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# Impact of inorganic UV filters contained in sunscreen products on tropical stony corals (*Acropora spp.*)

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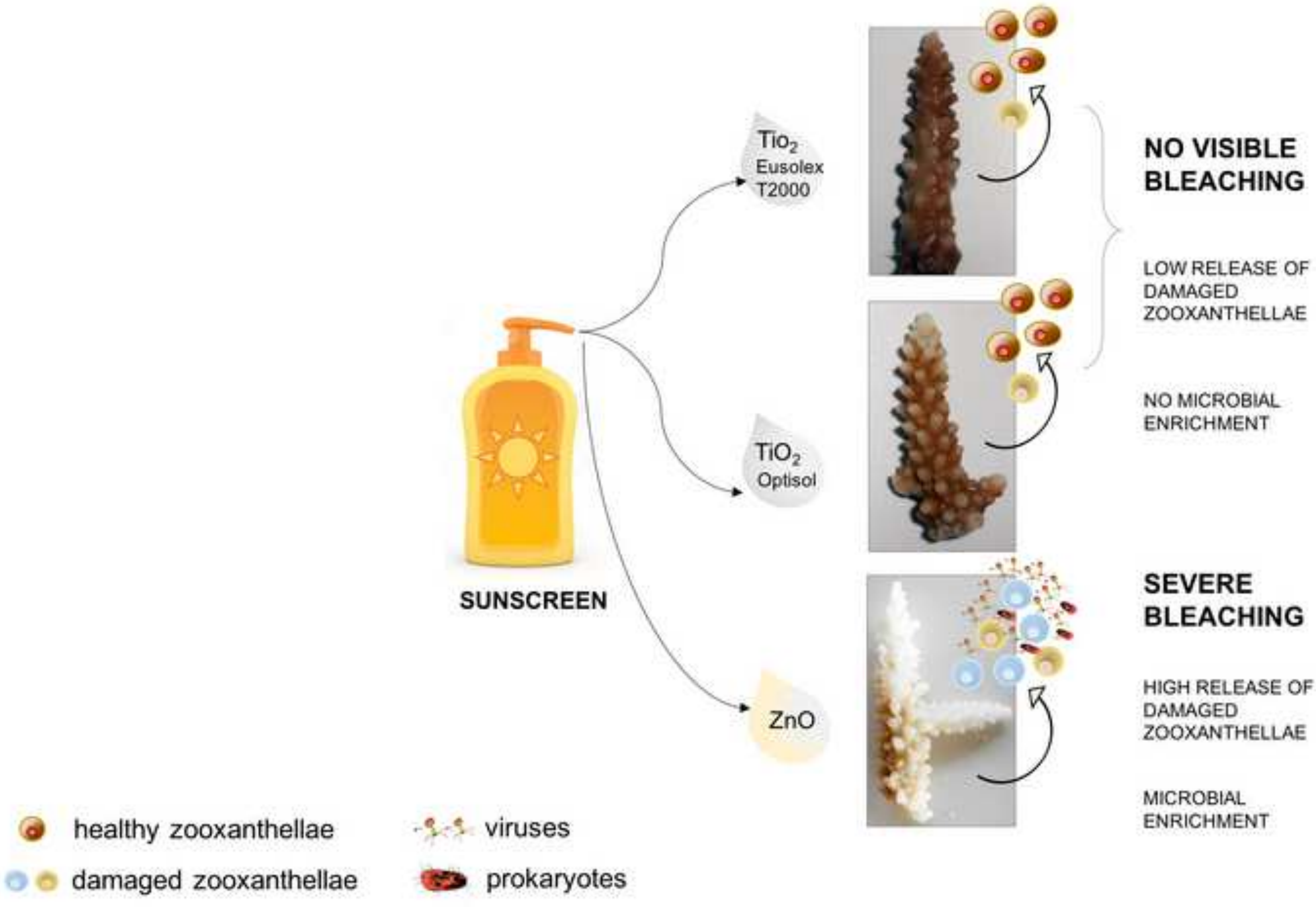
*Graphical Abstract



**Highlights**

- Organic UV-filters and preservatives in sunscreens can harm coral reefs worldwide.
- Among the inorganic UV filters tested in the Maldives, ZnO caused the bleaching of *Acropora spp*.
- Bleaching induced by ZnO was determined by its impact on symbiotic algae and was associated with a microbial enrichment.
- The inorganic filters Eusolex® T2000 and OptisolTM did not cause evident bleaching, resulting more eco-compatible.
- The use of eco-compatible filters in sunscreens is highly recommended to protect coral reef health in the future.

## Abstract

Most coral reefs worldwide are threatened by natural and anthropogenic impacts. Among them, the release in seawater of sunscreen products commonly used by tourists to protect their skin against the harmful effects of UV radiations, can affect tropical corals causing extensive and rapid bleaching. The use of inorganic (mineral) filters, such as zinc and titanium dioxide (ZnO and $TiO_2$) is increasing due to their broad UV protection spectrum and their limited penetration into the skin. In the present study, we evaluated through field experiments, the impact on the corals *Acropora spp*. of uncoated ZnO nanoparticles and two modified forms of $TiO_2$ (Eusolex® T2000 and Optisol™), largely utilized in commercial sunscreens together with organic filters. Our results demonstrate that uncoated ZnO induces a severe and fast coral bleaching due to the alteration of the symbiosis between coral and zooxanthellae. ZnO also directly affects symbiotic dinoflagellates and stimulates microbial enrichment in the seawater surrounding the corals. Conversely, Eusolex® T2000 and Optisol™ caused minimal alterations in the symbiotic interactions and did not cause bleaching, resulting more eco-compatible than ZnO. Due to the vulnerability of coral reefs to anthropogenic impacts and global change, our findings underline the need to accurately evaluate the effect of commercial filters on marine life to minimize or avoid this additional source of impact to the life and resilience ability of coral reefs.

**Keywords:** sunscreens, coral bleaching, inorganic filters, titanium dioxide, zinc oxide

## 1. Introduction

Coral reefs are amongst the most diverse and productive ecosystems on Earth supporting a huge biodiversity (around 830,000 multi-cellular species, Fisher et al., 2015), and providing ecosystem goods and services to half a billion people including food provision, financial incomes and protection against natural hazards (Hughes et al., 2012, Teh et al., 2013, Ferrario et al., 2014). Approximately, 70% of coral reefs are currently threatened by several natural and anthropogenic impacts including overfishing, urban-coastal development, pollution and tourism (Krieger and Chadwick 2013; Spalding & Brown, 2015; Nepote et al., 2016, Tsui et al., 2016). It has been estimated that every year, millions of tourists travel to tropical destinations (UNTWO, 2015) enhancing the risk of important consequences on marine life and ecosystems (Danovaro et al., 2008, Giglio et al. 2015). In the last decades, production and consumption of sunscreens containing active organic (e.g. cinnamates, camphor derivatives, benzophenones) and/or inorganic (e.g. $TiO_2$ and ZnO) ingredients to protect human skin from UV radiation, have increased in the cosmetic market on a global scale (Osterwalder et al. 2014, Sánchez-Quiles & Tovar-Sánchez, 2014).

Despite organic filters dominates the market of sunscreen products, the combined use of inorganic compounds, such as zinc oxide (ZnO) and titanium dioxide ($TiO_2$), is constantly increasing due to the broad UV spectrum of protection, and their limited penetration into the skin (Lu et al. 2015;). However, the potential of these compounds to generate reactive oxygen species (ROS) and release metal ions into the aquatic environment has been recently demonstrated, with consequent possible negative effects on aquatic organisms (Wong et al. 2010, Minetto et al., 2017; Hu et al., 2017, Blaise et al., 2008; Hayens et al., 2017). At the same time, investigations on the impact of ZnO and $TiO_2$ on marine life, being mostly focused on microalgae, are still too limited to draw general conclusions (Miller et al. 2010, Hazeem et al., 2016).

Previous studies have also shown that sunscreen products and their organic ingredients (e.g., organic UV filters such as ethylhexyl methoxycinnamate, benzophenone-3, benzophenone-2 and preservatives such as butylparaben) can harm tropical reefs worldwide contributing to coral bleaching (Danovaro et al., 2008; Downs et al., 2014).

It has also been hypothesised that inorganic filters, such as $TiO_2$ and ZnO, depending on their specific physical characteristics (i.e. size, crystal form, morphology of particles; Peng et al., 2011; Sendra et al., 2017), can produce different effects on marine algae. Indeed, uncoated ZnO and $TiO_2$ molecules are both known to generate reactive oxygen species (ROS) and release metal ions into the aquatic environment (Hazeem et al., 2016; Minetto et al., 2017).

In the present study, we tested the hypothesis that these filters can also harm stony corals, possibly through the impact on their symbiotic microalgae. For this purpose, we evaluated the impact of inorganic UV filters, largely utilised in commercial sunscreens, on the stony corals of the genus *Acropora* of the Maldivian Lhaviyani Atoll (Vavvaru Island). We conducted field experiments based on the addition of ZnO nanoparticles and of two forms of $TiO_2$ (Eusolex T2000 and Optisol). The genus *Acropora* was selected as it is the dominant stony coral in tropical coral reefs worldwide, and their symbiotic algae (i.e. *Symbiodinium sp*.) can be easily recognised, investigated and cultured. The findings obtained here can expand our knowledge on the impact of inorganic UV filters on coral reefs in order to understand the best tools and practices for minimising the impacts of tourism and recreational activities and preserving these corals and their ecosystems.

## 2. Materials and methods

### 2.1 *Inorganic UV filters*

In the present study, we tested the impact of zinc oxide nanoparticles (SIGMA) characterised by uncoated particles of size ranging from 20 to 200 nm (nanoparticles > 50% of the total particles), as observed by Scanning Electronic Microscopy and two forms of titanium dioxide: Optisol™ (Oxonica Ltd and UK Nanotechnology Company) and Eusolex® T2000 (Merck KGaA). Eusolex®

T2000 is represented by the crystal form "rutile" with particles size of 20 nm and by the surface coated with alumina and dimethicone. Optisol$^{TM}$ is another modified form of titanium dioxide in which a small amount of manganese is incorporated into the structural lattice conferring free radical scavenging power, thus minimising the formation of free radicals (Wakefield et al., 2004). These modifications (surface coatings and metal doping) have the scope to reduce the potential reactivity of photo-activated $TiO_2$ particles by quenching and/or reducing the reactive species generated before they can interact with the other ingredients in a formula and with skin components itself (Tiano et al., 2010).

*2.2 Sampling area and experimental design*

Coral nubbins (3-6 cm) belonging to the genus *Acropora spp*. were collected from different donor colonies at ca. 5 m water depth in the front reef area of Vavvaru Island (Lhaviyani Atoll, Maldives). Nubbins were immediately placed in experimental mesocosms located at ca. 50 m from the sampling site and supplied with a continuous seawater flow, which allowed us to keep the same conditions present *in situ*. Corals were acclimatised in aquarium for 48 h at *in situ* conditions of temperature and salinity (28 °C and 35, respectively). After acclimatisation, the healthy corals (i.e. without any sign of bleaching or necrotic tissue, and showing open polyps) were washed in virus-free seawater (filtered onto 0.02 µm membranes Anotop syringe-filters; Whatman, Springfield Mill, UK). Replicate sets of nubbins (n=3, containing more than 300 polyps each) were divided and immersed each in separate experimental mesocosms. A final concentration of 6.3 mg $L^{-1}$ of each inorganic UV filter was added to the three replicate systems, except for the systems used as controls, which were incubated without sunscreen products. Such a concentration (established considering that typically the concentration of inorganic filters in sunscreen products is~12%) falls within the range of values of these inorganic compounds detected in the aquatic environment according to the available literature (Tovar-Sanchez et al. 2013; Ruszkiewicz et al. 2017).

*2.3 Release of zooxanthellae and their health status*

Zooxanthellae were analysed from seawater samples collected from the seawater of the experimental mesocosms in order to quantify the number of the symbiotic organisms released from the coral colonies. Ten mL of seawater were collected from treated (added with filters) and untreated systems immediately after the addition of UV filters ($t_0$= start of the experiment) and after 24 h ($t_{24}$) and 48 h ($t_{48}$) from the beginning of the experiment. Aliquots of seawater samples were filtered through 2.0-μm polycarbonate filters and mounted on glass slides. Zooxanthellae were counted under a Zeiss Axioplan epifluorescence microscope (Carl Zeiss Inc., Jena, Germany; ×400 and ×1,000). Based on the autofluorescence and gross cell structure, we discriminated the zooxanthellae released from coral colonies as pale (P, pale yellow colour, vacuolated, partially degraded zooxanthellae) and transparent (T, lacking pigmentations, an empty zooxanthellae) from healthy zooxanthellae (H, brown/bright yellow colour, intact zooxanthellae) (Mise & Hidaka 2003; Danovaro et al., 2008).

*2.4 Bleaching quantification*

According to Siebeck et al. (2006), we performed a colorimetric analysis of digital photographs of corals taken at the beginning of the experiments and after 48 h of treatment with UV-filters (specified above). Photographs were taken under identical illumination with a Canon EOS 400D digital camera (Canon Inc., Tokyo, Japan) with a scale meter on the background. The photographs were subsequently analysed with a photo-editing software for colour composition cyan, magenta, yellow, black (CMYK). Levels of bleaching were measured as the difference between the coral's colour at the beginning of the experiments ($t_0$) and after 48 h of exposure ($t_{48}$). Thirty random measurements of variables CMYK were carried out across the coral area. Variations in the percentage of the different colour components (CMYK) were analysed with one-way analysis of variance (ANOVA). To rank the bleaching effect due to the different sunscreens tested, we obtained Bray–Curtis similarity matrix and multidimensional scaling analysis of the shifts in CMYK colour composition of treated corals using Primer 5.0 software (Primer-E Ltd., Plymouth, UK). Bleaching rates were measured as the variation percentage in CMYK colour composition between treated and control corals using Primer 5.0 software (Primer-E Ltd). In addition, to the mean values obtained we attributed scores of the bleaching degree by means of a mathematical function, according to a scale organized in ranks (0 to > 60), i.e. from "no visible coral bleaching" (0-10) to "total bleaching" of 100% of coral nubbins surface (> 60).

*2.5 Prokaryotic and viral abundance*

Prokaryotic and viral abundance in seawater samples was determined according to the protocol described by Noble & Fuhrman (1998). Sub-samples (10 mL) from treated (added with filters) and untreated systems were collected immediately after the addition of sunscreen ($t_0$= start of the experiment) and after 24 h ($t_{24}$) and 48 h ($t_{48}$) from the beginning of the experiment. After collection, three replicate seawater samples were stored at -20 °C until the analysis. Sub-samples were filtered onto 0.02 μm pore size filter (Whatmann Anodisc; diameter, 25 mm; $Al_2O_3$) and stained with 100 μL of SYBR Gold (stock solution diluted 1:5000). The filters were incubated in the dark for 20 min, washed three times with 3 mL of prefiltered Milli-Q water and mounted onto glass slides with 20 μL of 50% phosphate buffer (6.7 mM phosphate, pH 7.8) and 50% glycerol (containing 0.5% ascorbic acid). Slides were stored at -20 °C. Prokaryotes and viruses’ counts were obtained by epifluorescence microscopy (Zeiss Axioskop 2). For each slide, at least 20 microscope fields were observed and at least 200 prokaryotes and viruses were counted per filter.

*2.6 Statistical analysis*

Differences in the investigated variables between controls and treatments were assessed using permutational analyses of variance (PERMANOVA; Anderson, 2005; McArdle and Anderson, 2001) on square root transformed data. The design included two fixed factors (time and treatment). When significant differences were encountered ($p < 0.05$) *post-hoc* pairwise tests were also carried out. Statistical analyses were performed using PRIMER 6 (Clarke and Gorley, 2006).

**3. Results and discussion**

The inorganic UV-filters tested here, ZnO and $TiO_2$ (especially in the rutile form) nanoparticles are commonly used in commercially sunscreen products for their UVA (320-400 nm) and UVB (290-320 nm) coverage and to increase the transparency of cosmetics applied on the skin (Smijs and Pavel 2011).

The analyses conducted in this study reveal that ZnO caused the strongest negative effects in terms of number of zooxanthellae released from the stony corals investigated ($p<0.001$, Figure 1A). In particular, the release of zooxanthellae after ZnO addition was significantly higher than in the control and in the corals treated with both $TiO_2$ forms (Eusolex T2000 and Optisol) with the strongest effect after 48 h of exposure (zooxanthealle release up to two orders of magnitude higher than in the control and other treatments; Figure 1A). In addition, ZnO determined the release of the highest fraction of damaged zooxanthellae (up to one order of magnitude higher than the other UV filters) suggesting that these nanoparticles can strongly affect hard corals impairing their symbiotic microalgae.

Previous eco-toxicological studies documented the negative effects of ZnO nanoparticles on marine organisms (including algae, crustaceans and fish, Wong et al. 2010, Peng et al. 2011). Here, we expand the evidence on the negative effect of ZnO nanoparticles, revealing their impact also on tropical corals and their symbiosis with microalgae.

The addition of both Eusolex T2000 and Optisol also caused an increase in the release of zooxanthellae in the seawater surrounding coral nubbins when compared to the control (Figure 1A). However, whereas Eusolex T2000 showed effects in the short term ($t_0$ and $t_{24,}$ $p<0.01$), Optisol acted only after 24-48 h of exposure ($p<0.01$). PERMANOVA analyses confirmed the significant differences in the responses of *Acropora* exposed to the two types of $TiO_2$ as a result of the treatment × time interaction (treatment x time, $p<0.01$).

In the zooxanthellae released from corals we reported a loss of photosynthetic pigments already 24 h after exposure to ZnO (Figure 1B). The abundance of damaged zooxanthellae, indeed, increased over time reaching values up to two orders of magnitude higher than in the controls and in the other treatments ($p<0.001$). The amount of damaged zooxanthellae released by corals treated with Eusolex T2000 increased significantly already after 24 h of exposure compared to the control ($p<0.05$) whereas the effect of Optisol was more evident after 48 h of exposure ($p<0.001$).

Previous studies revealed that inorganic $TiO_2$ nanoparticles are the major-oxidizing agents in coastal waters, producing very high rates of $H_2O_2$ in seawater and directly affecting the growth of phytoplankton (Tovar-Sanchez et al. 2013). Our findings indicate that the $TiO_2$ filters, Eusolex T2000 and Optisol, have a very low impact on corals and symbiont microalgae potentially due to their surface or structural modifications (manganese doping for Optisol and alumina and dimethicone coating for Eusolex), which minimise the potential reactivity of photo-activated particles and render them initially inert in water (Botta et al. 2011). At the same time, the different response time of corals to the two inorganic filters (immediate for Eusolex and delayed for Optisol) might be associated with the diverse characteristics of the $TiO_2$ filters, which once released in seawater could have a different behaviour and/or action mechanism (Tsui, et al., 2017). Since

Optisol determined a “delayed effect” on the symbiotic interaction between corals and zooxanthellae, we cannot exclude a long-term effect on the corals due to chronic exposure (Tsui et al. 2017).

The loss of zooxanthellae induced by ZnO resulted in a fast coral bleaching, which was evident after 24 h of exposure (Figure 2), and at the end of the experiment bleaching dominated for 67% of the corals’ surface (Figure 3). Conversely, after addition of the two different types of $TiO_2$ no visible bleaching was observed in the corals (Figure 2), which, indeed, resulted bleached only for 6-7% of their surface similarly to the control (3%, Figure 3).

The lower impact of $TiO_2$ on the corals when compared to ZnO was evident also in terms of microbial enrichment in the seawater surrounding the nubbins of *Acropora*. Previous studies demonstrated that tropical corals subjected to environmental stress regulate the abundance of their associated microbes, essential to coral immunity and health (Krediet et al., 2013), by increasing the amount of bacteria and viruses released directly in seawater and/or through mucus (Garren and Azam, 2012; Nguyen-Kim et al. 2015). In addition, previous investigations reported that sunscreen products and their UV filters increase virus proliferation in seawater as well as other environmental stressors (Davy et al., 2006, Danovaro and Corinaldesi, 2003; Danovaro 2008). Here, we observed that in systems treated with ZnO a strong enrichment of both prokaryotes and viruses was observed after 48 h of incubation ($3.0 \pm 0.4 \times 10^9$ cells $L^{-1}$ vs. $4.0 \pm 0.3\times10^8$ cells $L^{-1}$ in the control; $p<0.001$, Figures 4A and B). Conversely, the two types of $TiO_2$ did not determine any significant increase in microbial abundance over time ($1.3 \pm 0.1\times10^8$ and $0.9 \pm 0.1\times10^8$ cells $L^{-1}$ in the treatment with Eusolex T2000 and Optisol, respectively, Figure 4A and B), suggesting that their impact on *Acropora* corals was limited.

Concluding, our findings indicate that uncoated ZnO nanoparticles induce a complete and irreversible coral bleaching causing a significant rapid and widespread mortality of the symbiotic zooxanthellae of the stony corals and stimulating microbial enrichment in the seawater surrounding corals.

Market trends of sunscreen products indicate that ZnO filter utilization will overtake nano-titanium dioxide ($nTiO_2$) in the near future, especially after the approval of ZnO for cosmetic purposes in the EU since April 2016 (http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016 R0621). Indeed, ZnO offers high skin protection due to its greater broad-spectrum UV coverage and reduces opaqueness thanks to improved formulation technologies (Lademann et al. 2006; Smijs and Pavel, 2011). The use of ZnO in cosmetic and sunscreen products has been hypothesised to be a safer alternative to conventional organic-based filters due to several issues related to photoinstability, skin irritability and endocrine disrupting ability (Krause et al. 2012; Hojerova et al. 2011; Biebl et al. 2006). However, the results reported here demonstrate that the use of ZnO is extremely harmful for the organisms tested and should be prohibited in all personal care products that can be introduced in seawater, not only for sunscreen products. Since the negative impact of ZnO will be also present when it is used in combination with $TiO_2$, the ban should be extended also to sunscreen products using a combination of both inorganic filters. Although the use of coated/modified $TiO_2$ in sunscreens is not completely exempt of potential negative effects (Tanvir et al. 2015), the results of the present study indicate that when it is used alone (i.e., as a single ingredient) can have a limited impact on tropical stony corals. However, further investigation are needed to clarify if its use is fully eco-compatible, able to preserve the marine life, while protecting human skin from UV damage or can be harmful if used in specific conditions or combination with other products.

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**Conflict of interest**

The authors declare no competing financial interests.

**Figure legends**

**Figure 1.** Impact of the inorganic filters on symbiotic microalgae. Total abundance of zooxanthellae released from corals exposed to 6.3 $mgL^{-1}$ zinc oxide and titanium dioxide (Eusolex T2000 and Optisol) over time (A), and abundance of damaged zooxanthellae released from the corals (partially degraded, lacking pigmentations, and empty zooxanthellae (B) compared to control (corals unexposed to any filter).

**Figure 2.** *Acropora spp*. nubbins exposed to the inorganic filters. Photographs of the corals exposed to inorganic filters at the start ($t_0$) and at the end (48h) of the experiment and in the control. Reported are: controls (corals unexposed to inorganic filters; A and B) and corals treated with zinc oxide (C and D), with Eusolex T2000 (E and F) and Optisol (G and H).

**Figure 3.** Bleaching degree in *Acropora spp*. exposed to different inorganic UV filters. Percentage of bleaching in the corals exposed to 6.3 $mgL^{-1}$ zinc oxide and titanium dioxide (Eusolex T2000 and Optisol) and scale of bleaching severity.

**Figure 4.** Microbial enrichment in the seawater surrounding corals induced by inorganic filters. Prokaryotic (A) and viral (B) abundances in seawater surrounding corals exposed to 6.3 $mgL^{-1}$ zinc oxide and titanium dioxide (Eusolex T2000 and Optisol) over time.

Figure 1
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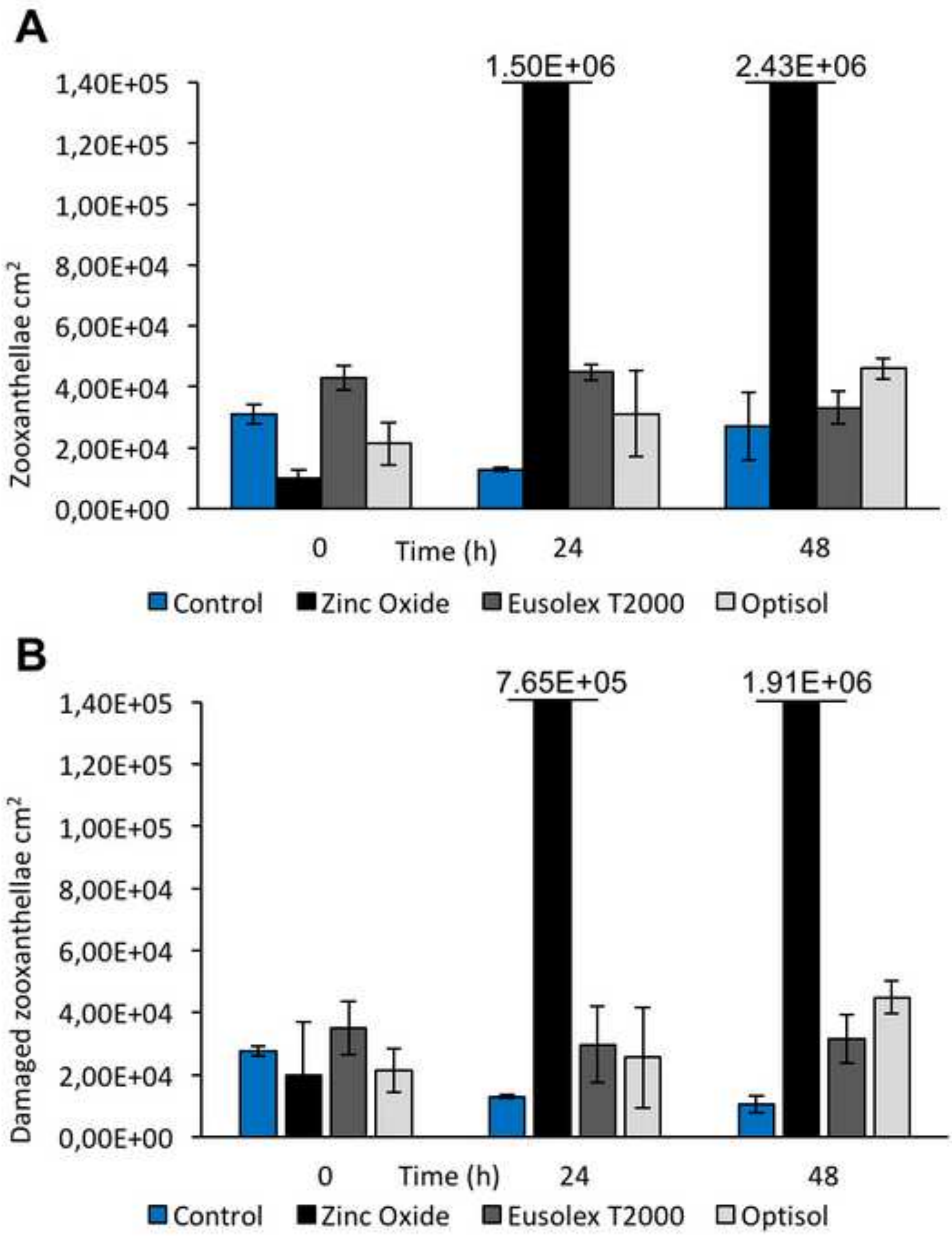

Figure 2

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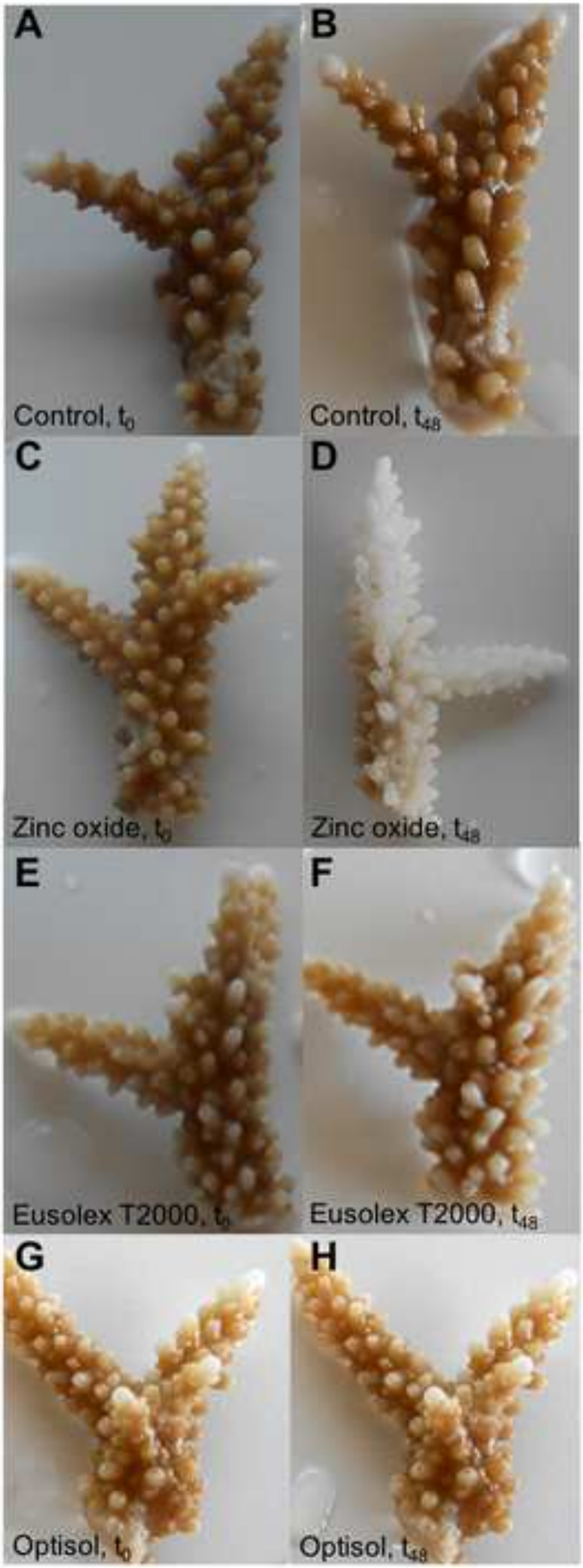

Figure 3

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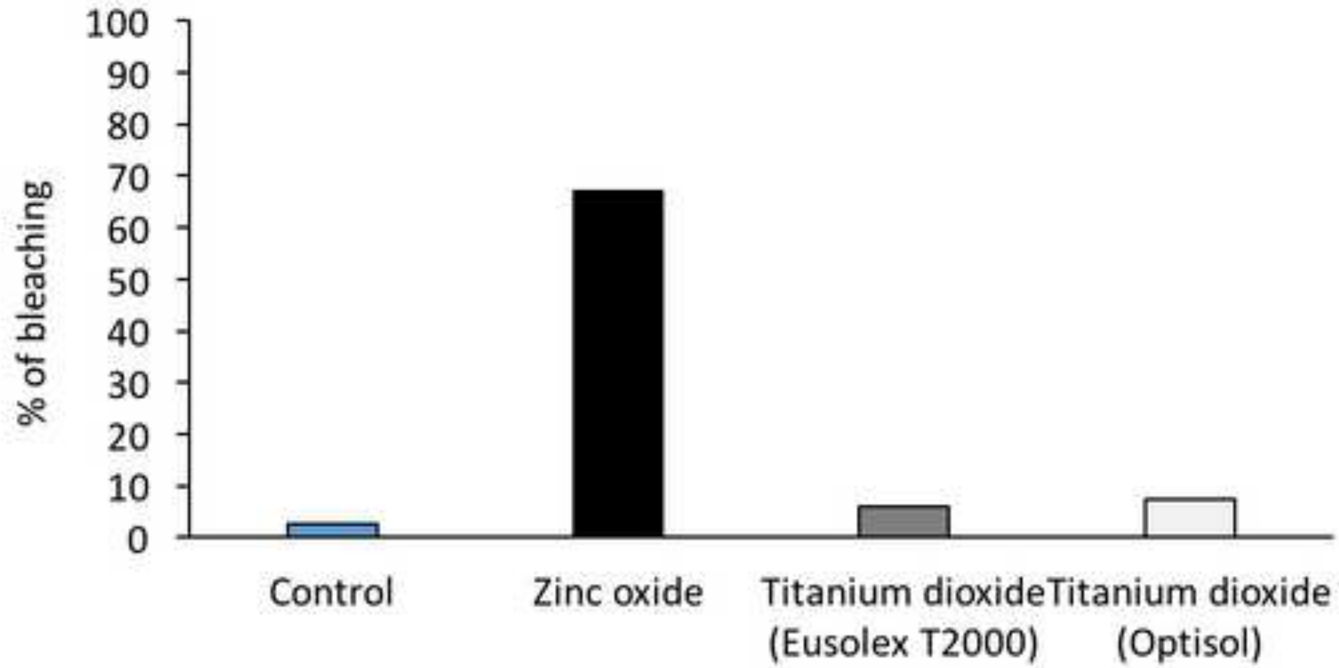


| Degree of bleaching (%) | Severity of coral bleaching |
|---|---|
| 0-10 | No visible bleaching |
| 10-20 | Slight color variation. No visible bleaching |
| 20-30 | Slight bleaching (<10% of coral nubbins surface) |
| 30-60 | Strong bleaching (50-60 % of coral nubbins surface) |
| >60 | Severe bleaching (> 60% of coral nubbins surface ) |

Figure 4
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