., 2022. Assessment cytotoxic assay of Rhizophora plants mangrove using brine shrimp (*Artemia salina L*) model. IOP Conf. Ser. Earth Environ. Sci. 951 https://doi.org/10.1088/1755-1315/951/1/012070.
Jayasekara, S., Drown, D.B., Sharma, R.P., 1986. Tolerance to cadmium and cadmium-binding ligands in great salt lake brine shrimp (*Artemia salina*). Ecotoxicol. Environ. Saf. 11, 23–30. https://doi.org/10.1016/0147-6513(86)90026-6.
Jennings, J.R., Rainbow, P.S., 1979. Accumulation of cadmium by *Artemia salina*. Mar. Biol. 51, 47–53. https://doi.org/10.1007/BF00389030.
Jeyavani, J., Sibiya, A., Bhavaniramya, S., Mahboob, S., Al-Ghanim, K.A., Nisa, Z., Riaz, M.N., Nicoletti, M., Govindarajan, M., Vaseeharan, B., 2022. Toxicity evaluation of polypropylene microplastic on marine microcrustacean *Artemia salina*: An analysis of implications and vulnerability. Chemosphere 296, 133990. https://doi.org/10.1016/j.chemosphere.2022.133990.
Yu, Jie, 2018. *Artemia* spp. Model -A Well-Established Method for Rapidly Assessing the Toxicityon an Environmental Perspective. Med. Res. Arch. 6, 1–15.
Jung, S.M., Bae, J.S., Kang, S.G., Son, J.S., Jeon, J.H., Lee, H.J., Jeon, J.Y., Sidharthan, M., Ryu, S.H., Shin, H.W., 2017. Acute toxicity of organic antifouling biocides to phytoplankton Nitzschia pungens and zooplankton *Artemia* larvae. Mar. Pollut. Bull. 124, 811–818. https://doi.org/10.1016/j.marpolbul.2016.11.047.
P., S. KV, J., Ebeneezar, S., Nair, V., 2021. Dietary supplementation of microalgae, Aurantiochytrium sp. and co-feeding with *Artemia* enhances the growth, stress tolerance and survival in P*enaeus monodon* (Fabricius, 1798) post larvae Aquaculture 533 https://doi.org/10.1016/j.aquaculture.2020.736176.
Kappas, I., Abatzopoulos, T.J., Van Hoa, N., Sorgeloos, P., Beardmore, J.A., 2004. Genetic and reproductive differentiation of *Artemia franciscana* in a new environment. Mar. Biol. 146, 103–117. https://doi.org/10.1007/s00227-004-1420-9.
Khaleel, R.I., 2019. Bio-toxicity study of some selected plant by *Artemia salina* (Leach) test. Plant Arch. 19, 2847–2850.
Khoshnood, R., Jaafarzadeh, N., Jamili, S., Farshchi, P., Taghavi, L., 2017. Acute toxicity of TiO2, CuO and ZnO nanoparticles in brine shrimp, *Artemia franciscana*. Iran. J. Fish. Sci. 16, 1287–1296.
Kim, L., Cui, R., Kwak, J.Il, An, Y.J., 2022. Sub-acute exposure to nanoplastics via two-chain trophic transfer: From brine shrimp Artemia franciscana to small yellow croaker *Larimichthys polyactis*. Mar. Pollut. Bull. 175, 113314 https://doi.org/10.1016/j.marpolbul.2021.113314.
Kim, L., Kim, S.A., Kim, T.H., Kim, J., An, Y.J., 2021. Synthetic and natural microfibers induce gut damage in the brine shrimp *Artemia franciscana*. Aquat. Toxicol. 232, 105748 https://doi.org/10.1016/j.aquatox.2021.105748.
Kokkali, V., Katramados, I., Newman, J.D., 2011. Monitoring the effect of metal ions on the mobility of *Artemia salina* Nauplii. Biosensors 1, 36–45. https://doi.org/10.3390/bios1020036.
Koutsaftis, A., Aoyama, I., 2008. Toxicity of Diuron and copper pyrithione on the brine shrimp, *Artemia franciscana*: The effects of temperature and salinity. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 43, 1581–1585. https://doi.org/10.1080/10934520802329794.
Koutsaftis, A., Aoyama, I., 2007. Toxicity of four antifouling biocides and their mixtures on the brine shrimp *Artemia salina*. Sci. Total Environ. 387, 166–174. https://doi.org/10.1016/j.scitotenv.2007.07.023.
Kumar, G.R., Babu, D.E., 2015. Effect of Light, Temperature and salinity on the growth of *Artemia*. Int. J. Eng. Sci. Invent. 4, 7–14.
Lapetaje, J.E., Creencia, E.C., 2019. Room-temperature synthesis of aryl-substituted benzimidazoles and their toxicity against *Artemia salina*. Mindanao J. Sci. Technol. 17, 31–55.
Lavtizar, V., Kimura, D., Asaoka, S., Okamura, H., 2018. The influence of seawater properties on toxicity of copper pyrithione and its degradation product to brine shrimp *Artemia salina*. Ecotoxicol. Environ. Saf. 147, 132–138. https://doi.org/10.1016/j.ecoenv.2017.08.039.
Lee, D.H., Eom, H.J., Kim, M., Jung, J.H., Rhee, J.S., 2017. Non-target effects of antifouling agents on mortality, hatching success, and acetylcholinesterase activity in the brine shrimp *Artemia salina*. Toxicol. Environ. Health Sci. 9, 237–243. https://doi.org/10.1007/s13530-017-0326-0.
Lee, J.M., Cho, B.C., Park, J.S., 2022. Transcriptomic analysis of brine shrimp *Artemia franciscana* across a wide range of salinities. Mar. Genomics 61, 100919. https://doi.org/10.1016/j.margen.2021.100919.
Libralato, G., 2014. The case of *Artemia* spp. in nanoecotoxicology. Mar. Environ. Res. 101, 38–43. https://doi.org/10.1016/j.marenvres.2014.08.002.
Libralato, G., Losso, C., Ghirardini, A.V., 2007. Toxicity of untreated wood leachates towards two saltwater organisms (*Crassostrea gigas* and *Artemia franciscana*). J. Hazard. Mater. 144, 590–593. https://doi.org/10.1016/j.jhazmat.2006.10.082.
Libralato, G., Prato, E., Migliore, L., Cicero, A.M., Manfra, L., 2016. A review of toxicity testing protocols and endpoints with *Artemia* spp. Ecol. Indic. 69, 35–49. https://doi.org/10.1016/j.ecolind.2016.04.017.
Libralato, G., Volpi Ghirardini, A., Avezzù, F., 2010. Seawater ecotoxicity of monoethanolamine, diethanolamine and triethanolamine. J. Hazard. Mater. 176, 535–539. https://doi.org/10.1016/j.jhazmat.2009.11.062.
Longo, C., Trani, R., Marzano, C.N., Mercurio, M., Lazic, T., Cotugno, P., Santobianchi, E., Gravina, M.F., 2021. Anti-fouling activity and toxicity of the Mediterranean alien sponge Paraleucilla magna Klautau, Monteiro & Borojevic, 2004 (Porifera, Calcarea). PeerJ 9, 1–18. https://doi.org/10.7717/peerj.12279.
Lu, J., Zhu, X., Tian, S., Lv, X., Chen, Z., Jiang, Y., Liao, X., Cai, Z., Chen, B., 2018. Graphene oxide in the marine environment: Toxicity to *Artemia salina* with and without the presence of Phe and Cd2+. Chemosphere 211, 390–396. https://doi.org/10.1016/j.chemosphere.2018.07.140.
Lu, Y., Xu, X., Li, T., Xu, Y., Wu, X., 2012. The use of a brine shrimp (*Artemia salina*) bioassay to assess the water quality in Hangzhou Section of Beijing-Hangzhou Grand Canal. Bull. Environ. Contam. Toxicol. 88, 472–476. https://doi.org/10.1007/s00128-011-0498-2.
Lu, Y., Xu, X.L., Meng, C., Zhou, J.Q., Sheng, J.J., Wu, C.K., Xu, S.W., 2013. The toxicity assay of *Artemia salina* as a biological model for the preliminary toxic evaluation of chemical pollutants. Adv. Mater. Res 726–731, 230–233. https://doi.org/10.4028/www.scientific.net/AMR.726-731.230.
Madhav, M.R., David, S.E.M., Kumar, R.S.S., Swathy, J.S., Bhuvaneshwari, M., Mukherjee, A., Chandrasekaran, N., 2017. Toxicity and accumulation of Copper oxide (CuO) nanoparticles in different life stages of *Artemia salina*. Environ. Toxicol. Pharmacol. 52, 227–238. https://doi.org/10.1016/j.etap.2017.03.013.
Magalhães, R.H.de P., Filho, Menezes, de, A.C.P., Ventura, M.V.A., Batista-Ventura, H.R. F., Castro, C.F.de S., Porfiro, C.A., 2021. Chemical profile and antioxidant, antibacterial, and cytotoxic activities on *Artemia salina* from the essential oil of leaves and xylopodium of Cochlospermum regium. Sci. Electron. Arch. 15 https://doi.org/10.36560/15120221506.
Manavi, P.N., Baniamam, M., 2011. Toxic effects of Nickel on *Artemia urmiana* and *Artemia franciscana*. Environ. Chem. 24, 53–56.
Manfra, L., Canepa, S., Piazza, V., Faimali, M., 2016. Lethal and sublethal endpoints observed for *Artemia* exposed to two reference toxicants and an ecotoxicological concern organic compound. Ecotoxicol. Environ. Saf. 123, 60–64. https://doi.org/10.1016/j.ecoenv.2015.08.017.
Manfra, L., De Nicola, E., Maggi, C., Zambianchi, E., Caramiello, D., Toscano, A., Cianelli, D., Cicero, A.M., 2011. Exposure of rotifers, crustaceans and sea urchins to produced formation waters and seawaters in the Mediterranean Sea. J. Mar. Biol. Assoc. United Kingdom 91, 155–161. https://doi.org/10.1017/S0025315410001037.
Manfra, L., Maggi, C., Bianchi, J., Mannozzi, M., Faraponova, O., Mariani, L., Onorati, F., Tornambè, A., Lamberti, C.V., Magaletti, E., 2010. Toxicity evaluation of produced formation waters after filtration treatment. Nat. Sci. 02, 33–40. https://doi.org/10.4236/ns.2010.21005.
Manfra, L., Savorelli, F., Di Lorenzo, B., Libralato, G., Comin, S., Conti, D., Floris, B., Francese, M., Gallo, M.L., Gartner, I., Guida, M., Leoni, T., Marino, G., Martelli, F., Palazzi, D., Prato, E., Righini, P., Rossi, E., Volpi Ghirardini, A., Migliore, L., 2015. Intercalibration of ecotoxicity testing protocols with *Artemia franciscana*. Ecol. Indic. 57, 41–47. https://doi.org/10.1016/j.ecolind.2015.04.021.
Maniatsi, S., Baxevanis, A.D., Kappas, I., Deligiannidis, P., Triantafyllidis, A., Papakostas, S., Bougiouklis, D., Abatzopoulos, T.J., 2011. Is polyploidy a persevering accident or an adaptive evolutionary pattern? The case of the brine shrimp *Artemia*. Mol. Phylogenet. Evol. 58, 353–364. https://doi.org/10.1016/j.ympev.2010.11.029.
Marta, S., Erica, S., Julián, B., Moreno-Garrido, I., Araujo Cristiano, V.M., 2020. Ingestion and bioaccumulation of polystyrene nanoplastics and their effects on the microalgal feeding of *Artemia franciscana*. Ecotoxicol. Environ. Saf. 188, 109853 https://doi.org/10.1016/j.ecoenv.2019.109853.
Mohamed, A.H., Sheir, S.K., Osman, G.Y., Abd-El Azeem, H.H., 2014. Toxic effects of heavy metals pollution on biochemical activities of the adult brine shrimp, *Artemia salina*. Can. J. Pure Appl. Sci. 8, 3019–3028.
Mutalipassi, M., Mazzella, V., Glaviano, F., Zupo, V., 2022. Constitutive and activated toxigenic activity of Halomicronema metazoicum (Cyanoprokaryota, Cyanophyta). Mar. Ecol. 1–11. https://doi.org/10.1111/maec.12697.
Nafisi Bahabadi, M., Torfi Mozanzadeh, M., Agh, N., Ahmadi, A., Yaghoubi, M., 2018. Enriched *Artemia* with L-lysine and DL-methionine on growth performance, stress resistance, and fatty acid profile of Litopenaeus vannamei postlarvae. J. Appl. Aquac. 30, 325–336. https://doi.org/10.1080/10454438.2018.1484838.
Ñañez Pacheco, G.K., Sanabio Maldonado, N.S., Pastrana Alta, R.Y., Aguilar Vitorino, H., 2021. Short exposure of *Artemia salina* to group-12 metals: Comparing hatchability, mortality, lipid peroxidation, and swimming speed. Ecotoxicol. Environ. Saf. 213 https://doi.org/10.1016/j.ecoenv.2021.112052.
Neves, R.A.F., Fernandes, T., Dos Santos, L.N., Nascimento, S.M., 2017. Toxicity of benthic dinoflagellates on grazing, behavior and survival of the brine shrimp *Artemia salina*. PLoS One 12, 1–17. https://doi.org/10.1371/journal.pone.0175168.
Nováková, J., Daňová, D., Strišková, K., Hromada, R., Mičková, H., Rabišková, M., 2007. Zinc and cadmium toxicity using a biotest with *Artemia franciscana*. Acta Vet. Brno 76, 635–642. https://doi.org/10.2754/avb200776040635.
Ntungwe, NE., Domínguez-Martín, E.M., Roberto, A., Tavares, J., Isca, V.M.S., Pereira, P., Cebola, M.-J., Rijo, P., 2020. *Artemia* species: An Important Tool to Screen General Toxicity Samples. Curr. Pharm. Des. 26, 2892–2908. https://doi.org/10.2174/1381612826666200406083035.
Nunes, B.S., Carvalho, F.D., Guilhermino, L.M., Van Stappen, G., 2006. Use of the genus *Artemia* in ecotoxicity testing. Environ. Pollut. 144, 453–462. https://doi.org/10.1016/j.envpol.2005.12.037.

Ocaranza-Joya, V.S., Manjarrez-Alcivar, I., Ruizgonzález, L.E., Guerrero-Galván, S.R., Vega-Villasante, F., 2019. Sensitivity of different stages of *Artemia franciscana* to potassium dichromate. Panam. J. Aquat. Sci. 14, 8–12.

Ozkan, Y., Altinok, I., Ilhan, H., Sokmen, M., 2016. Determination of TiO2 and AgTiO2 Nanoparticles in *Artemia salina*: Toxicity, Morphological Changes, Uptake and Depuration. Bull. Environ. Contam. Toxicol. 96, 36–42. https://doi.org/10.1007/s00128-015-1634-1.

Palácio, S.M., de Almeida, J.C.B., de Campos, É.A., Veit, M.T., Ferreira, L.K., Deon, M.T. M., 2021. Silver nanoparticles effect on *Artemia salina* and *Allium cepa* organisms: influence of test dilution solutions on toxicity and particles aggregation. Ecotoxicology 30, 836–850. https://doi.org/10.1007/s10646-021-02393-7.

Pati, A.C., Belmonte, G., 2003. Disinfection efficacy on cyst survival of *Artemia franciscana* (Crustacea), *Hexarthra fennica* (Rotifera) and *Fabrea salina* (Ciliophora). Mar. Biol. 142, 895–904. https://doi.org/10.1007/s00227-003-1026-7.

Peixoto, D., Amorim, J., Pinheiro, C., Oliva-Teles, L., Varó, I., de Medeiros Rocha, R., Vieira, M.N., 2019. Uptake and effects of different concentrations of spherical polymer microparticles on *Artemia franciscana*. Ecotoxicol. Environ. Saf. 176, 211–218. https://doi.org/10.1016/j.ecoenv.2019.03.100.

Pillard, D.A., Tapp, K.L., 2021. Influence of feeding and organism age on the acute toxicity of sodium bromide to *Artemia salina*. Ecotoxicology 30, 914–918. https://doi.org/10.1007/s10646-021-02417-2.

Qin, J.C., Zhang, Y.M., Gao, J.M., Bai, M.S., Yang, S.X., Laatsch, H., Zhang, A.L., 2009. Bioactive metabolites produced by Chaetomium globosum, an endophytic fungus isolated from Ginkgo biloba. Bioorganic Med. Chem. Lett. 19, 1572–1574. https://doi.org/10.1016/j.bmcl.2009.02.025.

Hamidi, R., Jovanova, M., Kadifkova Panovska, B., 2014. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina L.*) model. Maced. Pharm. Bull. 60, 9–18. https://doi.org/10.33320/maced.pharm.bull.2014.60.01.002.

Rahman, M.F., Pratama, A.A., 2019. Synthesis of Organonitrogen Compounds from Eugenol through the Ritter Reaction and the Toxicity Test on *Artemia salina* Leach. IOP Conf. Ser. Mater. Sci. Eng. 546 https://doi.org/10.1088/1757-899X/546/4/042036.

Rajabi, S., Ramazani, A., Hamidi, M., Naji, T., 2015. *Artemia salina* as a model organism in toxicity assessment of nanoparticles. DARU. J. Pharm. Sci. 23, 1–6. https://doi.org/10.1186/s40199-015-0105-x.

Rodd, A.L., Creighton, M.A., Vaslet, C.A., Rangel-Mendez, J.R., Hurt, R.H., Kane, A.B., 2014. Effects of surface-engineered nanoparticle-based dispersants for marine oil spills on the model organism *Artemia franciscana*. Environ. Sci. Technol. 48, 6419–6427. https://doi.org/10.1021/es500892m.

Rojo-Nieto, E., Smith, K.E.C., Perales, J.A., Mayer, P., 2012. Recreating the seawater mixture composition of HOCs in toxicity tests with *Artemia franciscana* by passive dosing. Aquat. Toxicol. 120–121, 27–34. https://doi.org/10.1016/j.aquatox.2012.04.006.

Rotini, A., Manfra, L., Canepa, S., Tornambè, A., Migliore, L., 2015. Can *Artemia* hatching assay be a (Sensitive) alternative tool to acute toxicity test? Bull. Environ. Contam. Toxicol. 95, 745–751. https://doi.org/10.1007/s00128-015-1626-1.

Rumbudzai Chikowe, G., 2021. Extracts of Lomandra hystrix Labill. Aerial Parts Lack Antibacterial Activity and are Non-toxic in vitro. Pharmacogn. Commun. 12, 23–27. https://doi.org/10.5530/pc.2022.1.5.

Saliba, L.J., Ahsanullah, M., 1973. Acclimation and tolerance of *Artemia salina* and *Ophryotrocha labronica* to cooper sulphate. Mar. Biol. 23, 297–302. https://doi.org/10.1007/BF00389337.

Saliba, L.J., Krzyz, R.M., 1976. Acclimation and tolerance of *Artemia salina* to copper salts. Mar. Biol. 38, 231–238. https://doi.org/10.1007/BF00388936.

Sánchez, M.I., Petit, C., Martínez-Haro, M., Taggart, M.A., Green, A.J., 2016. May arsenic pollution contribute to limiting *Artemia franciscana* invasion in southern Spain? PeerJ 2016, 1–22. https://doi.org/10.7717/peerj.1703.

Sarabia, R., Del Ramo, J., Varo, I., Diaz-Mayans, J., Torreblanca, A., 2002. Comparing the acute response to cadmium toxicity of nauplii from different populations of *Artemia*. Environ. Toxicol. Chem. 21, 437–444. https://doi.org/10.1002/etc.5620210229.

Sarabia, R., Varó, I., Amat, F., Pastor, A., Del Ramo, J., Díaz-Mayans, J., Torreblanca, A., 2006. Comparative toxicokinetics of cadmium in *Artemia*. Arch. Environ. Contam. Toxicol. 50, 111–120. https://doi.org/10.1007/s00244-005-7026-5.

Selvinsimpson, S., Eva Gnana Dhana Rani, S., Ganesh Kumar, A., Rajaram, R., Sharmila Lydia, I., Chen, Y., 2021. Photocatalytic activity of SnO2/Fe3O4 nanocomposites and the toxicity assessment of Vigna radiata, *Artemia salina* and *Danio rerio* in the photodegraded solution. Environ. Res. 195, 110787 https://doi.org/10.1016/j.envres.2021.110787.

Shaojie, D., Wenli, Z., 2012. Response of growth and development of *Artemia saline* to four kinds of heavy metals stress. Procedia Environ. Sci. 12, 1164–1171. https://doi.org/10.1016/j.proenv.2012.01.403.

Shokry, A., Khalil, M., Ibrahim, H., Soliman, M., Ebrahim, S., 2021. Acute toxicity assessment of polyaniline/Ag nanoparticles/graphene oxide quantum dots on *Cypridopsis vidua* and *Artemia salina*. Sci. Rep. 11, 1–9. https://doi.org/10.1038/s41598-021-84903-5.

Sindaarta, J., Cock, I.E., 2010. Effect of Aloe barbadensis Miller Juice on Oxidative Stress Biomarkers in Aerobic Cells using *Artemia franciscana* as a Model. Phyther. Res. 24, 360–364. https://doi.org/10.1002/ptr.

Soto-Jiménez, M.F., Arellano-Fiore, C., Rocha-Velarde, R., Jara-Marini, M.E., Ruelas-Inzunza, J., Páez-Osuna, F., 2011. Trophic transfer of lead through a model marine four-level food chain: *Tetraselmis suecica, Artemia franciscana, Litopenaeus vannamei*, and *Haemulon scudderi*. Arch. Environ. Contam. Toxicol. 61, 280–291. https://doi.org/10.1007/s00244-010-9620-4.

Sugantharaj David, E.M.D., Madurantakam Royam, M., Rajamani Sekar, S.K., Manivannan, B., Jalaja Soman, S., Mukherjee, A., Natarajan, C., 2017. Toxicity, uptake, and accumulation of nano and bulk cerium oxide particles in *Artemia salina*. Environ. Sci. Pollut. Res. 24, 24187–24200. https://doi.org/10.1007/s11356-017-9975-4.

Sujatha Devi, S., Sethu, M., Lalithambigai, P., Gomathi Priya, P., 2016. Study on the toxicokinetics of Ni(II) on *Artemia franciscana*. Desalin. Water Treat. 57, 10723–10729. https://doi.org/10.1080/19443994.2015.1043952.

Sukumaran, S., Grant, A., 2013a. Multigenerational demographic responses of sexual and asexual *Artemia* to chronic genotoxicity by a reference mutagen. Aquat. Toxicol. 144–145, 66–74. https://doi.org/10.1016/j.aquatox.2013.09.017.

Sukumaran, S., Grant, A., 2013b. Differential responses of sexual and asexual *Artemia* to genotoxicity by a reference mutagen: Is the comet assay a reliable predictor of population level responses? Ecotoxicol. Environ. Saf. 91, 110–116. https://doi.org/10.1016/j.ecoenv.2013.01.012.

Sukumaran, S., Grant, A., 2013c. Effects of genotoxicity and its consequences at the population level in sexual and asexual *Artemia* assessed by analysis of inter-simple sequence repeats (ISSR). Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 757, 8–14. https://doi.org/10.1016/j.mrgentox.2013.03.015.

Svensson, B.M., Mathiasson, L., Mårtensson, L., Bergström, S., 2005. *Artemia salina* as test organism for assessment of acute toxicity of leachate water from landfills. Environ. Monit. Assess. 102, 309–321. https://doi.org/10.1007/s10661-005-6029-z.

Tapia-Salazar, M., Diaz-Sosa, V.R., Cárdenas-Chávez, D.L., 2022. Toxicological effect and enzymatic disorder of non-studied emerging contaminants in *Artemia salina* model. Toxicol. Reports 9, 210–218. https://doi.org/10.1016/j.toxrep.2022.01.007.

Thiagarajan, V., Jenkins, D., Chandrasekaran, N., Mukherjee, A., 2020. Combined effects of nano-TiO2 and hexavalent chromium towards marine crustacean *Artemia salina*. Aquat. Toxicol. 225, 105541 https://doi.org/10.1016/j.aquatox.2020.105541.

Thiagarajan, V., Seenivasan, R., Jenkins, D., Chandrasekaran, N., Mukherjee, A., 2022. Mixture toxicity of TiO2 NPs and tetracycline at two trophic levels in the marine ecosystem: Chlorella sp. and *Artemia salina*. Sci. Total Environ. 812, 152241 https://doi.org/10.1016/j.scitotenv.2021.152241.

Tretyakova, M.O., Pikula, K.S., Yu Kirichenko, K., Golokhvast, K.S., 2021. Ecotoxicological Impact Assessment of Micro-Sized Coal Particles on Zooplanktonic Crustacean *Artemia Salina*. IOP Conf. Ser. Earth Environ. Sci. 720 https://doi.org/10.1088/1755-1315/720/1/012082.

Umarani, R., Kumaraguru, A.K., Nagarani, N., 2012. Investigation of acute toxicity of heavy metals in *Artemia salina* acclimated to different salinity. Toxicol. Environ. Chem. 94, 1547–1556. https://doi.org/10.1080/02772248.2012.713235.

Van Stappen, G., Sui, L., Hoa, V.N., Tamtin, M., Nyonje, B., de Medeiros Rocha, R., Sorgeloos, P., Gajardo, G., 2020. Review on integrated production of the brine shrimp *Artemia* in solar salt ponds. Rev. Aquac. 12, 1054–1071. https://doi.org/10.1111/raq.12371.

Varó, I., Navarro, J.C., Amat, F., Guilhermino, L., 2002. Characterisation of cholinesterases and evaluation of the inhibitory potential of chlorpyrifos and dichlorvos to *Artemia salina* and *Artemia parthenogenetica*. Chemosphere 48, 563–569. https://doi.org/10.1016/S0045-6535(02)00075-9.

Varó, I., Perini, A., Torreblanca, A., Garcia, Y., Bergami, E., Vannuccini, M.L., Corsi, I., 2019. Time-dependent effects of polystyrene nanoparticles in brine shrimp *Artemia franciscana* at physiological, biochemical and molecular levels. Sci. Total Environ. 675, 570–580. https://doi.org/10.1016/j.scitotenv.2019.04.157.

Venkateswara Rao, J., Kavitha, P., Jakka, N.M., Sridhar, V., Usman, P.K., 2007. Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina*. Arch. Environ. Contam. Toxicol. 53, 227–232. https://doi.org/10.1007/s00244-006-0226-9.

Viega, B.L., Rocha, A.M., Düsman, E., 2020. Cosmetics with hormonal composition for bioindicators *Artemia salina L.* and *Allium cepa L.* toxic potential. Environ. Sci. Pollut. Res. 27, 6659–6666. https://doi.org/10.1007/s11356-019-07392-0.

Wang, C., Jia, H., Zhu, L., Zhang, H., Wang, Y., 2017. Toxicity of α-Fe2O3 nanoparticles to *Artemia salina* cysts and three stages of larvae. Sci. Total Environ. 598, 847–855. https://doi.org/10.1016/j.scitotenv.2017.04.183.

Xu, X., Lu, Y., Zhang, D., Wang, Y., Zhou, X., Xu, H., Mei, Y., 2015. Toxic assessment of triclosan and triclocarban on *Artemia salina*. Bull. Environ. Contam. Toxicol. 95, 728–733. https://doi.org/10.1007/s00128-015-1641-2.

Yan, C., Yang, F., Wang, Z., Wang, Q., Seitz, F., Luo, Z., 2017. Changes in arsenate bioaccumulation, subcellular distribution, depuration, and toxicity in: *Artemia salina* nauplii in the presence of titanium dioxide nanoparticles. Environ. Sci. Nano 4, 1365–1376. https://doi.org/10.1039/c6en00621c.

Yi, X., Zhang, K., Liu, R., Giesy, J.P., Li, Z., Li, W., Zhan, J., Liu, L., Gong, Y., 2020. Transcriptomic responses of *Artemia salina* exposed to an environmentally relevant dose of Alexandrium minutum cells or Gonyautoxin2/3. Chemosphere 238, 124661. https://doi.org/10.1016/j.chemosphere.2019.124661.

Zhu, S., Luo, F., Chen, W., Zhu, B., Wang, G., 2017a. Toxicity evaluation of graphene oxide on cysts and three larval stages of *Artemia salina*. Sci. Total Environ. 595, 101–109. https://doi.org/10.1016/j.scitotenv.2017.03.224.

Zhu, S., Xue, M.Y., Luo, F., Chen, W.C., Zhu, B., Wang, G.X., 2017b. Developmental toxicity of Fe3O4 nanoparticles on cysts and three larval stages of *Artemia salina*. Environ. Pollut. 230, 683–691. https://doi.org/10.1016/j.envpol.2017.06.065.