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## (54) ECO-COMPATIBLE SUNSCREEN COMPOSITIONS

ECO-KOMPATIBLE SONNNENSCHUTZZUSAMMENSETZUNGEN COMPOSITIONS D'ÉCRAN SOLAIRE ECO-COMPATIBLES

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#### Description

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**[0001]** The present description refers to eco-compatible sunscreen compositions, in particular to sunscreens compatible with the marine environment and the life inhabiting it, with specific reference to coral reefs.

## STATE OF THE PRIOR ART

[0002] Coral reefs are among the most biologically productive and diverse ecosystems in the world, representing hot spots of marine biodiversity, and directly sustaining half a billion people (Moberg and Folke 1999, Wilkinson 2004). Approximately 60% of coral reefs are currently threatened by several natural and anthropogenic impacts (Hughes et al 2003; Pandolfi et al 2003). Over the last 20 years, coral bleaching (loss of symbiotic zooxanthellae hosted within scleractinian corals) has increased dramatically, both in frequency and spatial extent (Hoegh-Guldberg 1999; Hughes et al 2003; Knowlton 2001). This phenomenon has been associated with temperature anomalies, excess ultraviolet (UV) radiation or altered availability of photosynthetic radiation, and presence of bacterial pathogens and pollutants (Brown et al 2000; Bruno et al 2007; Douglas 2003; Glynn 1996; Jones 2004).

[0003] Production and consumption of personal care and cosmetic sun products are increasing worldwide, with potentially important consequences related to environmental contamination. The release of these products is also linked with the rapid expansion of tourism in marine coastal areas (Wilkinson 2004). Chemical compounds contained in sunscreens and other personal care products can reach detectable levels in seawater (Daughton and Ternes 1999; Giokas et al 2007). These compounds might be potentially harmful to the environment; hence the use of sunscreen products is now banned in a few of the most popular tourist destinations, for example, in marine ecoparks in Mexico (Xcaret 2007; Xel-ha 2007). Because sunscreens are lipophilic, their UV filters can accumulate in aquatic animals (Giokas et al, 2007) and cause effects similar to those reported for other xenobiotic compounds (Balmer et al 2005; Daughton and Ternes 1999).

[0004] Paraben preservatives and some UV absorbers contained in sunscreens have estrogenic activity (Daughton and Ternes 1999; Schlumpf et al 2004). In addition, it has been demonstrated that sunscreen ingredients can undergo various photodegradations, with the entailed transformation of these agents into toxic byproducts (Giokas et al. 2007, and literature therein). It has also been demonstrated that sunscreens have an impact on marine bacteria (Danovaro and Corinaldesi 2003). Recently, a tight relationship among sunscreens and bleaching of hard corals was observed through specific tests worldwide (Danovaro et al. 2008). In particular, in this study the harmful effects on corals of some UV filters used in sunscreens and of some commercially available sunscreen compositions have been analyzed.

**[0005]** A product manufactured by the company MyChelle is a non-toxic reef-safe sunscreen composition comprising the Zinc Oxide as a sunblock.

**[0006]** Object of the present invention is that of solving the above-mentioned drawbacks by providing a sunscreen composition that is eco-compatible with the marine ecosystem of the coral reef.

## SUMMARY OF THE INVENTION

**[0007]** Commercially available sunscreens comprise a variety of chemical compounds, synthetic and natural ones, all potentially harmful to the marine environment of the coral reef. The Inventors have surprisingly selected some substances that, both alone and in combination among them, cause no harm in this type of marine environment, but concomitantly are highly effective in screening the skin from solar radiations.

[0008] Therefore, object of the present invention is an eco-compatible sunscreen composition according to claim 1

## 45 BRIEF DESCRIPTION OF THE FIGURES

## [0009]

**Figure 1.** Viral enrichment factors in water surrounding coral nubbins ("small branches with alive polyps") in treated systems and in control systems (controls). The various classes of different treatments tested are shown: a) Fragrances; b) Creams; c) Antioxidants; d) UV filters; e) Controls.

**Figure 2.** Prokaryotic enrichment factors in water surrounding coral nubbins in treated and control systems. The various classes of different treatments tested are shown: a) Fragrances; b) Creams; c) Antioxidants; d) UV filters; e) Controls.

**Figure 3.** Total zooxanthellae released in water surrounding nubbins during the incubation test. The various classes of different treatments tested are shown: a) Fragrances; b) Creams; c) Antioxidants; d) UV filters; e) Controls.

**Figure 4.** Level of damage of zooxanthellae released after addition of sunscreen Creams; c) Antioxidants; d) UV filters; e) Controls.

**Figure 5.** Coral nubbins at different incubation times. Panels a and b show control systems at time 0 and after 72 h of incubation. Panels c and d show nubbins of treated corals at time 0 and after 72 h of incubation.

Figure 6. Photostability of UV-tested filters.

## 5 DETAILED DESCRIPTION OF THE INVENTION

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[0010] The present invention refers to sunscreen compositions eco-compatible with the marine ecosystem of coral reefs.

**[0011]** In the present description, by the definition: "eco-compatible with the marine ecosystem" of the coral reefs it is meant a product causing no harm nor alteration to the marine organisms or to the communities or habitats as a whole. An eco-compatible product is therefore usable without restrictions, as it protects the health of environment and biodiversity. In the tests reported herein, the health conditions of marine organisms exposed to the various chemical components were verified in order to select the components absolutely innocuous. Note that a biodegradable product (declaration associated with some marketed products) can be harmful to the environment and therefore can be not eco-compatible, as the concept of biodegradation refers only to the decomposition times of the compound.

[0012] The compositions of the present invention comprise:

- one or more UV filters selected from MBBT, DHHB and EHT;
- one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea, and which are free from preservatives belonging to the paraben family.

**[0013]** In the present description, by "parabens" are meant the esters of 4-hydroxybenzoic acid used as preservatives in the cosmetics industry. The most common parabens are methylparaben, ethylparaben, propylparaben and butylparaben.

[0014] An example of polyphenols extracted from green tea is polyphenone-60 (CAS N° 138988-88-2) commercially available from Sigma.

**[0015]** In an embodiment, the compositions of the present invention may comprise one or more fragrances ecocompatible with the marine environment, selected from the group consisting of fragrances of orange, lavender, grapefruit, guava and coconut.

[0016] Examples of fragrances suitable for the present invention are the fragrances commercially available from FAROTTI SRL company, such as guava and coconut, natural lavender, natural grapefruit, preferably in concentrations equal to or lower than 0.3% of the product.

**[0017]** In order to increase the shelf life times, in an embodiment the compositions comprise as preservative sorbic acid, which from the testing reported herein did not prove harmful to coral reefs in conjunction with the other compounds.

**[0018]** The compositions could be prepared in different cosmetic forms, such as, e.g., cream, lotion, ointment, spray, lipstick. The selected UV filters, antioxidants, fragrances and preservatives could be mixed with different carriers suitable (according to test results) for the determined cosmetic form that is to be manufactured.

**[0019]** Preferably, carriers having an emollient function will be used, selected in the group consisting of propylene glycol dicaprylate/dicaprate, olive oil, beeswax.

[0020] Propylene glycol dicaprylate/dicaprate (CAS number: 68583-51-7) is a derivative of propylene glycol, and is a diester of propylene glycol and fatty acids, In particular, it is a mixture of propylene glycol dicaprylate and propylene glycol dicaprate. This product is commercially available under the name Labrafac.

[0021] Beeswax (CAS number:8012-89-3) suitable for the present invention is e.g. commercially available under the name Apifil.

[0022] The data reported in the experimental section demonstrate that the filters MBBT, DHHB, the antioxidants tocopheryl acetate and tocopherol and the fragrance of guava and coconut are the compounds having a greater ecocompatibility with corals. Preferably, the compositions of the present invention will therefore comprise MBBT or DHHB, tocopheryl acetate or Tocopherol and optionally the fragrance of guava and coconut.

**[0023]** The present invention is based on the selection of compounds suitable to be used in compositions for use as sunscreens compatible with the marine environment and life inhabiting it, with particular reference to coral reefs.

[0024] The majority of compounds commonly used in sunscreens proved harmful to these environments, compounds such as, e.g.:

- preservatives belonging to the paraben family
- ascorbic acid derivatives;
  - UV filters selected from bis ethylhexyloxyphenol methoxyphenyl triazine (BEMT), cinnamates, benzophenones, camphor derivatives, titanium oxide;
  - fragrances selected from melaleuca essential oil, ylang ylang flower extracts, Bambousa Arundinacea extracts,

Karitè:

- carriers selected from animal-derived fats;
- Argan oil.
- 5 **[0025]** In an embodiment, the sunscreen compositions comprise:
  - one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phe-nol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
  - one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
  - one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax

#### and are free from:

- preservatives belonging to the paraben family or ascorbic acid derivatives;
  - UV filters selected from bis ethylhexyloxyphenol methoxyphenyl triazine, cinnamates, benzophenones, camphor derivatives, titanium oxide;
  - fragrances selected from melaleuca essential oil, ylang ylang flower extracts, Bambousa Arundinacea extracts, Karitè:
- carriers selected from animal-derived fats and argan oil.

**[0026]** In an embodiment with a very high eco-compatibility, they will further comprise sorbic acid and/or one or more fragrances selected from orange, lavender, grapefruit, guava and coconut.

[0027] In a further highly eco-compatible embodiment the compositions are essentially consisting of:

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- one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phe-nol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
- one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
- one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
  - water.

[0028] In a further highly eco-compatible embodiment, the compositions are essentially consisting of:

- one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
  - one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
  - sorbic acid;
- one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
  - water.

[0029] In a further embodiment, the compositions are essentially consisting of:

- 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol and/or 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate;
  - tocopheryl acetate and/or tocopherol;
  - sorbic acid;
  - one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
- 50 water;
  - one or more fragrances selected from orange, lavender, grapefruit, guava and coconut.

[0030] In a further embodiment, the compositions are essentially consisting of:

- 55 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol and/or 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate;
  - tocopheryl acetate and/or tocopherol;
  - sorbic acid;

- one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
- water:
- one or more fragrances selected from orange, lavender, grapefruit, guava and coconut;
- hydroxyethyl cellulose and/or EDTA and/or sodium hydroxide.

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[0031] In a further embodiment, the compositions are essentially consisting of:

- 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol,
   2-[4-(diethylamine)-2-hydroxy-benzoyl]hexyl benzoate and ethylhexyl triazone;
- tocopheryl acetate;
  - sorbic acid;
  - propylene glycol dicaprylate/dicaprate, olive oil and beeswax;
  - water:
  - hydroxyethyl cellulose;
- 15 EDTA;
  - sodium hydroxide.

**[0032]** All embodiments listed above are exclusively consisting of compounds tested individually and as a whole to have a full eco-compatibility with the coral reef and (with no need of addition of other compounds potentially harmful for the marine ecosystem) have a high photostability and prolonged storage in a suitable cosmetic formulation.

**[0033]** In the compositions of the present invention, the MBBT, DHHB and EHT could be in a percentage comprised between 3 and 6% by weight of the composition.

## **EXAMPLES**

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**[0034]** The following examples are merely illustrative and do not limit the invention (e.g., the weighings and concentrations are merely indicative, as the SPFs and the UVA/UVB absorbance ratios have to be specifically formulated to conform to the nominal SPFs of the product). Amounts of individual compounds are expressed as weight percentages.

30 Example 1: In a specific embodiment, the invention has the following formulation in the form of cream:

## [0035]

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Ingredients	w/w
MBBT	4%
DHHB	4%
EHT	1%
APIFIL	12%
LABRAFAC	15.5%
Olive oil	7%
Tocopheryl acetate	0.5%
Hydroxyethyl cellulose	0.2%
EDTA	0.05%
Sodium hydroxide	0.4%
Sorbic acid	1%
Guava and coconut	0.3%
H <sub>2</sub> O	remaining %

 $\underline{\text{Example 2:}} \text{ In a specific embodiment, the invention has the following formulation in the form of cream:}$ 

## [0036]

Ingredients w/w **MBBT** 6% DHHB 6% **EHT** 2% **APIFIL** 11% LABRAFAC 14.5% Olive oil 6% Tocopheryl acetate 0.5% Hydroxyethyl cellulose 0.2% **EDTA** 0.05% Sodium hydroxide 0.4% Sorbic acid 1% Lavender 0.3%  $H_2O$ remaining %

Example 3: In a specific embodiment, the invention has the following formulation in the form of cream:

## [0037]

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Ingredients	w/w
MBBT	8%
DHHB	8%
EHT	3%
APIFIL	10%
LABRAFAC	13.5%
Olive oil	5%
Tocopheryl acetate	0.5%
Hydroxyethyl cellulose	0.2%
EDTA	0.05%
Sodium hydroxide	0.4%
Sorbic acid	1%
Orange	0.3%
H <sub>2</sub> O	remaining %

## Example 4: preparing the compositions

[0038] Compositions are prepared by the process described hereinafter:

Phases 1 and 3, indicated in Table 1 are dissolved together at 65°C using a stirrer.

Phase 2 is dissolved separately at the same temperature, always under continuous stirring. Upon reaching the same temperature, phase 2 is added to phases 1+3 and the whole is mixed for some minutes at 65°C. To obtain a product having homogeneous consistency, it is mixed at room temperature and only at the end, then phase 4 is added. The three compositions thus prepared were tested as described in the experimental section of the present description.

Table 1. Composition of the 3 preparations of examples 1,2 and 3 having a different SPF.

	Base	1 - low SPF	2 - medium SPF	3 - high SPF
Phase	Apifil 13%	Apifil 12%	Apifil 11%	Apifil 10%
1	Labrafac 18.5%	Labrafac 15.5%	Labrafac 14.5%	Labrafac 13.5%
	Olive oil 8%	Olive oil 7%	Olive oil 6%	Olive oil 5%
	Tocopheryl acetate 0.5%	Tocopheryl acetate 0.5%	Tocopheryl acetate 0.5%	Tocopheryl acetate 0.5%
		DHHB4%	DHHB 6%	DHHB8%
		EHT 1%	EHT 2%	EHT 3%
		MBBT4%	MBBT 6%	MBBT 8%
Phase	Water 57.22	Water 53.05 %	Water 51.05%	Water 49.05%
2	Hydroxyethyl cellulose 0.2%	Hydroxyethyl cellulose 0.2%	Hydroxyethyl cellulose 0.2%	Hydroxyethyl cellulose 0.2%
	EDTA 0.05%	EDTA 0.05%	EDTA 0.05%	EDTA 0.05%
	Sorbic acid 1%	Sorbic acid 1%	Sorbic acid 1%	Sorbic acid 1%
Phase	Labrafac 1 %	Labrafac 1 %	Labrafac 1 %	Labrafac 1 %
3	Sodium hydroxide 0.4%	Sodium hydroxide 0.4%	Sodium hydroxide 0.4%	Sodium hydroxide 0.4%
Phase 4	Fragrance 0.3%	Fragrance 0.3%	Fragrance 0.3%	Fragrance 0.3%

## METHODOLOGY AND EXPERIMENTAL DATA

## 3.1. Study area

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[0039] Field activities were conducted at the Marine Biology Laboratory of Onong Resort, Siladen Island, Indonesia. The study area is located in Bunaken National Park, considered one of the most important Marine Protected Areas in the world. Bunaken National Park is located in the centre of a reef-building coral biodiversity triangle, which roughly encompasses the Philippines, Indonesia and the Great Barrier Reef. In total, about 605 zooxanthellate coral species were recorded in the Coral Triangle (Veron et al., 2009), of which 66% are common to all ecoregions of the area. This diversity amounts to 76% of the world's total species. About 450 coral species have been described so far in the Indonesian archipelago. Bunaken National Park and adjacent waters support diverse hermatypic (reef-building) coral communities, composed of more than 390 species, 63 genera and 15 families of hard corals, including also some species yet undescribed. The corals form different community types, broadly distributed in relation to depth, slope angle and other environmental factors. The area supports various regionally important populations of a wide array of coral species, including some 20 species considered to be globally rare. Bunaken National Park exhibits exceptional levels of withinlocation diversity. Richest locations host more than 25 - 30 % of the reef-building coral species complement of the entire Indo-Pacific region. In particular, in the study area, recent surveys reported that the reef-building corals accounted for about 90% of total coral species, with highest coral diversity occurring in shallow waters, likely related to the relatively higher habitat heterogeneity produced by changes in slope angle, exposure and illumination among other factors, from the shallow reef slope to the crest to the reef flat. In the study area, the occurrence of many endemic species of the genus Acropora were recently reported (Wallace et al., 2001). Acropora represent one of the larger taxonomic group in the reefs surrounding Siladen Island. Acropora is the most diverse hermatypic coral genus, with 114 species recognised worldwide and 91 species being identified for the Indonesian archipelago (Wallace, 1999). "Structural species" such as Acropora are a vital ecosystem component, and variations in their abundance are critical to the dynamics of entire reef communities (Connell et al., 1997). Acropora spp. plays a dominant role in the species composition and abundance of many modern day Indonesian reefs (Suharsono, 1992).

## 3.2. Experimental design

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[0040] In situ tests were performed on nubbins (i.e., small branches of about 3-6 cm) of the stony coral Acropora sp. The nubbins were incubated in microcosms in presence of known concentrations of various ingredients potentially to be included in the composition of the eco-compatible sunscreen cream. In particular, coral nubbins (including more than 3000 polyps each) were collected by scuba divers from the same colony, located at a depth of about 3 m on the edge of the reef slope. Sampling of nubbins was performed by means of forceps. The nubbins were washed with seawater filtered onto 0.02-µm filters to eliminate viruses and prokaryotes. Each microcosm was prepared with a replicate set of nubbins (n=3) immersed in sterile polyethylene bags (Whirl-pack, Nasco, Fort Atkinson, WI, USA) filled with 2 L filtered seawater. A total of 27 microcosms were prepared, in order to test 20 ingredients with the respective positive and negative controls. The list of treatments, the concentrations of compounds used and the control systems are reported in Table 2. The microcosms were incubated at in situ temperature and light conditions for 72 hours.

Table 2. List of microcosms, treatment types, concentrations of compounds used and incubation times.

15	Code	Compound	Concentration	Туре
	D-01	Natural grapefruit fragrance	0.3 %	
	D-02	Tea Tree oil (Melaleuca) fragrance	0.3 %	
	D-03	Ylang-ylang fragrance I	0.3 %	
20	D-04	Natural orange fragrance	0.3 %	
	D-05	Natural lavender fragrance	0.3 %	<b>F</b> /
	D-06	Argan oil	0.3 %	Essences/ Fragrances
	D-07	Guava & Coconut 22661	0.3 %	Fragrances
25	D-08	Bamboo 21842	0.3 %	
20	D-09	Karite 20471	0.3 %	
	D-10	Mare d'inverno 4/381 MC	0.3 %	
	D-11	Joyful 60029MC	0.3 %	
	D-13	Type-1 base cream	50 μL <sup>-1</sup>	
30	D-14	Type-2 base cream	50 μL <sup>-1</sup>	Base cream
	D-15	Type-3 base cream	$50~\mu L^{-1}$	
	D-16	Alpha-tocopherol (SIGMA code T3251-5G)	3%	Antioxidant
	D-17	DL-tocopherol-acetate (SIGMA code T3376-5G)	3%	
35	D-18	2-phospho-ascorbic acid trisodium salt (SIGMA code 49752-10G)	3%	
	D-19	retinyl palmitate (SIGMA code R3375-1G)	3%	
	D-20	Retinol (SIGMA code R7632-250MG)	3%	
40	D-21	Polyphenol 60 from green tea (SIGMA code P1204-25G)	3%	
	D-22	MBBT (filter)	7%	
	D-23	BEMT (filter)	7%	Filters
45	D-24	EHT (filter)	3%	Fillers
45	D-25	DHHB (filter)	7%	
	Mito	Mitomycin C (agent inducing the lytic cycle in viral infections).	1 μg mL <sup>-1</sup>	Control
	BLK	-		Control systems
50	BLK PG	PG		

**[0041]** Both negative and positive control systems (controls) were used. The negative ones are systems in which a substance has been used, the antibiotic mitomycin C, which triggers viral infections, as it happens with use of all creams currently marketed. Therefore, it serves to indicate the levels of undesired response of a compound. The positive ones, denoted by BLK PG and BLK, are simply microcosms without addition of any compound.

Table 3. Concentrations of tested filters

FILTERS	Max concentration	Low SPF	Medium SPF	High SPF
MBBT	10%	4	6	8
DHHB	10%	4	6	8
EHT	5%	1	2	3

#### 3.3. Determination of viral and prokaryotic abundances

**[0042]** Subsamples (15 mL) of seawater surrounding coral nubbins were collected at the start of the experiment (TO) and after 3 and 6 hours of incubation. After subsampling, sea water was transferred into sterile test tubes without use of fixatives and stored at -20°C until laboratory analysis for quantification of viral and prokaryotic abundance. Once in laboratory, the water samples were immediately processed. Briefly, 200 μL sea water were diluted 1:10 with prefiltered MilliQ water, through 0.02-μm pore filters (Anodisc filter; 25-mm diameter,  $Al_2O_3$ ; Whatman) and stained with 20 μL SYBR Green (stock solution diluted 1:20; Invitrogen, Carlsbad, CA, USA). Filters were incubated in the dark for 15 min and mounted on glass slides with a drop of antifade consisting of 50% phosphate buffer (6.7 mM, pH 7.8) and 50% glycerol containing 0.25% ascorbic acid (Shibata et al. 2006; Helton et al. 2006; Wen et al. 2004). Slides were stored at -20°C until microscopy analysis. Virus and prokaryote counts were obtained by epifluorescence microscopy (magnification, ×1000; Zeiss Axioplan) by examining at least 10 optical fields, that is, at least 200 cells or viruses per replicate. Viral (and prokaryotic) enrichment was calculated as ratio between virus (and prokaryote) abundances and virus (and prokaryote) abundance at the end of the experiment.

### 3.4 Zooxanthellae counts

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[0043] Subsamples (50 mL) of seawater surrounding coral nubbins were collected at the start of the experiment (TO) and after 6, 12, 24, 36, 48 and 72 hours of incubation. The subsamples were fixed in 3% glutaraldehyde and then about 6.0 ml of each replicate was filtered through 2.0- $\mu$ m polycarbonate filters. Filters were mounted on glass slides and zooxanthellae counted under epifluorescence microscopy (Zeiss Axioplan, Carl Zeiss Inc., Jena, Germany;  $\times$  400 and  $\times$ 1000) using standard and UV light. Based on color, autofluorescence and gross cell structure, zooxanthellae released or extracted from nubbins were classified as a) healthy (H, brown/bright yellow color, intact/in good conditions zooxanthellae; b) pale (P, pale yellow color, vacuolated, partially degraded zooxanthellae; transparent (T, lacking pigmentations, empty zooxanthellae; Mise and Hidaka 2003).

## 3.5 Quantification of coral bleaching

[0044] In order to quantify the levels of coral bleaching (Siebeck et al. 2006) a colorimetric analysis was performed on digital photographs of corals, taken at the beginning of the experiment and after 6, 12, 24, 36, 48 and 72 hours of incubation. Photographs were taken under identical illumination with a Canon PowerShot A620 digital camera (Canon Inc., Tokyo, Japan) with a scale meter on the background. The photos were successively analyzed with a photo-editing software (Adobe Photoshop CS2) for color composition [cyan, magenta, yellow, black (CMYK)]. Levels of bleaching were measured as the difference between the corals' color at the beginning of the experiment and after treatments. Variations in the percentage of the different color components (CMYK) were analyzed with one-way analysis of variance (ANOVA). To rank the bleaching effect due to the compounds tested, a Bray-Curtis similarity matrix was prepared and a multidimensional scaling analysis of the shifts in CMYK color composition of treated corals was performed using Primer 5.0 software (Primer-E Ltd., Plymouth, UK). Bleaching rates were measured as the dissimilarity percentage in CMYK color composition between treated and control corals using the SIMPER tool of Primer 5.0 software (Primer-E Ltd).

## 4. Results

## 4.1. Viral and prokaryotic abundance

**[0045]** Viral abundances significantly increased of about 1 order of magnitude during the incubation experiments. Viral enrichment ranged from 1.9 to 37.4, in the systems BLK PG and D08 (Figure 1). Viral abundances at the end of the experiment ranged from 0.44 to 31.2 x 10<sup>5</sup> viruses ml<sup>-1</sup> in systems BLK PG and D15, respectively. Prokaryotic abundances increased of 1-2 orders of magnitude during the incubation experiment. Prokaryiotes enrichment factors fluctuated within a wide range (Figure 2). The lowest enrichment factor (1.88) was observed in the BLK PG system, whereas in system

D11 the enrichment factor was 78.16. A slight reduction of ratios between viral and prokaryotic abundance (VPR) after incubations was observed in all systems, with the exception of systems D07 and BLK, where VPR remained stable over time. VPR decreased up to 10 times in system D22.

## 5 4.2. Zooxanthellae abundance

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**[0046]** At the start of the incubations, in the microcosms no released zooxanthellae were found. The number of zooxanthellae increased over time in all microcosms considered. The final abundance of zooxanthellae found in water surrounding the coral nubbins ranged from 0.85 to 4.45 x 10<sup>4</sup> cell ml<sup>-1</sup>, respectively in systems D11 (inoculated with Joyful 60029MC) and D18 (inoculated with 2-phospho-L-ascorbic acid trisodium salt), (Figure 3a, b, c, d, e).

**[0047]** In some cases, abundance of released zooxanthellae was lower in systems treated than in controls. Statistical analyses revealed that only in the case of systems D15, D16, D17 and D18 the abundance of released cells was significantly higher than in the BLK system (P<0.01). For all other compounds no significant differences were found between control and treated systems, suggesting that the presence of the compounds tested in the experimental systems (with the exception of D15, D16, D17 and D18) causes no increase in the rate of zooxanthellae release from corals.

[0048] During microscopic analyses, observed zooxanthellae were split into three main groups (healthy, pale and transparent) as described in the Materials and methods section. At the end of the incubations, each group exhibited different abundances in the water surrounding the coral nubbins (Figure 4). Pale and transparent zooxanthellae were, in all experimental systems, the dominant groups, on average representing 44% and 50% of total released zooxanthellae. Healthy zooxanthellae always represented the lesser fraction, ranging from 0 to 21%.

**[0049]** The low abundance of healthy cells suggests that the incubation time of the experiments causes a high level of damage to zooxanthellae in all systems considered, negative controls (BLK) included.

### 4.3. Coral bleaching

[0050] In all replicates of every microcosm (control systems included), the release of coral mucous (comprised of zooxanthellae and coral tissue) was observed within the first 48 h of incubation. This observation is in accordance with the results of the release of zooxanthellae reported in section 4.2. Mucous release by coral fragments could be related to the mechanical stress occurred during sampling and preparation of microcosms. In all microcosms (BLK systems included) after 72 h of incubation a slight change in color was observed in coral nubbins, potentially occurred as a consequence of the stress induced by incubations. The most evident changes were observed in systems D18 and MITO, where all 4 color channels were significantly altered at the end of the incubations. In systems D05, D08, and BLK PG, 3 out of 4 color channels showed alterations. In all other systems, 2 or 1 color channels showed significant changes. The systems D03, D09, D10, D11, D21, D21 showed changes in 2 color channels, whereas systems D01, D04, D05, D13, D14, D19, D20 and BLK showed changes only in 1 color channel. In all other experimental systems, no color changes were detected in tested nubbins.

## 4.4 Photostability of the sunscreen cream exposed to UVA rays (~ 275 kJ/m²)

- [0051] 2 mg cm<sup>-2</sup> of cream were spread on glass slides (5 x 5 cm) and exposed to UVA rays.
  - UVA absorbance, 320-400 nm (peak corresponding to the DHHB filter) is too high with respect to the UVB one,
     290-320 nm (peak corresponding to the EHT filter). UVA absorbance should be about 1/3 of the UVB one.
  - The spectrum of the wide-spectrum filter, MBBT, is not visible since it has an extinction coefficient lower than DHHB
    and EHT, therefore is covered by the absorbance of the latter. MBBT should appear as a wide spectrum without
    maximum and minimum peaks.
  - At the moment, obtained absorbance falls within the range of a sunscreen cream with a high and low SPF.
  - According to the results, it can be concluded that the filters are highly photostable.
- 4.5 Summary of criteria used to test eco-compatibility of tested products with coral reefs:

[0052] In order to select the most eco-compatible products for each group of compounds (i.e., base cream, fragrance, antioxidants and filters) the following criteria were selected:

- 1. coral bleaching level;
- 2. degree of release of symbiotic zooxanthellae;
- 3. zooxanthellae health conditions;
- 4. viral enrichment in the incubation medium with respect to controls.

**Table 4.** Summary of eco-compatible compounds grouped by category.

Compound group	Ingredients	Eco-compatibility
Base cream	Type 1	Highly compatible
	Type 2	Compatible
	Type 3	Compatible
Fragrances	Guava & Coconut	Highly compatible
	Orange	Compatible
	Lavender	Compatible
	Grapefruit	Compatible
Antioxidants	Tocopheryl acetate	Highly compatible
	Tocopherol	Highly compatible
	Retinol	Compatible
	Polyphenone-60	Compatible
	Retinyl palmitate	Compatible
UV filters	MBBT	Highly compatible
	DHHB	Highly compatible
	EHT	Compatible

**Table 5.** Viral abundance and enrichment during the incubations. Incubation times (Incub.), total viral abundances (TVN) and the viral enrichment factor (VEF) are also reported. Standard deviation values (STD) and variation coefficients are reported for all data.

System	Incub.	TVN	STD	cv	VEF	STD
D01	0	9.50E+04	2.69E+04	2.83E+01	19.62	2.00
	12 h	1.83E+06	3.39E+05	1.85E+01		
D02	0	8.13E+04	2.11E+04	2.60E+01	19.87	4.43
	12 h	1.57E+06	3.34E+05	2.12E+01		
D03	0	9.82E+04	2.19E+04	2.23E+01	15.48	0.81
	12 h	1.51E+06	2.99E+05	1.98E+01		
D04	0	1.71E+05	3.08E+04	1.80E+01	11.01	0.80
	12 h	1.87E+06	2.18E+05	1.17E+01		
D05	0	9.74E+04	1.94E+04	1.99E+01	12.44	2.28
	12 h	1.20E+06	2.29E+05	1.92E+01		
D06	0	1.14E+05	2.67E+04	2.34E+01	9.37	4.06
	12 h	1.02E+06	3.88E+05	3.79E+01		
D07	0	2.81E+05	2.48E+04	8.82E+00	8.50	1.08
	12 h	2.38E+06	3.09E+05	1.30E+01		
D08	0	7.49E+04	2.30E+04	3.08E+01	37.39	13.49
	12 h	2.60E+06	1.45E+05	5.58E+00		
D09	0	3.56E+05	6.28E+04	1.77E+01	5.55	0.93
	12 h	1.94E+06	1.96E+05	1.01E+01		

(continued)

	System	Incub.	TVN	STD	cv	VEF	STD
5	D10	0	2.20E+05	5.34E+04	2.43E+01	7.18	1.80
J		12 h	1.52E+06	1.31E+05	8.58E+00		
	D11	0	1.14E+05	5.77E+04	5.08E+01	30.89	14.56
		12 h	2.95E+06	5.30E+05	1.80E+01		
10	D13	0	1.34E+05	3.42E+04	2.56E+01	14.29	2.60
		12 h	1.87E+06	4.29E+05	2.29E+01		
	D14	0	2.35E+05	5.20E+04	2.21E+01	11.03	6.65
15		12 h	2.36E+06	1.03E+06	4.35E+01		
	D15	0	3.79E+05	8.90E+04	2.35E+01	8.21	0.28
		12 h	3.12E+06	7.64E+05	2.45E+01		
	D16	0	3.24E+05	7.97E+04	2.46E+01	4.54	0.48
20		12 h	1.46E+06	2.87E+05	1.97E+01		
	D17	0	3.49E+05	6.79E+04	1.94E+01	8.36	2.46
		12 h	2.83E+06	6.23E+05	2.20E+01		
25	D18	0	3.70E+05	5.71E+04	1.55E+01	7.09	3.00
		12 h	2.51E+06	6.73E+05	2.69E+01		
	D19	0	1.03E+05	1.37E+04	1.33E+01	18.92	4.43
		12 h	1.98E+06	6.60E+05	3.33E+01		
30	D20	0	2.47E+05	7.76E+04	3.14E+01	8.09	1.18
		12 h	1.97E+06	4.92E+05	2.50E+01		
	D21	0	3.23E+05	9.01E+04	2.79E+01	8.56	3.32
35		12 h	2.57E+06	1.94E+05	7.54E+00		
	D22	0	5.06E+05	1.12E+05	2.21E+01	6.16	1.77
		12 h	2.99E+06	3.50E+05	1.17E+01		
	D23	0	1.87E+05	3.30E+04	1.77E+01	4.80	2.60
10		12 h	8.65E+05	3.77E+05	4.36E+01		
	D24	0	9.58E+04	3.38E+04	3.53E+01	12.58	5.97
		12 h	1.08E+06	1.96E+05	1.81E+01		
<b>1</b> 5	D25	0	3.67E+05	8.46E+04	2.30E+01	7.44	1.79
		12 h	2.63E+06	8.45E+04	3.21E+00		
	MITO	0	1.11E+05	2.20E+04	1.98E+01	14.33	4.09
		12 h	1.53E+06	1.45E+05	9.46E+00		
50	BLK	0	2.03E+04	3.61E+03	1.78E+01	3.68	1.62
		12 h	4.45E+04	4.01E+04	9.00E+01		
	BLK PG	0	2.42E+04	4.26E+03	1.76E+01	1.88	0.64
55		12 h	4.37E+04	8.06E+03	1.84E+01		

**Table 6.** Bacterial abundance (total bacterial number, TBN) during incubations, and related standard deviations and coefficient of variation.

System	Incubation	TBN	STD	CV
D01	0	1.93E+04	3.42E+03	1.77E+01
	12 h	1.21E+06	1.66E+05	1.37E+0
D02	0	2.17E+04	6.39E+03	2.94E+0
	12 h	8.48E+05	1.42E+05	1.67E+0
D03	0	3.14E+04	4.83E+03	1.54E+0
	12 h	9.20E+05	1.04E+05	1.13E+0
D04	0	3.54E+04	1.09E+04	3.07E+0
	12 h	8.43E+05	9.84E+04	1.17E+0
D05	0	7.25E+03	2.42E+03	3.33E+0
	12 h	3.19E+05	2.59E+04	8.10E+0
D06	0	3.94E+04	5.03E+03	1.27E+0
	12 h	4.99E+05	5.03E+04	1.01E+0
D07	0	2.74E+04	7.76E+03	2.84E+0
	12 h	1.11E+06	2.42E+05	2.18E+0
D08	0	4.75E+04	2.79E+03	5.87E+0
	12 h	1.22E+06	2.74E+05	2.25E+0
D09	0	2.58E+04	1.39E+03	5.41E+0
	12 h	1.18E+06	1.87E+05	1.58E+0
D10	0	1.69E+04	2.42E+03	1.43E+0
	12 h	9.74E+05	1.12E+05	1.15E+0
D11	0	2.50E+04	6.08E+03	2.44E+0
	12 h	1.88E+06	2.83E+05	1.50E+0
D13	0	2.82E+04	6.08E+03	2.16E+0
	12 h	1.35E+06	1.73E+05	1.28E+0
D14	0	4.67E+04	3.69E+03	7.90E+0
	12 h	1.92E+06	3.52E+05	1.83E+0
D15	0	2.01E+04	5.03E+03	2.50E+0
	12 h	1.05E+06	2.11E+05	2.02E+0
D16	0	3.78E+04	1.01E+04	2.66E+0
	12 h	1.18E+06	2.51E+04	2.13E+0
D17	0	5.80E+04	1.05E+04	1.82E+0
	12 h	1.42E+06	3.82E+05	2.70E+0
D18	0	3.86E+04	6.39E+03	1.65E+0
	12 h	1.39E+06	1.22E+05	8.73E+0
D19	0	3.62E+04	1.45E+04	4.00E+0
	12 h	1.54E+06	1.58E+05	1.02E+0
D20	0	3.62E+04	9.66E+03	2.67E+0

(continued)

System	Incubation	TBN	STD	cv
	12 h	1.71E+06	4.32E+05	2.53E+01
D21	0	3.06E+04	6.08E+03	1.99E+01
	12 h	1.57E+06	1.99E+05	1.27E+01
D22	0	5.47E+04	9.14E+03	1.67E+01
	12 h	9.18E+05	2.90E+05	3.16E+01
D23	0	4.67E+04	3.69E+03	7.90E+00
	12 h	2.03E+06	3.31E+05	1.63E+01
D24	0	2.09E+04	3.69E+03	1.76E+01
	12 h	7.41E+05	2.13E+05	2.87E+01
D25	0	3.67E+05	8.46E+04	2.30E+01
	12 h	2.63E+06	8.45E+04	3.21E+00
MITO	0	1.11E+05	2.20E+04	1.98E+01
	12 h	1.53E+06	1.45E+05	9.46E+00
BLK	0	2.03E+04	3.61E+03	1.78E+01
	12 h	4.45E+04	4.01E+04	9.00E+01
BLK PG	0	2.42E+04	4.26E+03	1.76E+01
	12 h	4.37E+04	8.06E+03	1.84E+01

5		<u>ვ</u>	31.1	24.1	3.7	29.0	10.8	13.5	5.1	14.1	0.0	12.4	13.4	10.6	13.5	10.1	18.2	4.0	8.2	21.4	20.6	13.5	6.2	10.8	22.4	12.1	10.0
10	m.	STD	1.07E+03	2.82E+03	2.01E+02	1.22E+03	8.13E+02	1.22E+03	3.07E+02	1.21E+03	0.00E+00	8.13E+02	7.26E+02	6.47E+02	1.37E+03	1.31E+03	2.62E+03	4.03E+02	1.98E+03	1.94E+03	1.23E+03	9.22E+02	5.33E+02	1.14E+03	2.62E+03	1.23E+03	7.26E+02
15	y, pale and transparent (i.e., dead) zooxanthellae (in cells ml-1) released in each microcosm.	Transparent	3.42E+03	1.17E+04	5.43E+03	4.23E+03	7.51E+03	9.06E+03	5.97E+03	8.59E+03	4.03E+02	6.57E+03	5.43E+03	6.11E+03	1.01E+04	1.29E+04	1.44E+04	1.01E+04	2.42E+04	9.06E+03	5.97E+03	6.84E+03	8.65E+03	1.06E+04	1.17E+04	1.02E+04	7.25E+03
	leased ir	<u>ج</u>	14.0	6.9	26.9	18.8	27.7	21.8	21.7	14.4	15.3	18.5	42.6	12.9	29.0	15.6	19.9	15.2	8.3	14.7	21.6	17.1	17.3	8.4	8.1	7.3	17.8
20	ı cells ml <sup>-1</sup> ) re	STD	9.51E+02	4.19E+02	2.62 E+03	1.11E+03	7.26E+02	1.84E+03	1.01E+03	7.07E+02	1.80E+03	1.01E+03	1.23E+03	7.26E+02	1.83E+03	1.07E+03	1.86E+03	1.91E+03	1.56E+03	1.60E+03	1.14E+03	1.23E+03	1.48E+03	5.81E+02	6.15E+02	6.15 E+02	1.57E+03
25	xanthellae (ir	Pale	6.78E+03	6.11E+03	9.73E+03	5.90E+03	2.62E+03	8.45E+03	4.63E+03	4.90E+03	1.18E+04	5.43 E+03	2.88E+03	5.64E+03	6.31E+03	6.84E+03	9.33E+03	1.25E+04	1.88E+04	1.09E+04	5.30E+03	7.18E+03	8.59E+03	6.91E+03	7.58E+03	8.39E+03	8.86E+03
30	dead) zoc	<u>ک</u>	65.5	34.6	20.2	33.8	0.0	2.99	26.6	24.7	22.9	17.6	0.0	173.2	45.8	25.0	28.6	15.8	28.4	36.7	10.8	33.3	28.6	24.7	15.7	43.3	34.6
35	ısparent (i.e.,	STD	9.22E+02	1.16E+02	1.42E+02	9.08E+02	0.00E+00	4.03E+02	2.32E+02	2.32E+02	3.07E+02	3.07E+02	0.00E+00	1.16E+02	3.07E+02	6.04E+02	3.07E+02	6.47E+02	4.19E+02	4.19E+02	1.16E+02	2.01E+02	3.07E+02	1.16E+02	1.16E+02	1.16E+02	1.16E+02
40	pale and trar	Healthy	1.41E+03	3.35E+02	7.04E+02	2.68E+03	2.01E+02	6.04E+02	8.72E+02	9.39E+02	1.34E+03	1.74E+03	2.01E+02	6.71E+01	6.71E+02	2.42E+03	1.07E+03	4.09E+03	1.48E+03	1.14E+03	1.07E+03	6.04E+02	1.07E+03	4.70E+02	7.38E+02	2.68E+02	3.35E+02
,,	healthy,	ટ	25.3	13.9	18.7	4.5	14.1	17.2	6.3	14.8	5.3	3.4	23.4	5.5	16.5	8.2	13.7	6.5	2.0	6.4	19.5	4.4	12.5	3.4	10.1	9.0	8.0
45	Table 7. Number of health	STD	2.93E+03	2.51E+03	2.92E+03	5.81 E+02	1.45 E+03	3.12E+03	7.26E+02	2.13E+03	6.97E+02	4.65E+02	1.98E+03	6.47E+02	2.82E+03	1.82E+03	3.39E+03	1.74E+03	8.77E+02	1.34E+03	2.40E+03	6.47E+02	2.29E+03	6.15E+02	2.03E+03	1.69E+03	1.31E+03
50	Table	Total	1.16E+04	1.81E+04	1.56E+04	1.28E+04	1.03E+04	1.81E+04	1.15E+04	1.44E+04	1.31E+04	1.38E+04	8.45E+03	1.18E+04	1.71E+04	2.22E+04	2.48E+04	2.67E+04	4.45E+04	2.11E+04	1.23E+04	1.46E+04	1.83E+04	1.80E+04	2.00E+04	1.89E+04	1.64E+04
55		Systems	D01	D02	D03	D04	D05	900	D07	D08	60 <b>0</b>	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	D25

5	

(continued)

Systems Total	Total	STD	CV	Healthy STD	STD	CV Pale		STD	CV	CV Transparent	STD	c
MITO	2.20E+04	2.20E+04 4.22E+03 19.2	19.2	0.00E+00 0.00E+00	0.00E+00	0.0	5.25E+03	0.0 5.25E+03 4.33E+02 8.2	8.2	1.68E+04	3.88E+03 23.2	23.2
BLK	1.64E+04	1.64E+04 1.31E+03	8.0	3.35E+02	3.35E+02 1.16E+02	34.6	8.86E+03	34.6 8.86E+03 1.57E+03 17.8	17.8	7.25E+03	7.26E+02 10.0	10.0
BLK PG	2.66E+04	2.66E+04 1.54E+03	5.8	8.05E+02	2.01E+02	25.0	1.03E+04	8.05E+02 2.01E+02 25.0 1.03E+04 8.13E+02 7.9	7.9	1.55E+04	8.77E+02	5.7

**Table 8.** Percentage of healthy, pale and transparent zooxanthellae released in the microcosms.

Systems	Total	Healthy	Pale	Trasparent
D01	100	<u>12</u>	<u>58</u>	<u>29</u>
<u>D02</u>	<u>100</u>	<u>2</u>	<u>34</u>	<u>64</u>
<u>D03</u>	<u>100</u>	<u>5</u>	<u>62</u>	<u>35</u>
<u>D04</u>	<u>100</u>	<u>21</u>	<u>46</u>	<u>33</u>
<u>D05</u>	<u>100</u>	<u>2</u>	<u>25</u>	<u>73</u>
<u>D06</u>	<u>100</u>	12 2 5 21 2 3 8 7 10 13 2 4 11 4	46 25 47 40 34 90 40	<u>50</u>
<u>D07</u>	<u>100</u>	<u>8</u>	<u>40</u>	<u>52</u>
<u>D08</u>	<u>100</u>	<u>7</u>	<u>34</u>	<u>60</u>
<u>D09</u>	<u>100</u>	<u>10</u>	<u>90</u>	<u>3</u>
<u>D10</u>	<u>100</u>	<u>13</u>	<u>40</u>	<u>48</u>
<u>D11</u>	<u>100</u>	<u>2</u>	<u>34</u>	<u>64</u>
<u>D13</u>	<u>100</u>	<u>4</u>	<u>37</u>	<u>59</u>
<u>D14</u>	<u>100</u>	<u>11</u>	<u>31</u>	<u>58</u>
<u>D15</u>	<u>100</u>	4	34 37 31 38 47 42 52	<u>58</u>
D16	<u>100</u>	<u>15</u>	<u>47</u>	<u>38</u>
<u>D17</u>	<u>100</u>	<u>3</u>	<u>42</u>	<u>54</u>
<u>D18</u>	<u>100</u>	<u>5</u>	<u>52</u>	<u>43</u>
<u>D19</u>	<u>100</u>	<u>9</u>	<u>43</u>	<u>48</u>
<u>D20</u>	<u>100</u>	<u>4</u>	43 49	<u>47</u>
<u>D21</u>	<u>100</u>	15 3 5 9 4 6 3	<u>47</u>	<u>47</u>
<u>D22</u>	<u>100</u>	3		<u>59</u>
<u>D23</u>	<u>100</u>	4	<u>38</u>	<u>58</u>
<u>D24</u>	<u>100</u>	<u>1</u>	44	<u>54</u>
<u>D25</u>	<u>100</u>	<u>2</u>	<u>54</u>	<u>44</u>
MITO	<u>100</u>	<u>0</u>	38 38 44 54 24 54	<u>76</u>
<u>BLK</u>	<u>100</u>	1 2 0 2 3	<u>54</u>	64 315 313 73 50 51 60 3 48 64 59 58 58 38 54 47 47 59 58 54 44 76 44
BLKPG	<u>100</u>	<u>3</u>	<u>39</u>	<u>58</u>

<u>Table 9</u>. Output of one-way ANOVA analysis for the different color components of corals. Probability level reported (\*\*=P<0.01; \*=P<0.05; NS = not significant).

Sample code	Cyan (C)	Magenta (M)	Yellow (Y)	Black (K)
D01	NS	NS	NS	*
D02	NS	*	NS	NS
D03	NS	*	NS	*
D04	NS	**	NS	NS
D05	*	NS	NS	NS
D06	*	**	NS	**
D07	NS	NS	NS	NS
D08	*	**	NS	**
D09	*	NS	NS	*
D10	NS	**	NS	**
D11	NS	NS	*	*
D13	**	NS	NS	NS

(continued)

Sample code	Cyan (C)	Magenta (M)	Yellow (Y)	Black (K)
D14	NS	NS	NS	*
D15	NS	NS	NS	NS
D16	NS	NS	NS	NS
D17	NS	NS	NS	NS
D18	**	**	*	**
D19	NS	*	NS	NS
D20	*	NS	NS	NS
D21	NS	NS	NS	**
D22	NS	NS	NS	NS
D23	**	NS	*	NS
D24	NS	NS	NS	NS
D25	NS	NS	NS	NS
BLK	NS	*	NS	NS
BLK PG	**	**	NS	**
MITO	**	**	**	**

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## [0053]

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## Claims

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- 1. An eco-compatible sunscreen composition comprising:
  - one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethyl-butyl)-phenol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
  - one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
  - one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax.

## and free from:

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- preservatives belonging to the paraben family;
- ascorbic acid derivatives;
- UV filters selected from bis ethylhexyloxyphenol methoxyphenyl triazine, cinnamates, benzophenones, camphor derivatives, titanium oxide;
- fragrances selected from melaleuca essential oil, ylang ylang flower extracts, Bambousa Arundinacea extracts, Karitè:
- carriers selected from animal-derived fats and argan oil.
- 2. The composition according to claim 1, further comprising one or more fragrances selected from orange, lavender, grapefruit, guava and coconut.
  - 3. The composition according to claim 1 or 2, further comprising sorbic acid.
  - 4. The composition according to claim 1, consisting of:

- one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethyl-butyl)-phenol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
- one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
- one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
- water.
- 5. The composition according to claim 1, consisting of:

- one or more UV filters selected from 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethyl-butyl)-phenol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate, ethylhexyl triazone;
- one or more antioxidants selected from tocopheryl acetate, tocopherol, retinol, polyphenolic compounds extracted from green tea;
- sorbic acid;
- one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
- water.

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- 6. The composition according to claim 1, consisting of:
  - 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol and/or 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate;
  - tocopheryl acetate and/or tocopherol;
  - sorbic acid:
  - one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
  - water:
  - one or more fragrances selected from orange lavender, grapefruit, guava and coconut.
- 7. The composition according to claim 1, consisting of:
  - 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol and/or 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate;
  - tocopheryl acetate and/or tocopherol;
  - sorbic acid;
  - one or more carriers selected from propylene glycol dicaprylate/dicaprate, olive oil, beeswax;
  - water:
  - one or more fragrances selected from orange, lavender, grapefruit, guava and coconut;
  - hydroxyethyl cellulose and/or EDTA and/or sodium hydroxide.
- 30 **8.** The composition according to claim 1, consisting of:
  - 2,2'-methylene-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol, 2-[4-(diethylamine)-2-hydroxybenzoyl]hexyl benzoate and ethylhexyl triazone;
  - tocopheryl acetate;
  - sorbic acid;
  - propylene glycol dicaprylate/dicaprate, olive oil and beeswax;
  - water
  - hydroxyethyl cellulose;
  - EDTA;
  - sodium hydroxide.
    - **9.** The composition according to any one of the claims 1 to 6, wherein said UV filters are present in a concentration comprised between 3 and 6% by weight.
- **10.** The composition according to any one of the claims 1 to 9, in the form of cream, lotion, ointment, spray, lipstick.
  - **11.** The composition according to any one of the claims 1 to 10, **characterized in that** it is not harmful to the marine environment and in particular to the coral reef.

## Patentansprüche

- 1. Eine öko-kompatible Sonnenschutzzusammensetzung, umfassend:
- einen oder mehrere UV-Filter, ausgewählt aus 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetrame-thylbutyl)-phenol, 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat, Ethylhexyl-triazon;
  - ein oder mehrere Antioxidationsmittel, ausgewählt aus Tocopherylacetat, Tocopherol, Retinol, polyphenolischen Verbindungen, die von grünem Tee extrahiert sind;

- einen oder mehrere Träger, ausgewählt aus Propylenglykoldicaprylat/dicaprat, Olivenöl, Bienenwachs;

und frei von:

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- Konservierungsmitteln, die zur Paraben-Familie gehören;
  - Ascorbinsäurederivaten;
  - UV-Filtern, ausgewählt aus Bis-Ethylhexyloxyphenol Methoxyphenyl Triazin, Cinnamaten, Benzophenonen, Campherderivaten, Titaniumoxid;
  - Duftstoffen, ausgewählt aus Melaleuca ätherischen Ölen, Extrakten der Ylang-Ylang Blume, Bambousa Arundinacea Extrakten, Karitebaum;
  - Trägern, ausgewählt aus von Tieren abstammenden Fetten und Arganöl.
  - 2. Die Zusammensetzung gemäß Anspruch 1, ferner umfassend einen oder mehrere Duftstoffe, ausgewählt aus Orange, Lavendel, Grapefruit, Guave und Kokosnuss.
  - 3. Die Zusammensetzung gemäß Anspruch 1 oder 2, ferner umfassend Sorbinsäure.
  - 4. Die Zusammensetzung gemäß Anspruch 1, bestehend aus:
- einem oder mehreren UV-Filtern, ausgewählt aus 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetra-methylbutyl)-phenol, 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat, Ethylhexyl-triazon;
  - einem oder mehreren Antioxidationsmitteln, ausgewählt aus Tocopherylacetat, Tocopherol, Retinol, polyphenolischen Verbindungen, die von grünem Tee extrahiert sind;
  - einem oder mehreren Trägern, ausgewählt aus Propylenglykoldicaprylat/dicaprat, Olivenöl, Bienenwachs;
  - Wasser.
  - 5. Die Zusammensetzung gemäß Anspruch 1, bestehend aus:
    - einem oder mehreren UV-Filtern, ausgewählt aus 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetra-methylbutyl)-phenol, 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat, Ethylhexyl-triazon;
    - einem oder mehreren Antioxidationsmitteln, ausgewählt aus Tocopherylacetat, Tocopherol, Retinol, polyphenolischen Verbindungen, die von grünem Tee extrahiert sind;
    - Sorbinsäure;
    - einem oder mehreren Trägern, ausgewählt aus Propylenglykoldicaprylat/dicaprat, Olivenöl, Bienenwachs;
    - Wasser.
  - 6. Die Zusammensetzung gemäß Anspruch 1, bestehend aus:
    - 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol und/oder 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat;
    - Tocopherylacetat und/oder Tocopherol;
    - Sorbinsäure;
    - einem oder mehreren Trägern, ausgewählt aus Propylenglykoldicaprylat/dicaprat, Olivenöl, Bienenwachs;
    - Wasser;
    - einem oder mehreren Duftstoffen, ausgewählt aus Orange, Lavendel, Grapefruit, Guave und Kokosnuss.
  - 7. Die Zusammensetzung gemäß Anspruch 1, bestehend aus:
    - 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol und/oder 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat;
    - Tocopherylacetat und/oder Tocopherol;
    - Sorbinsäure;
    - einem oder mehreren Trägern, ausgewählt aus Propylenglykoldicaprylat/dicaprat, Olivenöl, Bienenwachs;
    - Wasser:
    - einem oder mehreren Duftstoffen, ausgewählt aus Orange, Lavendel, Grapefruit, Guave und Kokosnuss;
    - Hydroxyethylcellulose und/oder EDTA und/oder Natriumhydroxid.
  - 8. Die Zusammensetzung gemäß Anspruch 1, bestehend aus:

- 2,2'-Methylen-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-phenol, 2-[4-(Diethylamin)-2-hydroxybenzoyl]hexyl-benzoat und Ethylhexyl-triazon;
- Tocopherylacetat;
- Sorbinsäure;
- Propylenglykoldicaprylat/dicaprat, Olivenöl und Bienenwachs;
- Wasser;
- Hydroxyethylcellulose;
- EDTA;
- Natriumhydroxid.

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- **9.** Die Zusammensetzung gemäß einem der Ansprüche 1 bis 6, wobei die UV-Filter in einer Konzentration zwischen 3 und 6 Gew.-% vorhanden sind.
- 10. Die Zusammensetzung gemäß einem der Ansprüche 1 bis 9 in Form von Creme, Lotion, Salbe, Spray, Lippenstift.
- **11.** Die Zusammensetzung gemäß einem der Ansprüche 1 bis 10, **dadurch gekennzeichnet, dass** sie für die Meeresumgebung und insbesondere für Korallenriffe nicht schädlich ist.

#### 20 Revendications

- 1. Composition d'écran solaire compatible avec l'environnement comprenant :
  - un ou plusieurs filtres UV choisis parmi le 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétramé-thylbutyl)-phénol, le benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle, l'éthylhexyltriazone ;
  - un ou plusieurs antioxydants choisis parmi l'acétate de tocophéryle, le tocophérol, le rétinol, les composés polyphénoliques extraits du thé vert ;
  - un ou plusieurs supports choisis parmi le dicaprylate/dicaprate de propylène glycol, l'huile d'olive, la cire d'abeille,

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### et exempte de :

- conservateurs appartenant à la famille des parabènes ;
- dérivés de l'acide ascorbique ;
- filtres UV choisis parmi la bis-éthylhexyloxyphénol méthoxyphényltriazine, les cinnamates, les benzophénones, les dérivés du camphre, l'oxyde de titane ;
- fragrances choisies parmi l'huile essentielle de melaleuca, des extraits de fleurs d'ylang-ylang, des extraits de Bambousa arundinacea, le karité ;
- supports choisis parmi les graisses animales et l'huile d'argan.

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- 2. Composition selon la revendication 1, comprenant en outre une ou plusieurs fragrances choisies parmi l'orange, la lavande, le pamplemousse, la goyave et la noix de coco.
- 3. Composition selon la revendication 1 ou 2, comprenant en outre de l'acide sorbique.

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- 4. Composition selon la revendication 1, se composant de :
  - un ou plusieurs filtres UV choisis parmi le 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétramé-thylbutyl)-phénol, le benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle, l'éthylhexyltriazone ;
  - un ou plusieurs antioxydants choisis parmi l'acétate de tocophéryle, le tocophérol, le rétinol, les composés polyphénoliques extraits du thé vert ;
  - un ou plusieurs supports choisis parmi le dicaprylate/dicaprate de propylène glycol, l'huile d'olive, la cire d'abeille ;
  - eau.

- **5.** Composition selon la revendication 1, se composant de :
  - un ou plusieurs filtres UV choisis parmi le 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétramé-

thylbutyl)-phénol, le benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle, l'éthylhexyltriazone;

- un ou plusieurs antioxydants choisis parmi l'acétate de tocophéryle, le tocophérol, le rétinol, les composés polyphénoliques extraits du thé vert ;
- acide sorbique ;
- un ou plusieurs supports choisis parmi le dicaprylate/dicaprate de propylène glycol, l'huile d'olive, la cire d'abeille ;
- eau.

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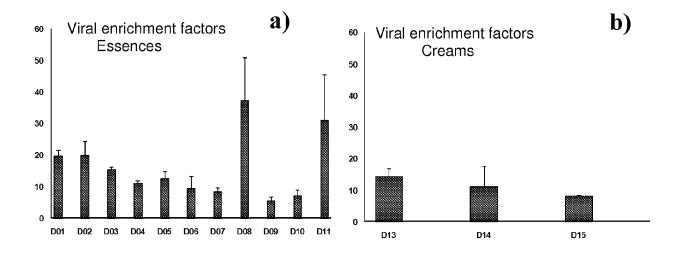
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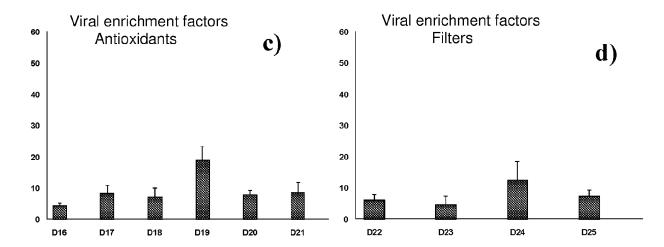
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- 6. Composition selon la revendication 1, se composant de :
  - 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétraméthylbutyl)-phénol et/ou benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle;
  - acétate de tocophéryle et/ou tocophérol ;
  - acide sorbique ;
  - un ou plusieurs supports choisis parmi le dicaprylate/dicaprate de propylène glycol, l'huile d'olive, la cire d'abeille ;
  - eau ;
  - une ou plusieurs fragrances choisies parmi l'orange, la lavande, le pamplemousse, la goyave et la noix de coco.
- 7. Composition selon la revendication 1, se composant de :
  - 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétraméthylbutyl)-phénol et/ou benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle ;
  - acétate de tocophéryle et/ou tocophérol ;
  - acide sorbique ;
    - un ou plusieurs supports choisis parmi le dicaprylate/dicaprate de propylène glycol, l'huile d'olive, la cire d'abeille ;
    - eau ;
    - une ou plusieurs fragrances choisies parmi l'orange, la lavande, le pamplemousse, la goyave et la noix de coco;
    - hydroxyéthylcellulose et/ou EDTA et/ou hydroxyde de sodium.
  - 8. Composition selon la revendication 1, se composant de :
    - 2,2'-méthylène-bis-(6-(2H-benzotriazole-2-yl)-4-(1,1,3,3-tétraméthylbutyl)-phénol, benzoate de 2-[4-(diéthylamine)-2-hydroxybenzoyl]hexyle et éthylhexyltriazone ;
    - acétate de tocophéryle ;
    - acide sorbique ;
    - dicaprylate/dicaprate de propylène glycol, huile d'olive, cire d'abeille ;
    - eau ;
    - hydroxyéthylcellulose;
    - EDTA ;
    - hydroxyde de sodium.
  - **9.** Composition selon l'une quelconque des revendications 1 à 6, dans laquelle lesdits filtres UV sont présents à une concentration comprise entre 3 et 6 % en poids.
  - **10.** Composition selon l'une quelconque des revendications 1 à 9, sous la forme d'une crème, d'une lotion, d'une pommade, d'un pulvérisateur, d'un rouge à lèvres.
- 11. Composition selon l'une quelconque des revendications 1 à 10, caractérisée en ce qu'elle n'est pas nuisible pour l'environnement marin et en particulier le récif de corail.





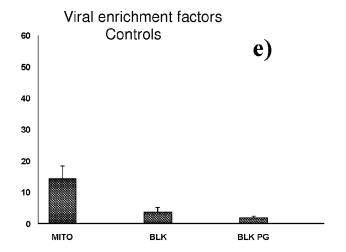
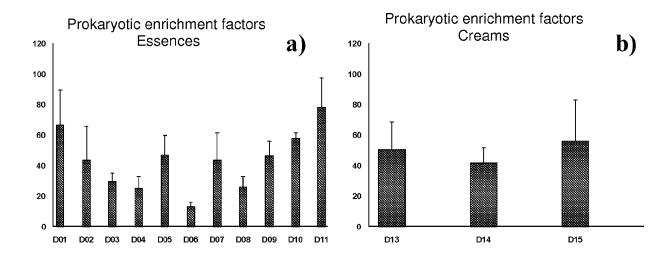
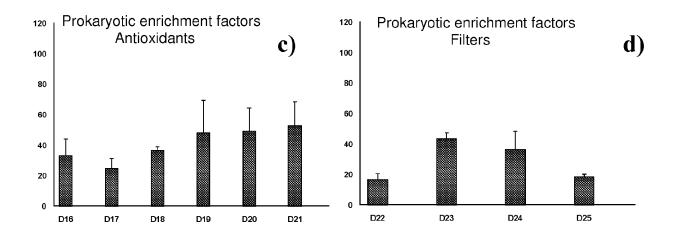


Figure 1





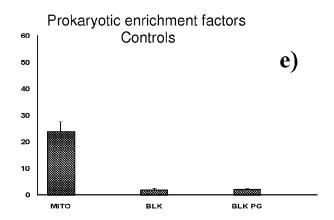


Figure 2

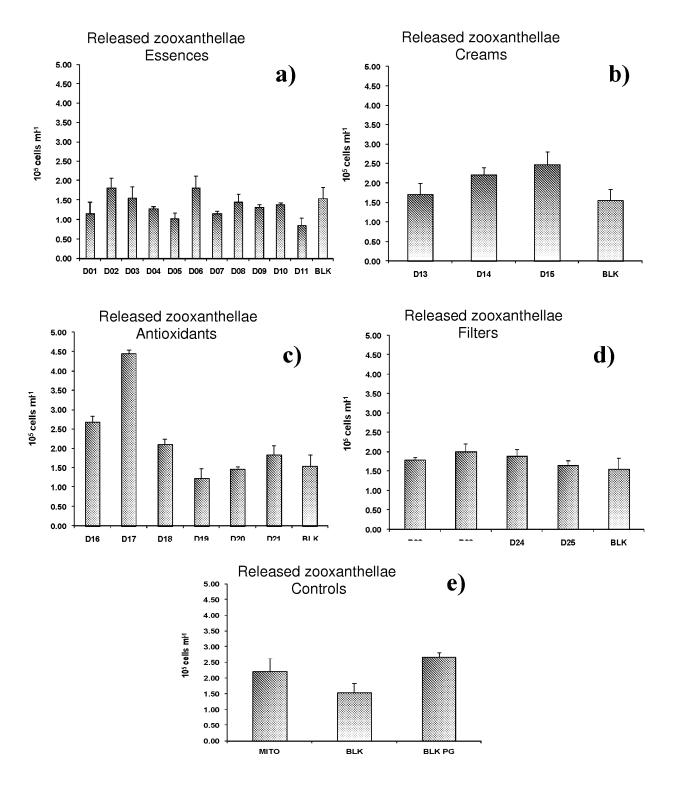


Figure 3

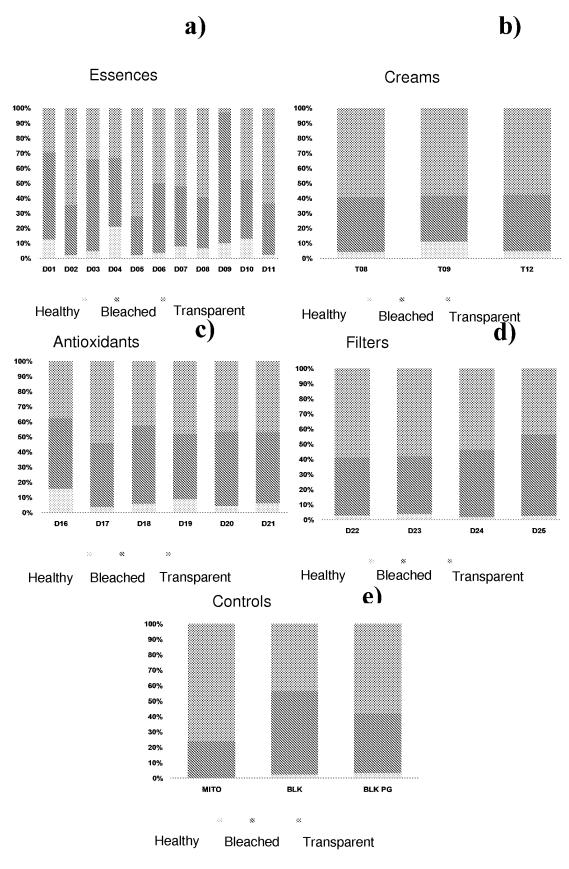


Figure 4

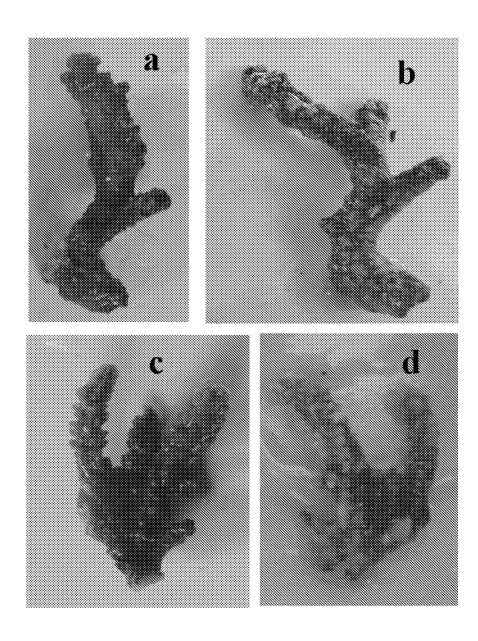
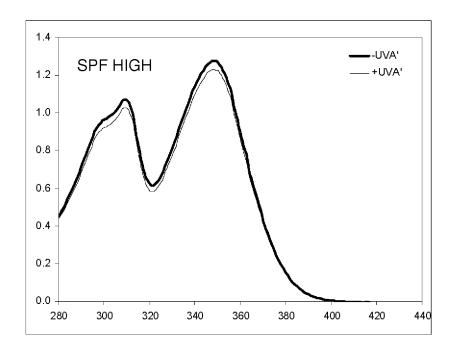


Figure 5



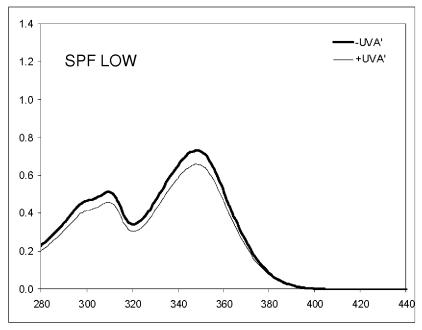


Figure 6

#### REFERENCES CITED IN THE DESCRIPTION

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