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Review

Could Contamination Avoidance Be an Endpoint That Protects the Environment? An Overview on How Species Respond to Copper, Glyphosate, and Silver Nanoparticles

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Abstract: The use of non-forced multi-compartmented exposure systems has gained importance in the assessment of the contamination-driven spatial avoidance response. This new paradigm of exposure makes it possible to assess how contaminants fragment habitats, interfering in the spatial distribution and species' habitat selection processes. In this approach, organisms are exposed to a chemically heterogeneous scenario (a gradient or patches of contamination) and the response is focused on identifying the contamination levels considered aversive for organisms. Despite the interesting results that have been recently published, the use of this approach in ecotoxicological risk studies is still incipient. The current review aims to show the sensitivity of spatial avoidance in non-forced exposure systems in comparison with the traditional endpoints used in ecotoxicology under forced exposure. To do this, we have used the sensitivity profile by biological groups (SPBG) to offer an overview of the highly sensitive biological groups and the species sensitive distribution (SSD) to estimate the hazard concentration for 5% of the species (HC_5). Three chemically different compounds were selected for this review: copper, glyphosate, and Ag-NPs. The results show that contamination-driven spatial avoidance is a very sensitive endpoint that could be integrated as a complementary tool to ecotoxicological studies in order to provide an overview of the level of repellence of contaminants. This repellence is a clear example of how contamination might fragment ecosystems, prevent connectivity among populations and condition the distribution of biodiversity.

Keywords: environmental heterogeneity; multi-compartment exposure system; non-forced exposure; sensitive profile; species sensitivity distribution

1. Ecotoxicology and Avoidance in a Chemically Heterogeneous Landscape

During the last 50 years since ecotoxicology was proposed as a new science [1], there has been a continuous advance regarding the number of methods, test species, and responses employed to assess the effects of contamination on organisms and ecosystems. The search for the most sensitive species has led researchers to test numerous species from different biological groups, trophic levels, and geographic distribution [2,3]. However, the concept of the most sensitive species has become obsolete as it is a rather theoretical concept since one species can be very sensitive to a given class of contaminants, but less sensitive to another one [2,4–6]. Alongside the need to standardize the test procedures adopted by industries and governments as a legal tool for the environmental risk assessments (ERAs) conducted; researchers need to use organisms that meet some basic requisites besides the sensitivity and ecological relevance [7]. For example, it should be relatively easy and cheap to culture organisms in a laboratory and the procedures to test toxicity should be simple and practical [3]. For this reason, ecotoxicology focused particularly on the growth inhibition tests with microalgae, and mortality/immobilization and reproduction tests with daphnids and fish. Over the years, organisms from temperate zones (mainly from Europe and United States) have been used widely, regardless of their importance for other ecosystems, because the experimental procedures were technically more developed and standardized [8,9]. However, many researchers from different geographic areas turned their focus to local key species, adapting or even creating new experimental procedures for the species considered of ecological importance. All these processes coupled with the rapid development of molecular biology (from the perspective of the sub-individual; [10]) and to higher integration of ecological concepts (from a perspective of ecosystem structure and functioning; [11]), have favored unprecedented advances in the field of ecotoxicology. Today, ecotoxicological studies are able to provide valuable information about the risk contaminants represent to organisms, although some limitations still exist regarding the extrapolation to natural ecosystems [12].

In this context, one classical paradigm of the ecotoxicity tests is the continuous and mandatory exposure of organisms to contaminants. Those tests assume that organisms in natural ecosystems are forcedly exposed to contaminants, with no possibility of fleeing. However, complementary methods, in which organisms are simultaneously exposed to several concentrations and can choose the most favorable one, have been proposed (see review by Jutfelt et al. [13]). Initially, these methods provided a bi-compartmentalization of the system (with and without contaminant), but with the limitation of not allowing the calculation of ACx (the concentration that triggers the avoidance of x% of the population) that is analog to the classical LCx (lethal concentration), ECx (effective concentration), etc. That, to some extent, prevents the comparison of data obtained from both approaches. However, new methods using non-forced, multi-compartmented linear exposure systems (linear 1-D system by Lopes et al. [14] and 2-D HeMHAS by Araújo et al. [15]) have been developed recently. The main benefit of the multi-compartmentalization exposure systems is the possibility of determining the concentrations of a contaminant in each zone (compartment) through which the organisms can move freely, providing an idea of the potential repellence or attractiveness of the contaminants [14,16,17]. It is important to bear in mind that this approach should be seen as a complementary tool to the classical forced exposure approach, as the non-forced approach provides information about how contamination could affect the spatial distribution of the organisms, but not about the toxic effects [17,18]. Thus, the concept of toxicity at the individual level is replaced by the effects on the dynamics of dispersion (spatial avoidance) and habitat selection, from a landscape (connected habitats) perspective [19–21]. Although non-forced exposure supposes no effect at the individual level, the fleeing of a species from an ecosystem could, ecologically, be considered similar to the death of the individuals [14]. Due to this methodological and conceptual particularity of the non-forced multi-compartmented approach, an important question arises: how sensitive is the avoidance response in highlighting the potential risk of a chemical compound?

Although the amount of data generated by ecotoxicology has been considerable over the last few years for many contaminants, especially the contaminants of emerging concern (new agrochemicals, nanoparticles, sunscreens, pharmaceutical products, plastic derivatives, etc.; [22]), information is still scarce. This seems to be highlighted when a new paradigm such as the non-forced multi-compartmented exposure is to be applied. Although this exposure approach has increased in ecotoxicological studies (see reviews by Araújo et al. [23] and Moreira-Santos et al. [24]), information about the real potential of contaminants to trigger avoidance in organisms and to change their habitat selection patterns is very limited. In addition, it is not clear whether toxicity and repellency are comparable in terms of sensitivity [17].

The current review aims to assess how sensitive the avoidance response measured in multi-compartmented exposure systems is in comparison with the various toxic responses used in ecotoxicology from forced exposure experiments. To this end, a sensitivity profile by biological groups (SPBG; [25] for three reference contaminants (copper, glyphosate, and silver nanoparticles—Ag-NPs) was created. The SPBG is a simple way to identify the biological groups that could be considered more susceptible and the groups of responses that could provide an idea about the main toxic effects expected to occur. Secondly, we assessed whether the concentrations that trigger an avoidance response for 50% of the population (AC_{50}) will be among the responses that are expected to occur at concentrations considered hazardous for 5% of the species (HC_5; [6]). Finally, we discuss: (i) the sensitivity of the avoidance response as an endpoint (focusing on the repellence of contaminants) from non-forced exposure approaches compared to toxicity data from forced exposure, (ii) the feasibility of using the avoidance response in multi-compartmented systems as a complementary tool in ERAs, and (iii) the ecological relevance and improvements that could result from integrating the avoidance response into ecotoxicological studies.

2. Chemicals Used as Reference Contaminants

To compare the sensitivities among avoidance response and other endpoints, three chemicals with completely different chemical characteristics and modes of action were chosen as the reference contaminants: copper, glyphosate, and Ag-NPs. Copper was selected as one of the most traditional chemical compounds used in ecotoxicology [26,27] with an ample amount of data available and because it is one of the most ubiquitous contaminants used in different sectors such as industry and agriculture. Glyphosate was also selected because it is one of the most widely used pesticides in the world and it is the object of widespread controversy concerning the effects it can produce on non-target organisms [28,29]. Finally, Ag-NPs are a contaminant of emerging concern chosen due to being one of the most common nanomaterials found in consumer products such as antimicrobial agents [30,31]. More than 100 results of ecotoxicological data were revised and included in the current study for each contaminant (Tables S1–S3). Particularly in the case of Ag-NPs, avoidance experiments were performed in multi-compartmented systems to compensate for the absence of data in the literature and make it possible to compare the results. The experiments are described briefly in the next section.

3. Avoidance Assays with Ag-NPs

Ag-NPs (<15 nm in aqueous suspension; US7140—US Research Nanomaterials, Inc., Houston, TX USA) described by Sendra et al. [32] were used. Avoidance assays were performed in the non-forced, six-compartmented exposure systems used by Islam et al. [33], and zebrafish (*Danio rerio*) were used as the test organisms. Initially, different concentrations of Ag-NPs (0, 5, 10, 20, 40, and 80 μg/L) were prepared and put into the system in the form of a gradient. Afterward, five juveniles of zebrafish (body size: 2.0 to 2.5 cm) were introduced in each concentration; therefore, 30 organisms were used in each replicate. The experiment was run in triplicate. The displacement of the fish was recorded at different time intervals: 30, 60, 90, 120, 150, and 180 min and after 24 h. A red light was used during observation to minimize the interference of the observer on the behavior of the fish. Exposure was performed at 22 °C and in the dark. More details about the assay are described in Islam et al. [33].

4. Sensitivity Profile by Biological Groups: Definition

SPBG is a representation of the potential toxicity of contaminants intended to provide information on the sensitivity of the ecotoxicological responses measured in different biological groups. This representation offers an overview that could help researchers to better identify the biological groups and the endpoints that could be more suitable to assess the toxicity of a specific, or class of, chemical (s). This representation was chosen because it provides a clear visual panorama of how sensitive the avoidance response might be when compared with other endpoints. All the information of the studies analyzed can be verified in Tables S1–S3.

The data collected to create the sensitivity profile were based on the concentrations that cause 50% of effect (EC_{50}). Although NOEC (the highest no-observed effective concentration) or LOEC (the lowest observed effective concentration) could be more protective environmentally, they were not chosen because both are dependent on the concentrations used in the studies. The use of the EC_{50} values did not have an environmental criterion, but instead, it was adopted to standardize the database.

The first step to creating the SPBG consisted in revising published papers looking for different species and different responses to create a sufficiently robust and diverse (as many species and responses as possible) database. The review of the literature (Google Scholar, Scielo, Scopus, and Web of Knowledge) was performed using various words like aquatic, ecosystems, EC or LC or IC, sensitivity, toxicity, and the name of the contaminant (copper, glyphosate, or Ag-nanoparticle). No selection about the year was made. Preferably, studies published in high impact factor journals (Q1 and Q2 according to JCR index) were selected, except if the study presented particular data for a determined species or response. When an imbalance regarding the amount of data for a given biological group or endpoint was identified, a more specific search taking into consideration the response or biological group of interest was performed, to provide more information about the specific biological group or endpoint. All the species were classified by biological groups in accordance with the groups described in Table 1 (see also Tables S1–S3). When the number of species in a group in the database was very high (like Crustacea), different subgroups (such as cladocerans, shrimps, crabs . . .) were created to discriminate any differences in the sensitivity of each subgroup. Data were not separated by species because the species used in a geographic region are not necessarily representative of another region; therefore, the organization by biological group made the selection of other species of the same biological group in a different region easier. Afterward, the ecotoxicological responses were classified as shown in Table 1. Finally, the data concerning toxicity were plotted according to biological groups and responses.

Table 1. Classification of the ecotoxicological responses used in the sensitivity profile of the three chemicals used in the current study by biological group.

Classification of the Effect	Ecotoxicological Responses
Mortality/Immobilization	Death; immobilization
Biochemical	Biomarkers of exposure and effects, enzymes, proteins
Physiological	Respiration rate; heartbeat
Feeding	Ingestion; excretion; post-exposure feeding
Growth/Reproduction	Increase in the body size; population growth (cell numbers)
Morphological	Any morphological alterations
Behavioral	Changes in the movement patterns; all the effects related to swimming; fleeing from a predator; sinking; burrowing
Spatial avoidance	Avoidance behavior related to the habitat selection response measured exclusively in multi-compartmented exposure systems

5. The Hazard Concentration (HC_5) Based on the Species Sensitive Distribution (SSD)

From the EC_{50} data used in the sensitivity profile for biological groups, the species sensitive distribution (SSD) [6] was also generated. When there were two results as the endpoint for the same species, the most sensitive data (lower EC_{50}) was used. Finally, the hazard concentration for 5% of the species (HC_5) was calculated from the SSD according to Posthuma et al. [6] to identify whether the avoidance response can be observed within the concentrations that suppose a risk for the most (5%) sensitive responses.

6. Results: Sensitivity Profile by Biological Group

As copper is one of the most widely used contaminants in ecotoxicology, the sensitivity profile was more diverse regarding biological groups and endpoints compared to the SPBG of glyphosate and Ag-NPs. In total, 19 biological groups were included for the SPBG of copper (Figure 1), while 9 biological groups were used for glyphosate (Figure 2) and only data for 5 biological groups concerning Ag-NPs were found (Figure 3). Similarly, the responses selected were more diverse for copper (8 groups of response), followed by glyphosate (6 groups of response) and Ag-NPs (5 groups of response). All the data described in the next sections, and their respective references, may be verified in Tables S1–S3. Any reference to the most or least sensitive organism or response discussed in the next sections should be viewed with caution due to intrinsic differences regarding the environmental conditions of the experiments (see more details in the "Avoidance response: relevance and final remarks" section). In addition, it should be taken into consideration that the database used in the current review has its limitation regarding the number of manuscripts revised.

6.1. Copper

From the bibliographic review, 160 results were selected for the SPBG to copper (Figure 1; see the complete database in Table S1), 79 for freshwater species, and 81 for marine/estuarine species. The most frequent response was immobility/mortality, which was selected for 13 out of 19 biological groups. The most sensitive biological group for considering the immobility/mortality response was the cladocerans *Bosmina longirostris* with EC_{50} values of 1.4 µg/L.

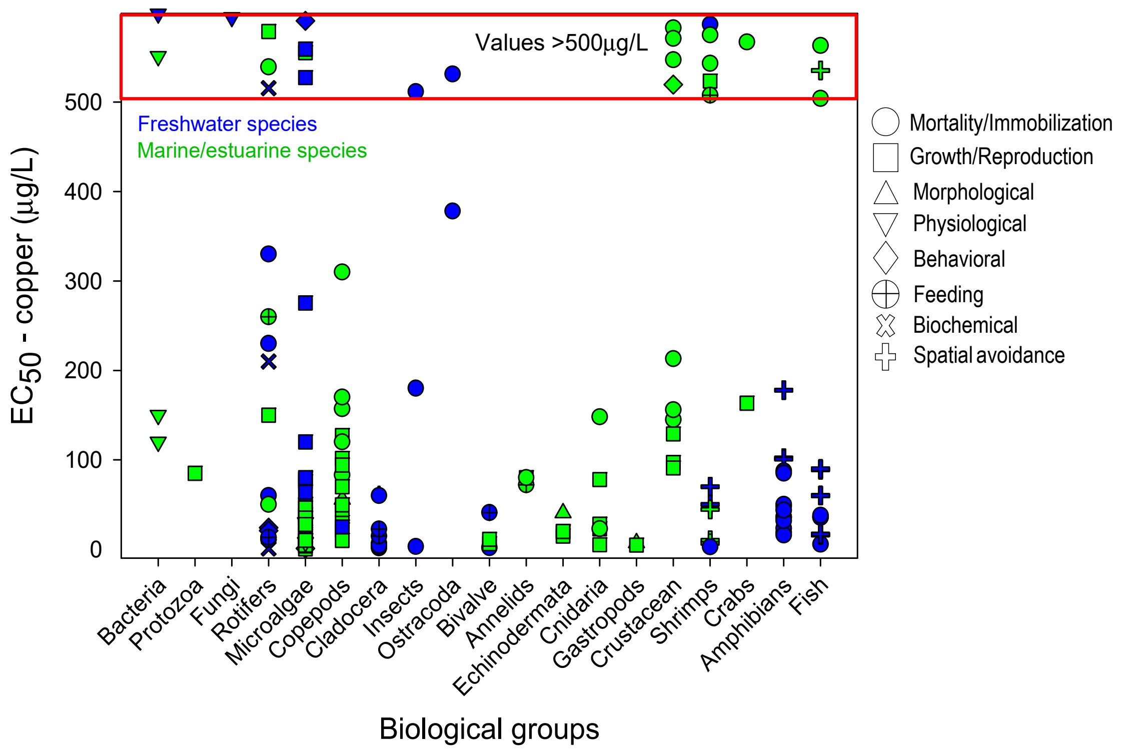

Figure 1. Sensitivity profile for the biological groups exposed to copper-based on the EC_{50} values. The effects, represented by different symbols, were classified according to Table 1. Data in blue and green represent, respectively, freshwater and estuarine/marine species. Data shown in the red zone represent results whose EC_{50} values are higher than 500 µg/L; therefore, the scale should not be considered in this zone (see real data in Table S1).

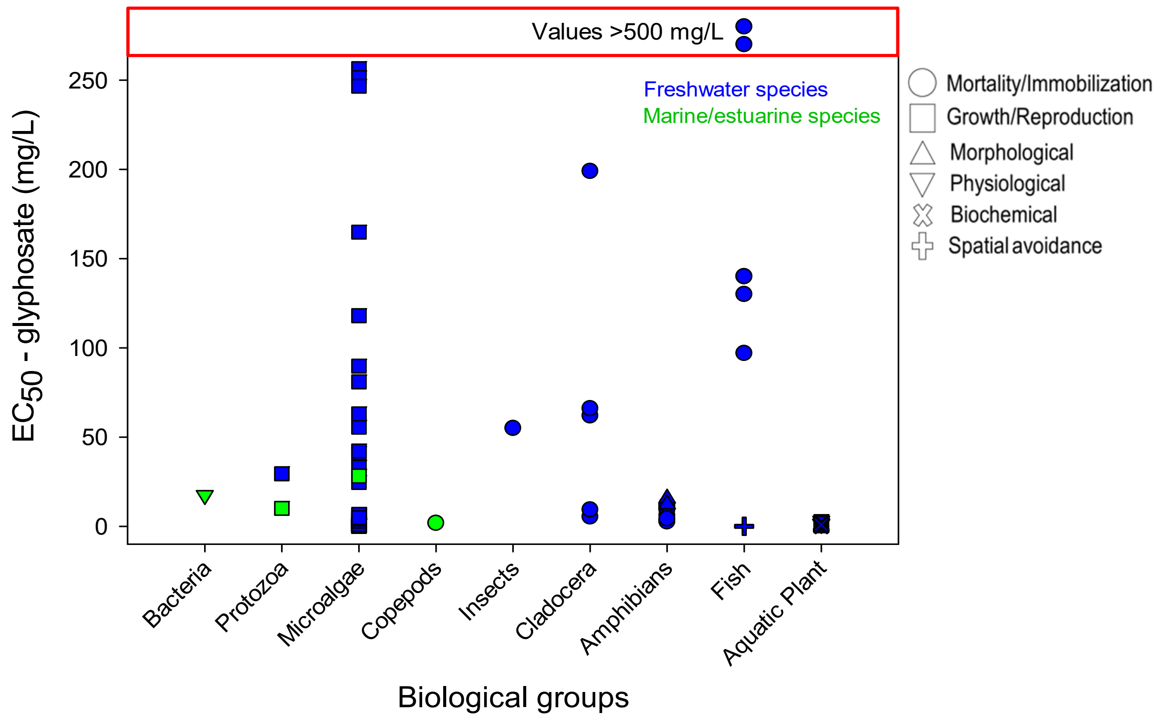

Figure 2. Sensitivity profile for the biological groups exposed to glyphosate-based on the EC_{50} values. The effects, represented by different symbols, were classified according to Table 1. Data in blue and green represent, respectively, freshwater and estuarine/marine species. Data shown in the red zone represent results whose EC_{50} values are higher than 500 mg/L; therefore, the scale should not be considered in this zone (see real data in Table S2).

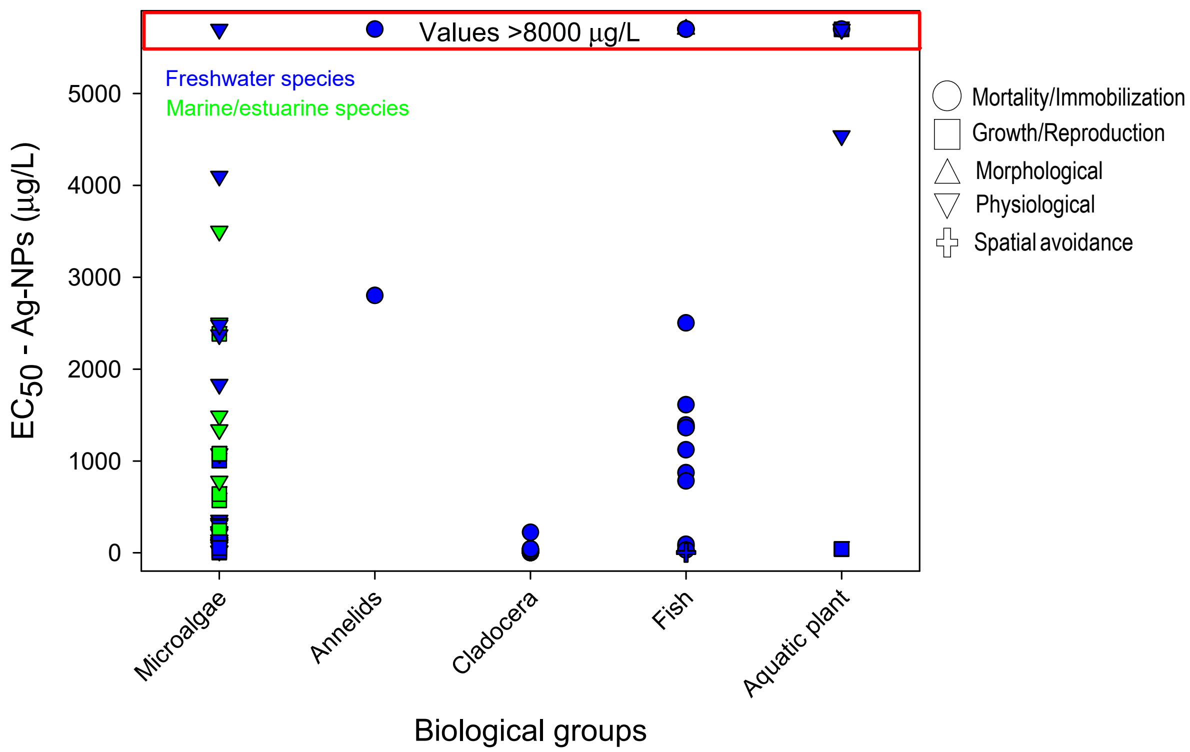

Figure 3. Sensitivity profile for biological groups exposed to Ag-NPs based on the EC_{50} values. The effects, represented by different symbols, were classified according to Table 1. Data in blue and green represent, respectively, freshwater and estuarine/marine species. Data shown in the red zone represent results whose EC_{50} values are higher than 8000 mg/L; therefore, the scale should not be considered in this zone (see real data in Table S3).

Growth/reproduction inhibition was the second most common response and was observed in 12 out of 19 biological groups. For this endpoint, microalgae and copepods were proven to be the most sensitive groups; however, microalgae presented the greatest number of sensitive species with EC_{50} values lower than 10 μg/L: e.g., *Isochrysis* aff. *galbana* clone T-ISO (EC_{50} of 0.4 μg/L), *Cylindrotheca closterium* (EC_{50} of 4.7 μg/L), *Selenastrum capricornutum* (EC_{50} of 6 μg/L) *Chlorella* sp. (EC_{50} of 6 μg/L) *Phaeodactylum tricornutum* (EC_{50} of 9 μg/L), *Chlorella autotrophica* (EC_{50} of 9.6 μg/L).

The gastropods *Nassarius dorsatus* (EC_{50} of 4.7 μg/L) and *Haliotis rubra* (EC_{50} of 7.1 μg/L) presented a high sensitivity to copper when growth/reproduction and morphological alterations were considered as the endpoints, respectively. The sensitivity of the rotifers to copper was represented by many different responses, but with ample variation regarding the sensitivity. Although the responses related to biochemical changes, feeding, behavior, and mortality/immobilization were relatively sensitive, the data of the sensitivity profile presented a great dispersion regarding the EC_{50} values. Some species of cnidarian and bivalves seem to be highly sensitive to copper (EC_{50} lower than 10 μg/L; see detail in Table S1).

Regarding avoidance, results were obtained for cladocerans, shrimps, amphibians, and fish. In general, the sensitivity of the organisms to avoiding copper is comparable to the most sensitive values observed in the other biological groups (Figure 1). The most sensitive values for avoidance response were observed for the estuarine/marine shrimp *Palaemon varians* (AC_{50} of 10 μg/L) and *Litopenaeus vannamei* (AC_{50} of 11 μg/L), followed by the freshwater fish *Danio rerio* and *Poecilia reticulata*, both with an AC_{50} of 16 μg/L. The cladoceran *Daphnia longispina* also proved to be able to avoid copper but at a higher concentration (AC_{50} of 65 μg/L). Finally, the amphibians were the groups tested with higher AC_{50} values: *Lithobates catesbeianus* (101 μg/L), *Leptodactylus latrans* (102 μg/L), *Pelophylax perezi* (178 μg/L).

6.2. Glyphosate

This sensitivity profile to glyphosate was formed by 105 results from 9 biological groups, the majority (97 results) were from freshwater; only 8 results were used for estuarine/marine species (Figure 2). Although the concentrations at which organisms respond to glyphosate are much higher than those for copper, a similarity was observed regarding the most common endpoints tested: growth/reproduction inhibition and mortality/immobilization.

Microalgae was the most common group included as test organisms, and the data collected (exclusively for growth inhibition) showed high variability in the sensitivity. High variation in the data has also been documented for fish, even considering the same response like mortality/immobilization. On the other hand, the results found for amphibians are very consistent and sensitive; the lowest EC_{50} value observed was for the mortality/immobilization response of *Rana clamitans* (2.7 mg/L). Similarly, the macrophyte *Lemna minor* was shown to be a very sensitive organism. Data of different responses have been found for this species (Table S2) and EC_{50} values as low as 0.09, 0.40, and 1.32 mg/L have been observed for biochemical effects, growth/reproduction, and physiological changes, respectively.

Considerations about the avoidance response must be made with caution because there is only one item of data for exposure to glyphosate. The avoidance shown by *Danio rerio* was observed at very low concentrations (AC_{50} of 0.0015 mg/L), much lower than the second most sensitive response for fish: mortality of *Pimpehales promelas* with EC_{50} of 97 mg/L. The complete database is described in Table S2.

6.3. Silver Nanoparticles

Few studies were found for this contaminant. Only five biological groups and five groups of respondents were represented (Figure 3). As observed in the previous sensitivity profile, microalgae presented the highest quantity of data and high variability in the results. In this group, two endpoints were recorded: physiological changes and growth/reproduction. The microalgae species that were shown to be highly sensitive (EC_{50} inferior a 20 µg/L) to Ag-NPs were: *Pseudokirchneriella subcapitata* (EC_{50} of 3.02 µg/L) and *Chlamydomonas reinhardtii* (EC_{50} of 19.8 µg/L). Data from cladocerans also showed a great sensitivity regarding the mortality/immobilization response. The most sensitive response was observed in *Daphnia magna* with an EC_{50} of 0.75 µg/L, followed by *Ceriodaphnia dubia* with an EC_{50} of 5 µg/L.

Regarding fish, the sensitivity to Ag-NPs seems not to be very high. An EC_{50} value as high as 12,600 µg/L was observed for morphological changes in *Oreochromis mossambicus*. On the other hand, the spatial avoidance measured in *D. rerio* with an AC_{50} of 2.5 µg/L (data from the current study) was the most sensitive response found for this biological group, and one of the most sensitive when compared to the other biological groups. Growth in aquatic plants did not show great sensitivity to Ag-NPs except for two results (see details of the EC_{50} in Table S3). The annelids seem to be the organisms with the lowest sensitivity to Ag-NP exposure.

7. Sensitivity of Avoidance Response According to SSD and HC_5

Due to the quantity of data collected for copper, the SSD was divided into two groups: freshwater and estuarine/marine organisms (Figure 4A,B, respectively). For glyphosate and Ag-NPs, only data from freshwater species were used, because very little data for estuarine/marine species were found (Figure 5A,B, respectively). For the four SSDs, the sigmoidal model was statistically significant ($p < 0.001$), and the coefficients of determination were always higher than 0.9 (Figures 4 and 5). Regarding the HC_5, the values calculated (and confidence intervals) were: 3.63 µg/L (3.22–4.10 µg/L) and 6.19 µg/L (5.67–6.77 µg/L) for freshwater and estuarine/marine species exposed to copper, respectively (Figure 4A,B), and 2.10 mg/L (1.59–2.74 µg/L) and 1.36 µg/L (8.96–2.04 µg/L) for freshwater species exposed to glyphosate and Ag-NPs, respectively (Figure 5A,B).

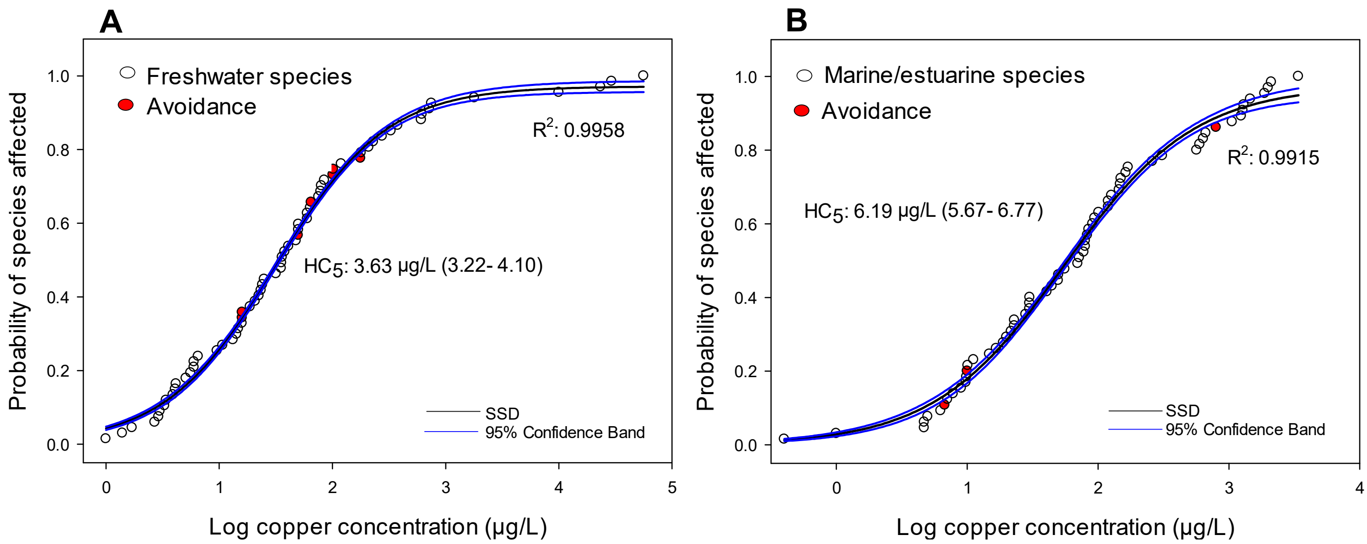

Figure 4. Species sensitive distribution (SSD), and 95% confidence intervals, expressed as the probability of the species being affected according to the log of the copper concentrations, considering the freshwater (**A**) and estuarine/marine (**B**) species. The spatial avoidance response is represented by red circles. Values of hazard concentrations for 5% of the species (HC_5) and the confidence intervals are also shown for each group of data.

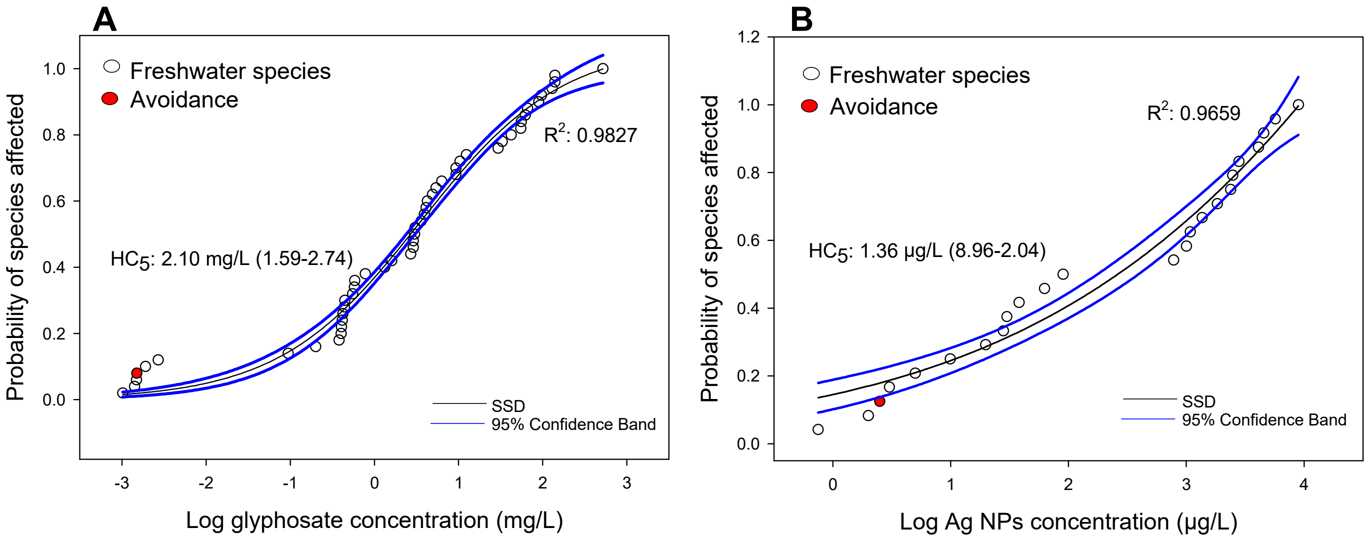

Figure 5. Species sensitive distribution (SSD), and 95% confidence intervals, expressed as the probability of the species being affected according to the log of the glyphosate (**A**) and Ag-NPs (**B**) concentrations, considering the freshwater species. The spatial avoidance response is represented by red circles. Values of hazard concentrations for 5% of the species (HC_5) and the confidence intervals are also shown for each group of data.

Considering the SSD curves, the avoidance response (red circles) of the freshwater species exposed to copper seems to be only moderately sensitive compared to other responses. The most sensitive avoidance response was observed at 16 µg/L (*D. rerio* and *P. reticulata*), which is higher than the HC_5 value (3.63 µg/L). For estuarine/marine species exposed to copper (Figure 4B), the avoidance responses were distributed at the extremes of the range of sensitivity, where some organisms seem to respond by avoiding low copper concentrations (*Palaemon varians* at 10 µg/L and *Litopenaeus vannamei* at 11 µg/L), while the fish *Rachycentron canadum* (AC_{50} of 800 µg/L) seems to be less responsive. The repellence of copper for the most responsive species (*P. varians* and *L. vannamei*) occurred at concentrations similar to those affecting the most sensitive species and close to the HC_5 value (6.19 µg/L).

Analyzing the SSD models for glyphosate and Ag-NPs (Figure 5A,B), the avoidance response appeared as one of the most sensitive endpoints. In the case of glyphosate, the AC_{50} for the fish *D. rerio* (0.0015 mg/L) is even lower than the HC_5 calculated (2.10 mg/L). For Ag-NPs, the AC_{50} for *D. rerio* (2.5 µg/L) was slightly higher than the HC_5 (1.36 µg/L).

8. Avoidance Response: Relevance and Final Remarks

This review is an attempt to situate the avoidance response (using the non-forced multi-compartmented exposure approach) into the sensitivity profile by biological groups to assess how sensitive it is. The data of the three contaminants (copper, glyphosate, and Ag-NPs) assessed here showed that avoidance may be considered a very sensitive response, even when compared with the most traditional endpoints such as growth/reproduction inhibition, physiological changes, feeding, mortality/immobilization, and others. As the use of avoidance in multi-compartmented exposure systems, first proposed by Lopes et al. [14], supposes a shift in the paradigm of how organisms are exposed to contaminants and which kind of response is measured (not toxicity, but repellence instead), this approach provides a complementary perspective concerning the risk that contamination may represent to ecosystems.

This response can be applied in different approaches, either by integrating the loss of population due to avoidance with mortality and reproduction [34,35] thereby assessing the decline of populations due to these three responses; simulating changes in the avoidance response and spatial distribution of organisms under scenarios of global changes [36] to predict the loss of populations due to the inhospitable environmental conditions or integrating it with other responses to assess how avoidance can be impaired if some toxic effects occur in organisms [37,38]. As a complementary tool to evaluate how contamination affects the spatial distribution of species, this approach allows researchers to apply a more ecological view of ecotoxicology by simulating some real scenarios, in which the chemical heterogeneity can generate attractive and repellent areas. This spatially broader perspective of the impact of contamination leads to new ecological concepts that can be included in ecotoxicological studies when the avoidance response is used: for instance, colonization [33,39]. This involves assessing the possibility of an area being colonized depending on the levels of contamination that make it attractive. Further, the concept of habitat connectivity/selection [15,16], which considers ecosystems as a spatially continuous landscape throughout which organisms can move. Another new aspect for consideration is habitat fragmentation [40], which integrates the concept of a chemical barrier that prevents the free displacement of species between areas separated by high levels of contamination. Taking into account that the loss of connectivity of habitats is a serious threat to biodiversity, the role of contamination in the chemical fragmentation of habitats should be considered by ecotoxicologists.

Although there is very little data on the avoidance response of organisms to contaminants, this review has shown the high sensitivity of this endpoint. The importance of avoidance as an ecotoxicological endpoint may be deduced from the sensitivity profiles and SSD for copper, glyphosate, and Ag-NPs. In order to protect the environment, it is not only important to know the toxic effect that contaminants produce, but also to what extent their repellence triggers the fleeing of organisms to more favorable areas. We encourage the use of the avoidance response employing non-forced exposure scenarios to make the integration of this response in SSD models more robust, reducing the uncertainties [41] and potentially providing environmentally more protective HC_5 values [42].

Finally, it is important to consider that, despite the high sensitivity of the avoidance response in non-forced exposure systems, any comparison of the sensitivities of organisms to contaminants should be viewed with great caution. This is necessary because the way organisms respond to chemicals not only depends on the species, their life stages used in the tests, and their origins; but also on several factors such as the environmental conditions under which the organisms are cultured and the tests performed (e.g., the chemical composition of the culture medium, levels of dissolved oxygen, pH, temperature, salinity) [43–45].

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/toxics9110301/s1, Table S1: copper, Table S2: glyphosate, Table S3: Ag-NPs.

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toxics MDPI

Could contamination avoidance be an endpoint that protects the environment? An overview on how species respond to copper, glyphosate and silver nanoparticles

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Table S1. copper.

Group	Species	Responses /Endpoint	Classification of the ecotoxicological responses	EC_{50} (µg/L)	References
Freshwater species					
Bacteria	*Pseudomonas fluorescens*	Respirometry	Physiological	56,100	Pérez-García et al., 1993
Fungi	*Saccharomyces cerevisiae*	Respirometry	Physiological	29,300	Pérez-García et al., 1993
Rotifers	*Brachionus calyciflorus*	Swimming alterations: Speed	Behavioral	24.2	Charoy and Janssen, 1999
		Swimming alterations: Periods of swimming	Behavioral	20.8	Charoy and Janssen, 1999
		Filtration rate	Feeding	10.75	Ferrando and Andreu, 1993
		Ingestion rate	Feeding	13.25	Ferrando and Andreu, 1993
	Lecane hamata	Esterases-inhibition	Biochemical	210	Pérez-Legaspi et al., 2002
		Mortality	Mortality/Immobilization	230	Pérez-Legaspi et al., 2002
	Lecane luna	Esterases-inhibition	Biochemical	620	Pérez-Legaspi et al., 2002
		Mortality	Mortality/Immobilization	60	Pérez-Legaspi et al., 2002
	Lecane quadridentata	Esterases-inhibition	Biochemical	1	Pérez-Legaspi et al., 2002
		Mortality	Mortality/Immobilization	330	Pérez-Legaspi et al., 2002
Microalgae	*Euglena gracilis*	Motility	Behavioral	23,400	Ahmed and Häder, 2010
		Growth inhibition	Growth/Reproduction	1800	Girling et al., 2000
	Selenastrum capricornutum	Cel division rate inhibition	Growth/Reproduction	6	Franklin et al., 2001
	Chlorella sp.	Cel division rate inhibition	Growth/Reproduction	6	Franklin et al., 2001
	Chlamydomonas reinhardi	Growth inhibition	Growth/Reproduction	79	Girling et al., 2000
	Scenedesmus subspicatus	Growth inhibition	Growth/Reproduction	120	Girling et al, 2000
	Scenedesmus quadricauda	Decrease in the surface area	Growth/Reproduction	275.5	Fawaz et al., 2019
		Cell density	Growth/Reproduction	14.29	Fawaz et al., 2019
	Ankistrodesmus angustus	Decrease in the surface area	Growth/Reproduction	75.65	Fawaz et al., 2019
		Cell density	Growth/Reproduction	720.3	Fawaz et al., 2019
	Oscillatoria prolifera	Decrease in the surface area	Growth/Reproduction	50.17	Fawaz et al., 2019
		Cell density	Growth/Reproduction	50.17	Fawaz et al., 2019
	Aphanizomenon gracile	Population growth	Growth/Reproduction	64	Lüderitz and Nicklish, 1989
	Oscillatoria redekei	Population growth	Growth/Reproduction	80	Lüderitz and Nicklish, 1989
	Chlorella autotrophyca	Population growth	Growth/Reproduction	9,6	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	19,3	Moreno-Garrido et al., 2000

		Population growth	Growth/Reproduction	38,3	Moreno-Garrido et al., 2000
Copepods	*Mesocyclops pehpeiensis*	Inhibition	Growth/Reproduction	25	Wong and Pak, 2004
Cladocera	*Bosmina longirostris*	Motility	Mortality/Immobilization	1.4	Koivisto et al., 1992
	Ceriodaphnia cornuta	Motility	Mortality/Immobilization	2.92	Bui et al., 2016
	Ceriodaphnia dubia	Immobilization	Mortality/Immobilization	4.16	Harmon et al., 2003
	Chydorus sphaericus	Motility	Mortality/Immobilization	3.3	Koivisto et al., 1992
	Daphnia ambigua	Immobilization	Mortality/Immobilization	6.53	Harmon et al., 2003
	Daphnia galeata	Motility	Mortality/Immobilization	4.1	Koivisto et al., 1992
	Daphnia longispina	Avoidance	Spatial avoidance	65	Lopes et al., 2004
		Motility	Mortality/Immobilization	60	Lopes et al., 2004
	Daphnia lumholtzi	Motility	Mortality/Immobilization	3.92	Bui et al., 2016
	Daphnia magna	Motility	Mortality/Immobilization	5.1	De Schamphelaere y Janssen, 2002
		Filtration rate	Feeding	14.75	Ferrando and Andreu, 1993
		Ingestion rate	Feeding	22.5	Ferrando and Andreu, 1993
	Daphnia pulex	Motility	Mortality/Immobilization	3.4	Koivisto et al., 1992
Insects	*Adenophlebia auriculata*	Survival	Mortality/Immobilization	180	Gerhardt and Palmer, 1998
	Drunella grandis	Survival	Mortality/Immobilization	3.0	Clements et al., 2013
	Tramea cophysa	Mortality	Mortality/Immobilization	611	Dos Santos Lima et al., 2019
Ostracoda	*Chlamydotheca* sp.	Mortality	Mortality/Immobilization	378	Dos Santos Lima et al., 2019
	Strandesia trispinosa	Mortality	Mortality/Immobilization	750	Dos Santos Lima et al., 2019
Bivalve	*Dreissena polymorpha*	Filtration rate	Feeding	41	Kraak et al., 1994
	Echyridella menziesii	Survival	Mortality/Immobilization	1.7	Clearwater et al., 2013
Shrimps	*Atyaephyra desmarestii*	Avoidance	Spatial avoidance	70	Vera-Vera et al., 2019
		Avoidance	Spatial avoidance	50	Araújo et al., 2019
	Macrobrachium amazonicum	Survival	Mortality/Immobilization	10,010	Soares et al., 2017
	Macrobrachium pantanalense	Survival	Mortality/Immobilization	2.7	Soares et al., 2017
Amphibians	*Ambystoma opacum*	Mortality	Mortality/Immobilization	18.76	Weir et al., 2019.
	Ambystoma talpoideum	Mortality	Mortality/Immobilization	47.88	Weir et al., 2019.
	Ambystoma tigrinum	Mortality	Mortality/Immobilization	35.3	Weir et al., 2019.
	Bufo arenarum	Mortality	Mortality/Immobilization	85	Herkovits y Helguero, 1998.
	Epidalea calamita	Larval mortality	Mortality/Immobilization	87.60	García-Muñoz et al., 2009
		Larval mortality	Mortality/Immobilization	31.85	García-Muñoz et al., 2009
		Larval mortality	Mortality/Immobilization	43.80	García-Muñoz et al., 2009
	Lithobates catesbeianus	Avoidance	Spatial avoidance	101	Araújo et al., 2014
	Leptodactylus latrans	Avoidance	Spatial avoidance	102	Araújo et al., 2014
	Pelophylax perezi	Avoidance	Spatial avoidance	178	Araújo et al., 2014
	Rhinella granulosa	Mortality	Mortality/Immobilization	23.48	Franco-de-Sá and Val, 2014
	Scinax ruber	Mortality	Mortality/Immobilization	36.37	Franco-de-Sá and Val, 2014
		Mortality	Mortality/Immobilization	50.02	Franco-de-Sá and Val, 2014
		Mortality	Mortality/Immobilization	15.9	Franco-de-Sá and Val, 2014
Fish	*Danio rerio*	Feeding Inhibition	Feeding	36	Abdel-moneim et al, 2015
		Avoidance	Spatial avoidance	17	Silva et al., 2018
		Avoidance	Spatial avoidance	60	Araújo et al., 2018
		Avoidance	Spatial avoidance	89	Araújo et al., 2018
		Avoidance	Spatial avoidance	90	Islam et al., 2019
		Avoidance	Spatial avoidance	60	Araújo et al., 2019
		Avoidance	Spatial avoidance	16	Moreira-Santos et al., 2008
	Rasbora sumatrana	Mortality	Mortality/Immobilization	5.6	Shuhaimi-Othman et al., 2015

	Oncorhynchus mykiss	Mortality	Mortality/Immobilization	35.5	Naddy et al., 2015
	Poecilia reticulata	Mortality	Mortality/Immobilization	37.9	Shuhaimi-Othman et al., 2010
		Avoidance	Spatial avoidance	16	Silva et., 2018
Marine/estuarine species					
Bacteria	*Vibrio fisherii* (Microtox)	Atenuation of light emission	Physiological	150	Moreno-Garrido et al., 1999
		Atenuation of light emission	Physiological	120	Moreno-Garrido et al., 1999
		Luminescence	Physiological	1300	Toussaint et al., 1995
Protozoa	*Pyrocystis lunula*	Growth rate inhibition	Growth/Reproduction	85	Stauber et al., 2008
Rotifers	*Brachionus plicatilis*	Mortality	Mortality/Immobilization	50	Rotini et al., 2018
	Proales similis	Mortality	Mortality/Immobilization	1060	Snell et al., 2019
		Reproduction	Growth/Reproduction	150	Snell et al., 2019
		Ingestion	Feeding	260	Snell et al., 2019
		Hatching	Growth/Reproduction	3400	Snell et al., 2019
Microalgae	*Cylindrotheca closterium*	Population growth	Growth/Reproduction	27.8	Araújo et al., 2010.
		Chlorophyll fluorescence	Growth/Reproduction	4.7	Araújo et al., 2010.
		Esterase activity	Physiological	7.8	Araújo et al., 2010.
		Population growth	Growth/Reproduction	10.1	Araújo et al.,2010.
	Dunaliella tertiolecta	Inhibition of oxygen rate production	Growth/Reproduction	1461	Franklin et al., 2001
	Gonyaulax tamarensis	Immobilisation	Mortality/Immobilization	1	Anderson and Morel, 1978
	Isochrysis aff. galbana Clone T-ISO	Population growth	Growth/Reproduction	0.4	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	3.6	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	4.4	Moreno-Garrido et al., 2000
	Nannochloris atomus	Population growth	Growth/Reproduction	16.7	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	27.3	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	46.2	Moreno-Garrido et al., 2000
	Phaeodactylum tricornutum	Population growth	Growth/Reproduction	9	Franklin et al., 2001
		Population growth	Growth/Reproduction	9.8	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	34.4	Moreno-Garrido et al., 2000
		Population growth	Growth/Reproduction	35	Moreno-Garrido et al., 2000
	Rhodomonas salina	Inhibition of oxygen rate production	Physiological	30	Moreno-Garrido et al., 1999
		Population growth	Growth/Reproduction	30	Moreno-Garrido et al., 1999
Copepods	*Acartia tonsa*	Egg production / reproduction	Growth/Reproduction	9.9	Lauer and Bianchini, 2010
	Tisbe battagliai	Mortality	Mortality/Immobilization	83.1	Diz, et al., 2009
		Mortality	Mortality/Immobilization	157	Diz, et al., 2009
		Fecundity	Growth/Reproduction	30.08	Diz, et al., 2009
		newborn production	Growth/Reproduction	44.5	Diz, et al., 2009
	Tigriopus fulvus	Mortality	Mortality/Immobilization	310	Biandolino et al., 2018
		Moult naupliar reduction	Morphological	55.8	Biandolino et al., 2018
		Development inhibition	Growth/Reproduction	21.7	Biandolino et al., 2018
		Mortality	Mortality/Immobilization	120	Rotini et al., 2018
		Release of the molt	Growth/Reproduction	70	Rotini et al., 2018
	Gladioferens pectinatus	Survival	Mortality/Immobilization	170	Charry et al., 2019
		Larval development ratio	Growth/Reproduction	49.8	Charry et al., 2019
		Realized offspring	Growth/Reproduction	101.5	Charry et al., 2019
		Potential offspring	Growth/Reproduction	127.1	Charry et al., 2019
		Total offspring	Growth/Reproduction	94.3	Charry et al., 2019

Bivalve	*Crassostera virginica*	Embryo-larval development	Growth/Reproduction	11.2	Arnold et al., 2010
	Mytilus galloprovincialis	Embryo-larval development	Growth/Reproduction	6.28	Arnold et al., 2010
Annelids	*Neanthes arenaceodentata*	Feeding rate	Feeding	72	Rosen and Miller, 2011.
	Ficopomatus enigmaticus	Sperm toxicity/Fertilization rate	Growth/Reproduction	80	Oliva et al., 2017
		Mortality	Mortality/Immobilization	80	Rosen y Miller, 2011.
Echinodermata	*Dendraster excentricus*	Embryo-larval development	Growth/Reproduction	18.9	Arnold et al., 2010
	Paracentrotus lividus	Embryonic malformations	Morphological	40.65	Morroni et al., 2018.
		Fertilization rate	Growth/Reproduction	20	Rotini et al., 2018.
	Strongylocentrotus purpuratus	Embryo-larval development	Growth/Reproduction	14.8	Arnold et al., 2010
Cnidaria	*Acropora aspera*	Inhibition in fertilisation	Growth/Reproduction	78	Gissi et al., 2017
	Aiptasia pallida	Inhibited development	Growth/Reproduction	5	Howe et al., 2014
		Mortality	Mortality/Immobilization	23	Howe et al., 2014
	Exaiptasia pallida	Reproduction	Growth/Reproduction	23	Trenfiled et al., 2017
		Mortality	Mortality/Immobilization	148	Trenfiled et al., 2017
	Platygyra daedalea	Inhibition in fertilisation	Growth/Reproduction	28	Gissi et al., 2017
Gastropods	*Haliotis rubra*	Morphological abnormalities	Morphological	7.10	Gorski and Nugegoda, 2006
	Nassarius dorsatus	Growth rate	Growth/Reproduction	4.7	Trenfield et al., 2016
Crustacean	*Artemia franciscana*	Mortality	Mortality/Immobilization	1280	Rotini et al., 2018
		Swimming speed alteration	Behavioral	638.54	Manfra et al., 2016
	Balanus amphitrite	Survivorship	Mortality/Immobilization	145	Qiu et al., 2005
		Molting success	Growth/Reproduction	97	Qiu et al., 2005
		Survivorship	Mortality/Immobilization	156	Qiu et al., 2005
		Molting success	Growth/Reproduction	91	Qiu et al., 2005
		Survivorship	Mortality/Immobilization	213	Qiu et al., 2005
		Molting success	Growth/Reproduction	129	Qiu et al., 2005
	Exosphaeroma gigas	Immobility	Mortality/Immobilization	6960	Giarratano et al., 2007
		Immobility	Mortality/Immobilization	2110	Giarratano et al., 2007
Shrimps	*Litopenaeus vannamei*	Avoidance	Spatial avoidance	11	Redondo-López et al. (*under review*)
	Palaemon varians	Avoidance	Spatial avoidance	10	Araújo et al., 2020.
		Avoidance	Spatial avoidance	43	Redondo-López et al. (*under review*)
	Penaeus monodon	Mortality	Mortality/Immobilization	1246	Chen and Lin, 2001
		Mortality	Mortality/Immobilization	3078	Chen and Lin, 2001
		Reduced length increase	Growth/Reproduction	667	Chen and Lin, 2001
		Reduced weight gain	Feeding	600	Chen and Lin, 2001
Crabs	*Carcinus maenas*	Mortality	Mortality/Immobilization	2000	Nonnote et al., 1993
	Chasmagnathus granulata	Loss of spawning	Growth/Reproduction	163.4	Zapata et al., 2001.
Fish	*Centropomus parallelus*	Mortality	Mortality/Immobilization	1880	Oliveira et al., 2014
	Pomatoschistus microps	Mortality	Mortality/Immobilization	568.1	Vieira et al., 2009
	Rachycentron canadum	Avoidance	Spatial avoidance	800	Araújo et al., 2016

Table S2. glyphosate.

Group	Species	Responses /Endpoint	Classification of the ecotoxicological responses	EC_{50} mg/L	References
Freshwater species					
Protozoa	*Tetrahymena pyriformis*	Growth	Growth/Reproduction	29.5	Tsui and Chu, 2003.
Microalgae	*Scenedesmus vacuolatus*	Growth rate	Growth/Reproduction	4.9	Iummato et al., 2019
		Growth	Growth/Reproduction	4.9	Lummato et al., 2019
	Microcystis aeruginosa	Growth	Growth/Reproduction	6.3	Zhang et el., 2018
		Growth	Growth/Reproduction	0.001486	Hernández-García and Martínez-Jerónimo, 2020
		Growth	Growth/Reproduction	6.7	Lipok et al., 2010
		Growth	Growth/Reproduction	251.4	Lipok et al., 2010
		Growth	Growth/Reproduction	81	Zhang et al., 2018
		Growth	Growth/Reproduction	63	Zhang et al., 2018

	Microcystis aeruginosa (nontoxic)	Growth	Growth/Reproduction	0.441	Smedbol et al., 2017
	Microcystis aeruginosa (toxic)	Growth	Growth/Reproduction	0.446	Smedbol et al., 2017
	Microcystis sp.	Growth	Growth/Reproduction	0.406	Smedbol et al., 2017
	Chlorella pyrenoidosa	Growth	Growth/Reproduction	0.396	Anton et al., 1993.
	Selenastrum capricornutum	Growth inhibition	Growth/Reproduction	5.81	Tsui and Chu, 2003
		Growth	Growth/Reproduction	24.7	Tsui and Chu, 2003
		Growth	Growth/Reproduction	41	Tsui and Chu, 2003
		Growth	Growth/Reproduction	3.92	Tsui and Chu, 2003
		Growth	Growth/Reproduction	5.81	Tsui and Chu, 2003
	Scenedesmus obliquus	Growth	Growth/Reproduction	0.422	Smedbol et al., 2017
	Oocystis lacustris	Growth	Growth/Reproduction	0.415	Smedbol et al., 2017
	Chlamydomonas reinhardtii	Growth	Growth/Reproduction	0.551	Smedbol et al., 2017
	Cryptomonas obovata	Growth	Growth/Reproduction	0.584	Smedbol et al., 2017
	Ankistrodesmus falcatus	Growth	Growth/Reproduction	0.649	Smedbol et al., 2017
		Growth	Growth/Reproduction	0.001411	Hernández-García and Martínez-Jerónimo, 2020
	Pseudokirchneriella subcapitata	Growth	Growth/Reproduction	0.001022	Hernández-García and Martínez-Jerónimo, 2020
	Chlorella vulgaris	Growth	Growth/Reproduction	0.001908	Hernández-García and Martínez-Jerónimo, 2020
		Growth	Growth/Reproduction	0.24	Rodriguez-Gil et al., 2017
		Growth	Growth/Reproduction	0.54	Rodriguez-Gil et al., 2017
		Growth	Growth/Reproduction	118.1	Lipok et al., 2010
		Growth	Growth/Reproduction	292	Lipok et al., 2010
	Scenedesmus incrassatulus	Growth	Growth/Reproduction	0.002702	Hernández-García and Martínez-Jerónimo, 2020
	Raphidocelis subcapitata	Growth	Growth/Reproduction	0.201	Rodriguez-Gil et al., 2017
		Growth	Growth/Reproduction	0.69	Rodriguez-Gil et al., 2017
	Oophila sp.	Growth	Growth/Reproduction	1.98	Rodriguez-Gil et al., 2017
		Growth	Growth/Reproduction	1.61	Rodriguez-Gil et al., 2017
	Chlorella pyrenoidosa	Growth	Growth/Reproduction	0.396	Anton et al., 1993
		Growth	Growth/Reproduction	0.38	Anton et al., 1993
		Growth	Growth/Reproduction	1.08	Anton et al., 1993
	Spirulina platensis	Growth	Growth/Reproduction	33.1	Lipok et al., 2010
	Nostoc puntiforme	Growth	Growth/Reproduction	42.3	Lipok et al., 2010
		Growth	Growth/Reproduction	598.4	Lipok et al., 2010
	Anabaena catenula	Growth	Growth/Reproduction	2.9	Lipok et al., 2010
		Growth	Growth/Reproduction	256.5	Lipok et al., 2010
	Synechocystis aquatilis	Growth	Growth/Reproduction	89.8	Lipok et al., 2010
		Growth	Growth/Reproduction	164.9	Lipok et al., 2010
	Leptolyngbya boryana	Growth	Growth/Reproduction	4.1	Lipok et al., 2010
		Growth	Growth/Reproduction	246.6	Lipok et al., 2010
	Chlorella kessleri (tolerant strain)	Growth	Growth/Reproduction	55.62	Romero et al., 2011
Insects	*Chironomus plumosus*	Immobility	Mortality/Immobilization	55	Folmar et al., 1979
Cladocera	*Ceriodaphnia dubia*	Mortality	Mortality/Immobilization	5.39	Tsui and Chu, 2003
	Daphnia magna	Mortality	Mortality/Immobilization	199	Demetrio et al., 2014
		Mortality	Mortality/Immobilization	9.34	Demetrio et al., 2014
		Immobility	Mortality/Immobilization	62	Alberdi et al., 1996
	Daphnia spinulata	Immobility	Mortality/Immobilization	66	Alberdi et al., 1996
Amphibians	*Lymnodynastes dorsalis*	Mortality	Mortality/Immobilization	3	Mann and Bidwell, 1999
		Mortality	Mortality/Immobilization	12	Mann and Bidwell, 1999
	Litoria moorei	Mortality	Mortality/Immobilization	2.9	Mann and Bidwell, 1999

		Mortality	Mortality/Immobilization	10.4	Mann and Bidwell, 1999
	Heleioporus eyrei	Mortality	Mortality/Immobilization	6.3	Mann and Bidwell, 1999
	Crinia insignifera	Mortality	Mortality/Immobilization	3.6	Mann and Bidwell, 1999
	Xenopus laevis	Mortality	Mortality/Immobilization	9.3	Perkins et al., 2009
	Rana temporaria	Mortality	Mortality/Immobilization	11.1	Wagner et al., 2017
		Mortality	Mortality/Immobilization	10.4	Wagner et al., 2017
		Mortality	Mortality/Immobilization	12.2	Wagner et al., 2017
		Teratogenic	Morphological	15.7	Wagner et al., 2017
		Teratogenic	Morphological	12.4	Wagner et al., 2017
	Rana clamitans	Mortality	Mortality/Immobilization	2.7	Wojtaszek et al., 2004
	Rana pipiens	Mortality	Mortality/Immobilization	4.25	Wojtaszek et al., 2004
Fish	*Salmo gairdneri*	Mortality	Mortality/Immobilization	140	Folmar et al., 1979
	Pimpehales promelas	Mortality	Mortality/Immobilization	97	Folmar et al., 1979
	Ictalurus punctatus	Mortality	Mortality/Immobilization	130	Folmar et al., 1979
	Lepomis macrochirus	Mortality	Mortality/Immobilization	140	Folmar et al., 1979
	Cyprinus carpio	Mortality	Mortality/Immobilization	620	Nešković et al., 1996
		Mortality	Mortality/Immobilization	520.77	Ma and Li, 2015
	Danio rerio	Avoidance	Spatial avoidance	0.0015	Mena et al. (*under review*)
Aquatic Plant	*Lemna minor*	Inhibition of growth rate	Growth/Reproduction	0.40222	Sikorski et al., 2019
		Inhibition in field (Iy)	Growth/Reproduction	0.47996	Sikorski et al., 2019
		Fresh mass of new fronds	Growth/Reproduction	0.57629	Sikorski et al., 2019
		Dry mass	Growth/Reproduction	2.72935	Sikorski et al., 2019
		Shikimic acid content	Biochemical	0.10985	Sikorski et al., 2019
		Chlorophyll SPAD	Physiological	1.47199	Sikorski et al., 2019
		Chlorophyll a content	Physiological	1.35876	Sikorski et al., 2019
		Chlorophyll b content	Physiological	1.32496	Sikorski et al., 2019
		Carotenoid content	Biochemical	1.69507	Sikorski et al., 2019
		Max. quantum efficiency (Fv/Fm)	Physiological	3.04538	Sikorski et al., 2019
		SAMDC activity - S-adenosylmethionine decarboxylase	Biochemical	0.09464	Sikorski et al., 2019
		ODC activity - ornithine decarboxylase	Biochemical	1.44833	Sikorski et al., 2019
		LDC activity - lysine decarboxylase	Biochemical	1.46354	Sikorski et al., 2019
		TDC activity - tyrosine decarboxylase	Biochemical	1.62071	Sikorski et al., 2019
		Tyramine content	Biochemical	0.92612	Sikorski et al., 2019
		Putrescine content	Biochemical	0.86528	Sikorski et al., 2019
		Cadaverine content	Biochemical	0.88894	Sikorski et al., 2019
		Spermidine content	Biochemical	0.78078	Sikorski et al., 2019
		Spermine content	Biochemical	0.90077	Sikorski et al., 2019
		Total biogenic amines content	Biochemical	0.8619	Sikorski et al., 2019
		Peroxidase activity	Biochemical	0.57798	Sikorski et al., 2019
		Catalase activity	Biochemical	0.9971	Sikorski et al., 2019
Marine/estuarine species					
Bacteria	*Vibrio fischeri*	Luminescence	Physiological	17.5	Tsui and Chu, 2003

Protozoa	*Euplotes vannus*	Growth	Growth/Reproduction	10.1	Tsui and Chu, 2003
Microalgae	*Skeletonema costatum*	Growth	Growth/Reproduction	1.85	Tsui and Chu, 2003
		Growth	Growth/Reproduction	5.89	Tsui and Chu, 2003
		Growth	Growth/Reproduction	3.35	Tsui and Chu, 2003
		Growth	Growth/Reproduction	2.27	Tsui and Chu, 2003
	Arthrospira fusiformis	Growth	Growth/Reproduction	28.2	Lipok et al., 2010
Copepods	*Acartia tonsa*	Mortality	Mortality/Immobilization	1.77	Tsui and Chu, 2003

Table S3. Ag-NPs.

Group	Species	Responses /Endpoint	Classification of the ecotoxicological responses	EC_{50} (ug/L)	References
Freshwater species					
Microalgae	*Raphidocelis subcapitata*	Photosynthetic efficiency	Physiological	21,200	Wang et al., 2012
		Photosynthetic efficiency	Physiological	4100	Wang et al., 2012
	Chlamydomonas reinhardtii	Photosynthetic yield	Physiological	20.3	Navarro et al., 2008
		Photosynthetic yield	Physiological	19.8	Navarro et al., 2008
		Photosynthetic yield	Physiological	21.5	Navarro et al., 2008
		Photosynthetic yield	Physiological	356	Navarro et al., 2008
		Photosynthetic yield	Physiological	113	Navarro et al., 2008
		Photosynthetic yield	Physiological	95	Navarro et al., 2008
		Photosynthetic yield	Physiological	86	Navarro et al., 2008
		Photosynthetic yield	Physiological	89	Navarro et al., 2008
		Photosynthetic yield	Physiological	321	Navarro et al., 2012
		Photosynthetic yield	Physiological	139	Navarro et al., 2012
		Photosynthetic yield	Physiological	231	Navarro et al., 2012
		Photosynthetic yield	Physiological	306	Navarro et al., 2012
		Photosynthetic yield	Physiological	84	Navarro et al., 2012
		Photosynthesis	Physiological	138	Piccapietra et al., 2012
		Photosynthesis	Physiological	62.6	Piccapietra et al., 2012
		Photosynthesis	Physiological	1078	Dewez & Oukarroum, 2012
	Thalassiosira weissflogii	Growth	Growth/Reproduction	1003	Bielmyer-Fraser et al., 2014
	Pseudokirchneriella subcapitata	Growth	Growth/Reproduction	3.02	Angel et al., 2013
		Growth	Growth/Reproduction	3.2	Angel et al., 2013
		Growth	Growth/Reproduction	32.4	Ribeiro et al., 2014
		Growth	Growth/Reproduction	9.9	Kennedy et al., 2014
		Growth	Growth/Reproduction	115.4	Tuominen et al., 2013
		Growth	Growth/Reproduction	51.8	Tuominen et al., 2013
	Synechococcus sp.	Growth	Growth/Reproduction	1079	Burchardt et al., 2012
	Mixed periphyton	PS yield	Physiological	8953	Gil - Allué et el., 2015
		respiration	Physiological	2373	Gil - Allué et el., 2015
		GLU activity	Physiological	1834	Gil - Allué et el., 2015
		LAP activity	Physiological	2480	Gil - Allué et el., 2015
Annelids	*Caenorhabditis elegans*	Mortality	Mortality/Immobilization	13400	Ellegaard-Jensen et al., 2012
		Mortality	Mortality/Immobilization	2800	Ellegaard-Jensen et al., 2012
Cladocera	*Daphnia magna*	Mortality	Mortality/Immobilization	4	Asghari et al., 2012
		Mortality	Mortality/Immobilization	2	Asghari et al., 2012
		Inmmobilization	Mortality/Immobilization	0.75	Lee et al., 2012
		Immobilization	Mortality/Immobilization	1	Kim et al., 2011
		Immobilization	Mortality/Immobilization	1.4	Kim et al., 2011
		Immobilization	Mortality/Immobilization	7	Hoheisel et al. 2012
		Immobilization	Mortality/Immobilization	10	Hoheisel et al. 2012

		Immobilization	Mortality/Immobilization	20	Hoheisel et al. 2012
		Immobilization	Mortality/Immobilization	30	Hoheisel et al. 2012
		Immobilization	Mortality/Immobilization	30	Hoheisel et al. 2012
		Immobilization	Mortality/Immobilization	10	Jo et al., 2012
		Immobilization	Mortality/Immobilization	10	Poynton et al., 2012
		Immobilization	Mortality/Immobilization	4	Poynton et al., 2012
		Immobilization	Mortality/Immobilization	30	Zhao and Wang, 2011
		Immobilization	Mortality/Immobilization	2	Zhao and Wang, 2011
		Immobilization	Mortality/Immobilization	1	Zhao and Wang, 2011
		Immobilization	Mortality/Immobilization	1	Kim et al., 2011
		Immobilization	Mortality/Immobilization	2	Kim et al., 2011
		Immobilization	Mortality/Immobilization	20	Blinova et al., 2012
		Immobilization	Mortality/Immobilization	40	Blinova et al., 2012
	Ceriodaphnia dubia	mortality and/or immobilization	Mortality/Immobilization	221	McLaughlin and Bonzongo, 2012.
		Immobilization	Mortality/Immobilization	5	Kennedy et al., 2012
		Immobilization	Mortality/Immobilization	30	Kennedy et al., 2012
	Daphnia pulex	Immobilization	Mortality/Immobilization	40	Griffitt et al. 2008
Fish	*Oryzias latipes*	Survival	Mortality/Immobilization	28	Kim et al. 2011
		Survival	Mortality/Immobilization	67	Kim et al. 2011
		Mortality	Mortality/Immobilization	1380	Wu and Zhou, 2013
		Mortality	Mortality/Immobilization	1120	Wu and Zhou, 2013
		Mortality	Mortality/Immobilization	870	Wu and Zhou, 2013
		Mortality	Mortality/Immobilization	10,000	Kwok et al., 2012
		Mortality	Mortality/Immobilization	2500	Kwok et al., 2012
		Mortality	Mortality/Immobilization	10,000	Kwok et al., 2012
		Mortality	Mortality/Immobilization	30	Chae et al., 2009
		Mortality	Mortality/Immobilization	1390	Kashiwada et al., 2012
	Pimephales promelas	Mortality	Mortality/Immobilization	90	Hoheisel et al., 2012.
	Danio rerio	Mortality	Mortality/Immobilization	1610	Wang et al., 2012
		Mortality	Mortality/Immobilization	1360	Wang et al., 2012
		Mortality	Mortality/Immobilization	780	Wang et al., 2012
		Avoidance	Spatial avoidance	2.5	The current study
	Oreochromis mossambicus	Morphological	Morphological	12,600	Govindasamy and Rahuman, 2012
	Pimephales promelas	Mortality	Mortality/Immobilization	9400	Laban et al., 2010

		Mortality	Mortality/Immobilization	11,250	Laban et al., 2010
		Mortality	Mortality/Immobilization	10,600	Laban et al., 2010
		Mortality	Mortality/Immobilization	1360	Laban et al., 2010
Aquatic plant	*Lemna minor*	Frond number	Growth/Reproduction	38.06	Gubbins et al., 2011
		Frond number	Growth/Reproduction	42.51	Gubbins et al., 2011
	Spirodela polyrhiza	Fresh weight	Growth/Reproduction	13,670	Jiang et al., 2012
		Dry weight	Mortality/Immobilization	13,670	Jiang et al., 2012
		ChlA	Physiological	16,100	Jiang et al., 2012
		Phosphate–phosphorus	Physiological	17,330	Jiang et al., 2012
		Nitrate–nitrogen	Physiological	4540	Jiang et al., 2012
Marine/estuarine species					
Microalgae	*Phaeodactylum tricornutum*	Growth	Growth/Reproduction	162,5	Sendra et al, 2017
		Growth	Growth/Reproduction	100,3	Pérez et al., 2014
		Growth	Growth/Reproduction	2384	Angel et al., 2013
		Growth	Growth/Reproduction	6925	Angel et al., 2013
	Chlorella autotrophica	Growth	Growth/Reproduction	570	Sendra et al., 2018
		Cell viability	Physiological	320	Sendra et al., 2018
		cell complexity	Physiological	1490	Sendra et al., 2018
		EQY	Physiological	1340	Sendra et al., 2018
		active Chlorophyll	Physiological	220	Sendra et al., 2018
		ROS	Physiological	200	Sendra et al., 2018
	Dunaliella salina	Growth	Growth/Reproduction	640	Sendra et al., 2018
		Chla	Physiological	3500	Sendra et al., 2018
		EQY	Physiological	2500	Sendra et al., 2018
		active Chlorophyll	Physiological	780	Sendra et al., 2018
	Cylindrotheca closterium	Growth	Growth/Reproduction	239.5	Pérez et al., 2014
	Nitzchia palea	Growth	Growth/Reproduction	76.6	Pérez et al., 2014
	Thalassiosira pseudonana	Growth	Growth/Reproduction	1079	Burchardt et al., 2012

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