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Revisão

TOXICITY OF ZINC OXIDE TO SCLERACTINIAN CORALS AND ZOOXANTHELLAE: A BRIEF REVIEW

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In recent years, the safety of sunscreens to coral reefs and the role these products would play in the process of coral bleaching has been a concern. However, the discussion centers almost exclusively on the organic components used as UV filters, with little attention given to inorganic UV filters, such as zinc oxide. Zinc oxide nanoparticles (ZnO-NPs) have been a common ingredient of sunscreen formulations for decades and are being discharged in coral reef environments. Even though there are data supporting that ZnO is toxic to certain species of fish and algae, regulatory agencies do not appear concerned with the possible outcome of the exposure of corals and their algal symbionts to this metal oxide. This review compiles the published work on the toxicity of ZnO and ZnO-NPs to corals and zooxanthellae, which strongly supports the notion that zinc oxide is toxic to both corals and microalgae. In view of this, concern has been raised by the use of the "reef safe" label to promote sunscreens that lack specific organic components, however containing ZnO as the major UV filter.

Keywords: zinc oxide; sunscreen; coral; zooxanthellae; reefs.

INTRODUCTION

Coral reefs are threatened in the current environmental scenario mainly due to the large load of anthropogenic carbon dioxide, which induces the acidification of marine environments, and increases sea surface temperatures.[1] Although such problems require great attention, they are not the only anthropogenic threats to reef biodiversity. Emission of pollutants into the aquatic environment, either localized or diffuse, can quickly degrade a region or conservation area. However, due to its very nature, it can be more easily controlled. A category of xenobiotics that has received interest due to the possible impact on the health of reef ecosystems is the sunscreens used by bathers in touristic areas. In fact, it is estimated that around 14000 tons of sunscreen are released in coral reef areas each year.[2]

Sunscreens are topical products that can protect the skin from the harmful effects of sunlight, such as burns and cancer. They include in their formulation ultraviolet (UV) filters, ingredients that have the ability to interact with solar radiation by absorption, reflection or dispersion, either organic (salicylates, PABA esters, cinnamates, benzimidazoles, cyanocrylates, benzophenones, anthranilates or dibenzoylmethanes) or inorganic (zinc oxide, titanium dioxide).[3] Some countries have banned the use of certain sunscreens due to the presence of organic compounds considered toxic to corals (e.g. oxybenzone and octinoxate), however, there are no bans regarding zinc oxide nanoparticles (ZnO-NPs), which are commonly used as UV filters.[4]

This implies that corals are being exposed to ZnO-NPs,[5,6] and their effects and risks to marine life have been the subject of recent studies which motivated this review. Initially, the context that led to the use of ZnO in sunscreens will be addressed, and then we will verify what results from its contact with fundamental organisms in reef areas: corals and their symbiotic algae.

Use of ZnO as a UV filter

Historians believe that ancient peoples already had some knowledge of the properties of ZnO as a protector against burns caused by excessive exposure to sunlight, since records of its use for this purpose were found in medical texts from ancient India.[7] Since then, ZnO has been applied in a wide variety of cosmetic and medicinal products; starting in the 1990s, it has been used in the composition of sunscreens as an inorganic filter to protect against UV light.[7–11] ZnO has a broad spectrum of coverage and can be effective against both UVA (320 - 400 nm) and UVB (290 - 320 nm) radiation. In addition, ZnO is photostable at these wavelengths, which is not always the case with organic filters, which absorb radiation in a short range of wavelengths and do not always remain stable after continuous exposure to light.[8,12] When applied at the nanometer scale (less than 100 nm), ZnO does not leave white blots or streaks on the skin, making the product more attractive to consumers without compromising the sun protection factor.[13–15]

Furthermore, a recent study demonstrated that such ZnO nanoparticles (ZnO-NPs) do not seem to penetrate deeper than the *stratum corneum*, the outermost layer of the epidermis, after local and repeated application, and that there is no change in the morphology of the cells or redox states caused by toxicity or apoptosis,[16] contradicting previous concerns regarding the safety of using this substance in topically applied products.[6,17]

The use of ZnO (and other components) in sun protection products is subject to regulatory aspects. In the United States of America, Food and Drug Administration (FDA) allows the use of 16 UV filters in the composition of sunscreens, 14 of which are organic compounds and only two inorganic: zinc oxide and titanium dioxide (TiO_2).[9] FDA further divides the 16 permitted active compounds into three groups according to the safety and efficacy of the application, with only the inorganic UV filters ZnO and TiO_2 generally recognized as safe and effective (GRASE determination).[5,13]

The European Commission, as well as FDA, allows the use of zinc oxide as an ingredient in sunscreen formulations in concentrations of up to 25% by mass.[7] In the European Union (EU) sunscreens are regulated as cosmetics, but in the US these products are more rigorously controlled, as they are considered over-the-counter (OTC) drugs. This difference in regulation results in 16 approved UV filters applicable in sunscreen formulations in the US against 29 in the EU.[4,18] As a consequence, even though the maximum allowed concentration of ZnO is the same between US and EU, the other components may vary considerably between these locations.

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Corals, zooxanthellae, and coral bleaching

Corals are marine invertebrates that form colonies of identical polyps, secreting an exoskeleton of calcium carbonate. Many corals live in symbiosis with unicellular, photosynthetic algae, usually of the genus *Symbiodinium,* colloquially known as zooxanthellae.[19] About 90% of the products of algal photosynthesis are used by the animal host and, in return, the symbiotic cells receive inorganic micronutrients, protection from predators and constant exposure to sunlight.[20]

Coral bleaching occurs when, under stress, there is partial or total release of microalgae from the endodermal tissue of the hosts, causing discoloration, which may be related to the loss of photosynthetic pigments.[21] In some instances the corals can switch their source of nutrients to a heterotrophic way, by capturing zooplankton,[22] however, in the long term this may not be sufficient for their survival.

In addition, corals regulate the abundance of other microorganisms associated with them, releasing bacteria and viruses directly into seawater or through their mucous membranes. Under stress, this release can be excessive, leading to an imbalance in coral immunity and increased susceptibility to infections, which can further contribute to the bleaching process.[23–25]

Bleaching can be avoided, and some organisms are naturally more resistant to this phenomenon. On the coast of the northern region of the Red Sea, for example, mass bleaching of corals has never been recorded. Deposition of sand from neighboring deserts in this ecosystem is a form of supplementation of nutrients such as nitrate, phosphate, and metal ions (especially iron and manganese), thus stimulating photosynthesis of zooxanthellae.[26]

Motivation for the review

On the one hand, zinc is an essential metal for biological systems, entering into the composition of about 10% of eukaryotic proteins; after iron, it is the most abundant trace element in the human body.[27] Zinc is the only metal that is represented in the six fundamental classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases), playing both catalytic and structural roles.[28]

In the case of corals and its symbionts, when does zinc stop being a micronutrient and become toxic? Could zinc released from the dissolution of ZnO-NPs present toxicity? Would the overload by dissolved zinc be the only way ZnO-NPs could be toxic? Such questions have been raised and, in this review, some of the works that brought answers and new insights into the relationship between sunscreens, zinc oxide nanoparticles and coral reefs will be addressed.

Several efforts have been made to study the physicochemical behavior of these nanoparticles under environmental conditions, and the antibacterial activity and toxicity of ZnO to several species of plants and macroalgae were already demonstrated.[29,30] There is also concern about other anthropogenic sources of zinc that, by chance, may increase the concentration of this metal in neritic environments, such as rivers that pass through heavily populated urban or industrial environments [31–33] or accidents with ships on coral reefs, as antifouling paints usually contain zinc oxide derivatives.[34–36] ZnO is, in principle, poorly soluble in water, however this behavior may be different at the nanometer scale. Also, in the surroundings of coral reefs there is a higher concentration of organic matter, which may allow leaching of zinc by the formation of zinc complexes.[10,11,37–45] This means that, in addition to the ZnO-NPs themselves, reefs can be exposed to the most diverse chemical species containing zinc, therefore it is necessary to consider the toxicity of the nanoparticles and the possible compounds derived from them.

In this sense, the keywords "zinc and (zooxanth* or symbiodin* or coral* or dinoflag*)" were used to search for pertinent references in the Web of Science® database. This search returned 409 papers whose abstracts were read. Three and six works were directly dedicated to the toxicity of zinc or zinc oxide to zooxanthellae and corals, respectively. In addition, other articles dealing with other microalgae (6) and cnidarians (4) discussed various possibilities of test organisms, methodologies, and chemical species used in toxicity assessment tests, therefore they were included as a source of comparative data and of recommendations that could be extended to the problematic of coral ecotoxicology.

TOXICITY OF ZINC OR ZnO-NPs TO ZOOXANTHELLAE AND OTHER MICROALGAE

In zooxanthellae, Zn^{2+} is present in enzymes involved in photosynthesis, in the capture and sequestration of inorganic carbon (e.g. carbonic anhydrase), in the assimilation of macronutrients (e.g. nitrate reductase), in antioxidant reactions (e.g. catalases, peroxidases and superoxide dismutase) and in several other cellular processes, such as DNA and RNA replication and repair.[46] In addition, studies indicate that zinc, after iron, is the most necessary metal for the growth of zooxanthellae (specifically the species *Fugacium kawagutii*), precisely because it is a cofactor in a large number of enzymes.[21,47]

However, even with its important role, it must be understood whether an overload of zinc or the presence of zinc oxide nanoparticles can pose threats to the health of the symbionts[48–50] (Table 1).

Table 1. Survey of the toxicity of Zn species to zooxanthellae and other microalgae

Organism	Zinc source	Evaluated Parameters	Reference
Symbiodinium microdriaticum	$ZnSO_4·7H_2O$	Zooxanthellae density, Specific growth rate	51
Symbiodinium sp.	$ZnSO_4$	Effects on key photosynthetic proteins and processes	52
Zooxanthellae from *Exaiptasia pallida*	$ZnCl_2$	Zinc accumulation in zooxanthellae and anemone tissue	53
Pseudokirchneriella subcapitata	$ZnSO_4·7H_2O$, ZnO-NPs and bulk ZnO	IC50[a], EC50[b], EC20[c], NOEC[d]	54,55
Thalassiosira pseudonana, Skeletonema marinoi	ZnO-NPs	NEC[e]	56
Skeletonema costatum	ZnO-NPs Bulk ZnO	Growth inhibition ratio, EC50, Intracellular zinc accumulation, Malondialdehyde (MDA) level	57
Thalassiosira pseudonana	Industry-grade ZnO-NP Sunscreen-grade ZnO-NP ZnO-GO[f] ZnO-CNT[g]	Growth inhibition, ROS[h] detection	58,59

[a]IC50: inhibitory concentration giving a 50% reduction in algal growth rate; [b]EC50: 50% effect concentration; [c]EC20: 20% effect concentration; [d]NOEC: no observed effect concentration; [e]NEC: no effect concentration; [f]ZnO-GO: zinc oxide and graphene nanohybrid; [g]ZnO-CNT: zinc oxide and carbon nanotube nanohybrid; [h]ROS: reactive oxygen species.

Goh and Chou,[51] in 1997, observed that *Symbiodinium microdriaticum* under a mixture of zinc and copper will only reach the stationary phase of growth when in the presence of chelators (Na_2EDTA and $Na_2SiO_3{\cdot}9H_2O$), indicating that free ions are more toxic than chelated ones. Growth rates were not affected when comparing controls and Zn (509 μg L^{-1}).[51]

In 2013, Kuzminov and colleagues[52] found that exposure to zinc led to damage and decreased levels of key proteins for the photosynthetic process, inhibition of electron transport between photosystems II (PSII) and I (PSI), alterations on the maximum rate of electron transport and decrease of *S. microadriaticum* growth rate.[52]

Hardefeldt and Reichelt-Brushett[53] studied the role zooxanthellae play in zinc uptake in the *Exaiptasia pallida* anemone, another cnidarian that can undergo bleaching. It was found that there was greater accumulation of Zn in the zooxanthellae when compared to the tissue of the anemones at the end of the experiment (32 days), showing that the microalgae have a greater capacity to absorb and accumulate zinc than the hosts. Interestingly, anemones treated with Zn lost more zooxanthellae, which could be a mechanism of host detoxification in case of metal overload.[53]

The previous references addressed the effects that dissolved zinc would have in zooxanthellae. However, to the best of our knowledge no studies of the effects of nanoparticulate zinc oxide were carried out on isolated *Symbiodinium* sp. For this reason, some of the vast body of work regarding the toxic effects of ZnO-NPs to other marine and freshwater microalgae is reported below. The following references were chosen based mainly on their discussion of the possible toxicity mechanisms of ZnO-NPs, or presenting methodologies that could be adapted on tests with zooxanthellae.

Franklin *et al.*[54] and Aruoja *et al.*[55] compared the toxicity of Zn^{2+}, ZnO-NPs and bulk ZnO (ie, ZnO whose properties do not depend on particle size) to the freshwater microalga *Pseudokirchneriella subcapitata*, with similar results (Table 1). The dissolution of both nanoparticulate and bulk ZnO was rapid and almost total in culture media, and the toxicities of ZnO-NPs or bulk zinc oxide were not significantly different from the toxicity of zinc chloride, suggesting that toxicity could be due to Zn^{2+} dissolution.[54] As seawater is usually more alkaline than freshwater, the dissolution rate of ZnO due to amphoterism could be increased and, consequently, raise its toxicity profile.

Miller and colleagues[56] studied the effect of ZnO-NPs over marine diatoms (however, no comparison to freshwater diatoms were conducted). *T. pseudonana* and *S. marinoi* were significantly affected at the highest concentration of ZnO-NPs, with the former having its growth rate reduced by a factor of 3 at the ppb scale. This clearly indicates that even low concentrations of nanoparticles in the marine environment can cause complications in the ecosystem, as ZnO-NPs could be a constant source of Zn.[56] Besides, the attachment of nanoparticles to the cells may promote physical damage and oxidative stress.[48,56]

Multiple modes of action were studied in the diatom *Skeletonema costatum* [57] Even though the dissolution levels of both ZnO-NPs and bulk ZnO were not significantly different, the former caused higher growth inhibition ratio (IR) (Table 1) by increased lipid peroxidation at the cellular membrane, which would lead to changes in its permeability and, therefore, favor higher zinc uptake.[57] This suggests that accumulation of intracellular zinc is also an interesting parameter to investigate when studying toxicity of zinc or ZnO-NPs to zooxanthellae.

Baek and collaborators[58] showed that nanohybrids of ZnO with graphene oxide (ZnO-GO) or carbon nanotubes (ZnO-CNT) (Table 1) were less toxic than pure ZnO-NPs. In all cases, high ROS production was detected. It was argued that hybrid nanoparticles aggregate less than pure NPs and, therefore, have greater surface area and reactive sites available for ROS formation, which can increase their toxic character.[58] Another work demonstrated that industry- or sunscreen-grade ZnO-NPs have similar toxicity characteristics (Table 1).[59] In both studies, however, extremely high NPs concentrations were used, which may not be relevant to real world concerns. For example, the Australian and New Zealand Guidelines for Freshwater and Marine Water Quality states that the trigger value to protection of 99% of marine species is only 7 μg Zn L^{-1}.[60] When testing in ecotoxicologically relevant concentrations the effects might even be absent. Besides, quantitative parameters to express toxicity such as EC50 were not calculated.

Studies with microalgae other than Symbiodiniaceae are relevant since they shed light on parameters and methodologies that can be valid to establish the toxicity of ZnO-NPs to zooxanthellae. It is important to be aware that the tested concentrations reflect environmental conditions. Also, there must be consensus on which parameters should be applied to evaluate the toxicity of metal ions and the nanoparticles. Lastly, it is recommended to assess the toxicity of not only ZnO-NPs to zooxanthellae, but also of other zinc compounds (e.g. zinc salts), which may help to establish the toxicity mechanism(s).

TOXICITY OF ZINC OR ZnO-NPs TO CORALS AND OTHER CNIDARIA

Table 2 summarizes the findings regarding toxicity of zinc and zinc oxide to corals and other cnidarians.

It is important to study the toxicity of possible contaminants in all life stages of the organisms of interest.[18] In the case of corals, early life stages may be more sensitive to stressors, especially for broadcast spawning corals, whose fertilization occurs externally with gametes and coral larvae directly exposed to the marine environment.[71]

Reichelt-Brushett and Harrison[61] studied the effects that copper, cadmium and zinc could have on the fertilization of *Goniastrea aspera* gametes (Table 2). In the case of zinc at concentrations up to 500 μg L^{-1}, no difference was observed in fertilization success compared to the control.[61] However, the fertilization of *Acropora tenius* was affected even at the lowest concentration of zinc tested. In controls, the average fertilization success rate was 91%, but in the treatment with 10 μg L^{-1} of zinc it was 70% and, with 100 μg L^{-1} of zinc, the success rate dropped to values below 1%. At the highest concentration tested, fertilization of *A. tenius* gametes was completely abolished.[62] There is a dramatic difference of behavior, depending on coral species. Therefore, for any general ecotoxicological analysis for regulation purposes, it is crucial to determine the effects that zinc (or any other contaminant) has on coral fertilization and, especially, which coral species should be used as standard in tests. This topic will be addressed later in this review.

Scleractinian corals, also known as stony corals, are the main builders of coral reefs. Zinc is an important micronutrient for the process of photosynthesis of zooxanthellae and calcification of hosts, and studies with *Stylophora pistillata* indicate that corals have good physiological adaptations to the low concentrations of Zn^{2+} in the oceans, and that symbiotic microalgae play an important role in the absorption of zinc by the hosts, since this absorption is stimulated by exposure to light.[72] However, several recent studies indicate that zinc excess is potentially toxic to corals from the genera *Seriatopora*, *Acropora* and *Stylophora*.

Tang and collaborators[66] studied the consequences that exposure of *Seriatopora cliendrum* to ZnO-NPs would have in the composition of the lipid membrane of coral cells. Interestingly, 34-38% dissociation of the nanoparticles (with free Zn^{2+} release) was observed. The authors observed significant changes in the lipids profile of the

Table 2. Survey of the toxicity of Zn species to corals and other cnidarians

Organism	Zinc source	Evaluated Parameters	Reference
		Corals	
Goniastrea aspera (gametes)	$ZnSO_4$	Fertilization success, NOEC[a]	61
Acropora tenuis (gametes)	$ZnSO_4$	Fertilization success, NOEC	62
Acropora aspera (adult)	$ZnCl_2$	DMSP[b] concentration, Zinc uptake, Symbiont density	63
Stylophora pistillata (adult)	Bulk ZnO	Photochemical response, Changes in the PSII[c]	64
Acropora spp. (adult)	ZnO-NPs	Zooxanthellae release, Coral bleaching, Prokaryotic and viral abundance	65
Seriatopora cliendrum (adult)	ZnO-NPs	Membrane lipid profiles	66
		Other	
Exaiptasia pallida (adult)	$ZnCl_2$	Zinc accumulation, Catalase activity	67
Aiptasia pulchella (adult)	$ZnCl_2$	28-day EC50[d] and EC10[e], LOEC[f]	68
Aiptasia pulchella (adult)	$ZnCl_2$	8- and 14-day-EC10, EC50, LC10[g] and LC50[h]	69
Aiptasia pulchella (adult)	$ZnCl_2$ (metal mixture containing also $CuCl_2$, $NiCl_2$ and $CdCl_2$)	Metal accumulation, Zooxanthellae density, Activities of catalase, carbonic anhydrase, and glutathione reductase	70

[a]NOEC: no observed effect concentration; [b]DMSP: dimethylsulfonipropionate; [c]PSII: photosystem II; [d]EC50: 50% effect concentration; [e]EC10: 10% effect concentration; [f]LOEC: lowest observed effect concentration; [g]LC10: concentration leading to 10% lethality; [h]LC50: concentration leading to 50% lethality.

plasma membranes and, as white spots of accumulation of ZnO-NPs were observed on the surface of the corals, they considered that the observed effects were due to the ZnO-NPs as particles and not due to the release of free Zn^{2+}. The changes observed in lipids are consistent with what would be expected of a cell that needed to accommodate the mechanical disturbances caused by nanoparticles, and could, in the long term, represent a chronic danger.[66] Importantly, this work focused on subcellular changes and does not necessarily translate into effects to the entire holobiont. On the other hand, the short exposure time (24 h) simulated a condition of acute contamination only; it remains to be evaluated the effects of these nanoparticles on corals in tourist regions during an entire holiday season with bathers.

The work of Corinaldesi *et al.*[65] addressed the influence in coral bleaching of the two inorganic oxides most used in sunscreen formulations, nanoparticulated ZnO and TiO_2. It was observed that ZnO caused an extensive release of zooxanthellae. With only 24 h of exposure, a considerable loss of photosynthetic pigments was already detected, which indicates that the bleaching induced by ZnO occurs very quickly. At the end of the exposure time (48 h), 67% of the coral surface was already bleached. Titanium dioxide did not cause similar effects. These observations indicate that ZnO-NPs can induce total and irreversible bleaching of corals and zooxanthellae mortality.[65] This is in agreement with other works that have suggested, for example, that Zn^{2+} can lead to manganese deficiency in higher algae,[56] damage to mitochondria and DNA,[73] oxidative stress[74] and damage to the plasma membrane [75] in concentrations that exceed physiological usefulness.

Neither of the two previous works evaluated the toxicity of dissolved zinc ions alone. This would be important to assess how the dissolution of zinc from ZnO-NPs played a part in its toxicity to the corals. Also, in some works very high concentrations of ZnO-NPs (mg L^{-1} level) are studied,[65] therefore their environmental relevance should be discussed.

Another approach to monitoring zinc toxicity to *Acropora aspera* corals was proposed by Deschaseaux and colleagues.[63] They evaluated the concentration of dimethylsulfonipropionate (DMSP), one of the compounds that may be involved in metal detoxification mechanisms both in the host and in the symbionts, after zinc overload. The decrease in DMSP levels occurred first in coral tissue and later in zooxanthellae, which is an indication that these microalgae may be more resistant to zinc than the cnidarians. DMSP is a possible marker of oxidative stress caused by zinc overload, since the decrease in DMSP concentration may be a consequence of an increase in the production of ROS or of the cessation of DMSP synthesis due to the consumption of its precursor cysteine for metal detoxification.[63] This study provides a possible parameter for monitoring zinc contamination in *A. aspera* corals, and it would be interesting to apply this method with other screlactinian corals to determine DMSP levels would also answer to zinc overload.

Fel and coworkers[64] studied the photochemical response of *Stylophora pistillata* after chronic exposure to several UV filters, including ZnO, pesticides, and herbicides. To monitor this response, the authors verified changes in PSII in a manner similar to the work already discussed by Kuzminov and collaborators[52]. However, in this case, corals and symbionts were treated at the same time. The main observations were that, while organic UV filters only caused changes in PSII at the highest concentrations, ZnO induced 38% reduction in its activity at *ca.* 100 µg L^{-1}. This suggests that the organic components of UV filters are less toxic than ZnO to the PSII of the symbionts, with possible consequences for other coral physiological parameters.[64]

Again, Tang *et al.*[66], Deschaseaux *et al.*[63] and Fel *et al.*[64] focused on subcellular alterations that may not be translated into cellular death or bleaching when considering the holobiont in macroscale. Yet, these works provide interesting aspects to be evaluated when studying the toxicity mechanisms of Zn^{2+} and ZnO-NPs to corals.

The study of the toxicity of zinc to anemones, which are also zooxanthelated organisms subject to bleaching, can serve as a basis for future works that address the toxicity of zinc to corals. Duckworth and collaborators[67] investigated the correlation between ocean acidification conditions and zinc and nickel absorption by *E. pallida*. Co-exposure of metals and high levels of carbon dioxide caused the anemone to accumulate more metals in its tissues. Since these levels of CO_2 caused a 0.2 unit decrease in pH, it was concluded that ocean acidification can make metal cations more bioavailable, which could potentiate their toxic effects.[67] However, in a similar work carried out with the coral *S. pistillata* lower pH values decreased zinc uptake in tissue and skeleton.[76] This difference in behavior between both organisms under similar conditions deserves to be further investigated in order to understand which biochemical mechanisms and strategies make corals more resistant than anemones to zinc absorption when this metal is more bioavailable.

Howe, Reichelt-Brushett and Clark published several works addressing the toxic effects of metals in solution for asexual reproduction of *A. pulchella* anemones (Table 2).[68,69,77] Under ca. 500 mg L^{-1} Zn after 22 days, *A. pulchella* expelled its zooxanthellae, bleached and died. As in other studies,[53,78] the loss of zooxanthellae was attributed to an attempt by the hosts to remove symbiotic cells with high metal loads.[68] Zinc caused a time-dependent inhibition in the development of new individuals.[69,77]

Brock and Bielmyer also worked with *A. pulchella*, exposing them to a mixture of metals (copper, zinc, nickel and cadmium) in solution. Zinc started to accumulate in the organisms at concentrations of 50 and 100 µg L^{-1}, but it was completely cleared after a recovery period, which may have been due to the loss of zooxanthellae. It is also interesting to note that GR activity increased in individuals exposed to concentrations of 50 and 100 µg L^{-1} of cations, which indicates an excess in the production of hydrogen peroxide. The authors suggest that the activity of this enzyme may be a possible marker of metal-induced stress in anemones.[70] This multi-metal exposure approach can offer valuable leads in identifying markers of zinc toxicity to corals.

Parameters such as effective concentrations (EC) are typically found for zooxanthellae (Table 1) or anemones (Table 2), but not for screlactinian corals, which may reflect the different time scale of the toxicity response and the difficulty to handle and determine death in the latter. Addressing these shortcomings would be important in order to have better comparative information to assess threats to reef communities. In fact, the most comprehensive studies on zinc toxicity to cnidarians were performed in anemones; hence, we suggest that further work with corals could benefit from the same multiparameter approach (EC10 and EC50, LOEC, enzyme inhibition, effect on growth). We believe this would allow for a better informed choice of the parameters relevant for adult corals.

THE USE OF THE TERM "REEF SAFE" FOR SUNSCREENS AND THE DIFFICULTY OF STANDARDIZING TOXICOLOGICAL TESTS FOR CORALS

In the last year, two reviews were published on personal care products for sun protection, the active compounds used as sunscreens, and their possible toxicity to corals.

Miller and coworkers[4] carried out a bibliographic survey about sunscreens banned in some countries on the grounds of containing compounds deemed toxic to corals. In addition, it was surveyed if there were studies on coral toxicity of the ingredients of sunscreens marketed as "reef safe" (or similar).[4] One of the motivators for their review was the recent, widespread claims of coral-safe formulas for sunscreens. Of all the products presented as "reef safe", 79% used inorganic UV filters as the main component and, of these, 78% used ZnO only and 20% used a mixture of ZnO and TiO_2. Moreover, the authors noticed that only organic compounds were included in any of the bans, especially oxybenzone (benzophenone-3; (2-hydroxy-4-methoxyphenyl)-phenylmethanone), whose dermatological and ecotoxicological risks, including bleaching of corals,[2,79] have already been extensively investigated. However, ZnO is classified as hazardous to aquatic life by the Globally Harmonized System of Classification and Labeling of Chemicals (GHS)[80] and the European Chemical Agency (ECHA).[81] In fact, as discussed above, there are abundant indications of the toxicity of ZnO-NPs specifically to corals.[64–66]

Therefore, it is debatable whether sunscreens should be considered "reef safe" if most of them contain zinc oxide filters. Furthermore, it would be necessary to encourage a reduction in the amount of sunscreen released into the seas, since in addition to the compounds that act as UV filters, there are several other ingredients that can contribute to the bioavailability of filters to corals. It was indicated the need to establish scientific criteria for the use of the terminology "*coral-/reef-safe*". Also, it would be important to review the banning of sunscreens due to the different treatments granted to organic and inorganic filters,[4] and to the possible risks to public health by decreased use of sunscreens, or by the use of less efficient or even dangerous home-made concoctions.[82]

In this context, the question arises whether there would be any substitute for metallic oxides in the formulations of safe sunscreens for coral reefs. The answer may be the defense strategies of marine microorganisms against UV radiation.[83] In addition to DNA repair mechanisms and behavioral patterns, dinoflagellates, diatoms and cyanobacteria produce photoactive substances and antioxidants that protect them from direct exposure to UV light. One class of compounds produced is the so-called mycosporine-like amino acids (MAAs), based on a cyclohexenone or cyclohexemine conjugated to an amino acid residue, which absorb in the UV between 310-362 nm.[84–86] Some cyanobacteria also produce scytonemin, a lipid-soluble dye with an indole and a phenolic subunit that absorbs mainly in the UVA region.[87,88] These two classes of compounds have the potential to be used in sun protection products, but they should be further studied regarding their extraction, stability and sun protection factor.[83]

The second review focuses on aspects necessary for the standardization and regulation of ecotoxicology tests of UV filters in scleractinian corals. Toxicological test results may not be adequate if obtained using non-standardized and non-validated methods.[18] For the practical and immediate purpose of preventing/reversing damage to reef ecosystems, this is probably the most urgent need. Besides the main points raised by the authors (appropriate choice of species and their life stage, appropriate exposure duration and appropriate choice of endpoints), which should be internationally agreed upon, we would like to add that the consideration of chemical speciation should also be included in such tests. As pointed out throughout the review, particle size matters, and zinc form (soluble or solid) affects bioavailability. In addition, standardization of proper concentration units such as molarity rather than mass per volume should be preferred. Although less intuitive for the general population, molarity-described toxicity parameters are directly comparable across different chemical substances.

In addition, zinc oxide is already considered toxic to aquatic life by agencies such as ECHA, meaning that it has been tested in fish, algae and daphnia, which are already part of standardized ecotoxicology methodologies. Therefore, it is likely that even to non-standard species, such as corals, the apparent toxicity would be like that of standard species. The authors then conclude by suggesting that all the data obtained on the toxicity of ZnO to corals are preliminary because they did not follow a standardized and validated method that is necessary to develop a specific test system.[18] Notwithstanding, not only the data referring to ZnO are preliminary. Burns and Davies,[89] applying reliability approach, assessed published results from ecotoxicity tests with corals and organic UV filters used for environmental risk assessment (ERA) and regulatory decisions. None of the then published works were considered reliable for higher tier ERA or regulatory decision making, hence all evidence for the toxicity of organic UV filters is also preliminary. This work brings up the urgency to achieve greater quality toxicity data regarding corals, whichever would be possible with robust standard methodologies.[4,18,49,89,90]

It is evident that the development of standardized toxicity assessment methods would facilitate the execution of tests on an international scale and aid the decision-making of health and regulatory authorities on, for example, which UV filters would be allowed or not, without departing from scientific rigor.[18] Efforts in this sense have been published by Miller *et al.* in 2022.[90] Using benzophenone-3 as a test compound, this work aimed to contribute to the standardization of an acute coral larvae toxicity test. The test system with larvae, test duration (48 h) and endpoints were aspects suitable for validation. Yet, the selection of representative species remains an obstacle, which is made more complicated by the high lipophilicity of sunscreens and subsequent difficulty in securing homogeneous distribution in the test compartments.[90]

CONCLUSIONS

Literature parameters for the determination of the toxicity of zinc or zinc oxide nanoparticles to zooxanthellae and corals are quite diverse. The species tested, the concentrations of zinc or ZnO-NPs, and exposure times are different among studies. This situation hinders scientific and/or regulatory consensus. However, even under these conditions, there is an indication that zinc ions and ZnO-NPs may present toxicity to some species of zooxanthellae and corals. Thus, it is urgent to validate and standardize methodologies for toxicological tests in these organisms, to establish the toxicity of ZnO-NPs (and other constituents of sunscreens) to corals and their maximum allowed levels in reef environments. A standardized ecotoxicological methodology would also give the "reef safe" label an evidence-based meaning. Until then, it is important to continue the work aimed at unraveling the mechanisms of Zn^{2+} and ZnO-NPs toxicity to corals and zooxanthellae, in addition to the development of models that predict the behavior and destination of compounds used in sunscreens in the marine environment.

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