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1 **Calcium phosphates from fish bones in sunscreen: an LCA and toxicity study of an**  
2 **emerging material for circular economy**

3  
4 **Serena Righi<sup>1,2</sup>, Ermelinda Prato<sup>3</sup>, Giulia Magnani<sup>4</sup>, Virginia Lama<sup>1,2</sup>, Francesca Biandolino<sup>3</sup>,**  
5 **Isabella Parlapiano<sup>3</sup>, Francesca Carella<sup>5</sup>, Michele Iafisco<sup>5\*</sup> and Alessio Adamiano<sup>5\*</sup>**

6  
7 **Correspondence:** [alessio.adamiano@istec.cnr.it](mailto:alessio.adamiano@istec.cnr.it), [michele.iafisco@istec.cnr.it](mailto:michele.iafisco@istec.cnr.it)

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9 <sup>1</sup>CIRSA (Interdepartmental Research Centre for Environmental Sciences), University of Bologna,  
10 via Sant'Alberto, 163, 48123 Ravenna, Italy;

11 <sup>2</sup>Department of Physics and Astronomy, University of Bologna, viale Berti Pichat, 6/2, 40127  
12 Bologna, Italy

13 <sup>3</sup>Institute for the Coastal Marine Environment of the Italian National Research Council (IAMC-CNR),  
14 Taranto, Italy

15 <sup>4</sup> Dipartimento di Chimica Giacomo Ciamician Università di Bologna, Via Selmi, 2, 40126 Bologna,  
16 Italy.

17 <sup>5</sup>Institute of Science and Technology for Ceramics (ISTEC), National Research Council (CNR), Via  
18 Granarolo 64, 48018 Faenza, Italy.

19  
20  
21 **Abstract**

22 The use of sustainable and natural materials is an ever-increasing trend in cosmetic. Natural calcium  
23 phosphate (CaP-N) from food by-products and especially from fisheries (i.e., bones), has been  
24 suggested as a sustainable option to chemicals commonly used in cosmetic products, in particular  
25 to UV-filters in sunscreens. However, the environmental benefits and impacts of its production and  
26 use are still uncertain as they have never been quantified. In this paper, we report on toxicological  
27 characterization of CaP-N produced from incineration of fish meal in a pilot scale plant.  
28 Furthermore, we quantified the environmental burdens linked to the partial substitution of UV-  
29 filters by CaP-N through the life cycle assessment (LCA) comparing CaP-N with zinc oxide  
30 nanoparticles (ZnO NPs) as alternative option. CaP-N consists in a biphasic mixture 53:47 of  
31 hydroxyapatite:β-tricalcium phosphate, and is made of round particles with a diameter in the range

32 of a few microns. Toxicity tests on 4 aquatic species (*Dunaliella tertiolecta*, *Tigriopus fulvus*,  
33 *Corophium insidiosum* and *Gammarus aequicauda*) revealed that CaP-N does not produce any  
34 adverse effect, all the species showing EC/LC50 values higher than 100 mg L<sup>-1</sup>. Moreover, during the  
35 96 hours acute toxicity test on *C. insidiosum*, which is a tube-building species, the specimens built  
36 their tubes with the available CaP-N, further attesting the non-toxicity of the material. The LCA study  
37 showed that the environmental performance of CaP-N is better than that of ZnO NPs for 11 out of  
38 16 impact categories analyzed in this study, especially for the categories Ecotoxicity and  
39 Eutrophication of freshwaters (an order of magnitude lower), and with the exception of fossil  
40 resources for which CaP-N has a significantly higher impact than ZnO NPs (+140%). Concluding, our  
41 study demonstrates that the replacement of ZnO NPs with CaP-N thermally extracted from fish  
42 bones in cosmetic products can increase their safety and sustainability.

43

## 44 **Introduction**

45 During the last 30 years, new green and sustainable chemicals capable to replace dangerous or  
46 brown ones have been sought by researchers worldwide. Among the plethora of alternative  
47 materials, hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  – HA) and more in general calcium phosphates (CaPs)  
48 have gained high interest due to their wide range of applications and to their intrinsic bio and eco-  
49 compatibility (Yoon et al., 2020). CaPs are a family of materials that are ubiquitous in the biosphere,  
50 as they can be found in water bodies, soils, vegetables and animals, where they mainly constitute  
51 the mineral part of bones and teeth. They are widely recognized as biologically safe and are  
52 considered standard materials for biomedical applications (Dorozhkin, 2021); moreover, they are  
53 employed in many other fields (Degli Esposti et al., 2022) (Adamiano et al., 2019; Pérez-Álvarez et  
54 al., 2021; Ghosh et al., 2022) including cosmetic (Carella et al., 2021a).

55 In view of their large-scale utilization, the recovery of CaPs from circular economy sources such as  
56 food by-products is gaining more and more attention (Ar, 2009). The extraction from fish bones is  
57 particularly interesting, as fishing companies produce massive quantities of this by-product without  
58 there being any ad hoc valorisation stream. In fact, fish bones are an excellent source of CaPs,  
59 mainly in the form of highly pure HA, that can constitute up to the 40 wt.% of bones depending on  
60 the fish species (Nam et al., 2019). In this respect, it has been estimated that fish processing by  
61 products accounts for approximately the 70 wt.% of fish weight, up to the 30 wt.% being composed  
62 of heads, skins and bones (Motalipassi et al., 2021), for a total of circa 125 Mt of fish by-products  
63 produced each year worldwide (Agriculture Organization of the United Nations, 2020).

64 Several works have proposed a circular economy approach for the extraction of chemicals from  
65 several by-products deriving from macroalgae, sea urchin and shrimps to be used in cosmetic as  
66 excipients, additives, preservatives, anti-aging and whitening agents, and photoactive ingredients  
67 (Lourenco-Lopes et al., 2020; Zilia et al. 2021; King et al. 2017). Among them, an increasing number

68 of papers reports on the use of CaPs extracted from fish bones as photo-protective materials, where  
69 they are claimed to be more eco-compatible and safer respect to UV-filters commonly used in  
70 commercial products (Piccirillo et al., 2014) (Teixeira et al., 2017). CaPs have been also proposed as  
71 boosters of the Sun Protection Factor (SPF), i.e., as materials that do not hold intrinsic UV-filtering  
72 properties but have the ability to increase the SPF by scattering light and increase the adsorption by  
73 UV-filters occurring in the formulation. In this case, the advantage of using CaPs is reducing the  
74 concentrations of UV-filters as these chemicals have been in the spotlight for their controversial  
75 toxic and reef-damaging potential (Moeller et al., 2021).

76 Among UV-filters, the inorganic ones such as zinc and titanium oxides (ZnO and TiO<sub>2</sub>) are generally  
77 considered safer respect to organic ones; nonetheless, their occurrence in cosmetic products is a  
78 matter of concern for consumers and the environment as they are typically used in the form of  
79 nanoparticles (NPs) (Musial et al., 2020). In fact, it has been reported that NPs can penetrate  
80 through the epidermis into the body in case of damaged or diseased skin (Monteiro-Riviere et al.,  
81 2011). Moreover, since increasing concentrations of TiO<sub>2</sub> and ZnO NPs derived from sunscreens in  
82 the environment have been detected, attention towards potential drawbacks for both aquatic and  
83 terrestrial ecosystem is increasing (Labille et al., 2020) (Gondikas et al., 2018) (Gomes et al., 2021).  
84 A number of studies showed the negative effects of ZnO and TiO<sub>2</sub> NPs used in sunscreen on stony  
85 corals in reefs across the world, with coral bleaching being significantly greater after ZnO NPs  
86 exposure compared with TiO<sub>2</sub> NPs, calling for further investigation of the potential harms of these  
87 NPs on marine ecosystems (Corinaldesi et al., 2018).

88 Overall, despite CaPs are widely renowned as highly biocompatible materials for humans, their  
89 potential toxicity on aquatic species, such as gastropods or crustaceans populating aquatic  
90 environments, has not been properly investigated. Moreover, whether the production and use of  
91 CaPs extracted from fish bones in cosmetic would be beneficial for the environment remains unclear

92 as incineration, calcination or pyrolysis, which are the most employed methods for CaPs recovery  
93 from circular economy due to their low cost and large-scale applicability (Carella et al., 2021b), are  
94 also high energy consuming processes, and their environmental footprint may pose serious issues  
95 on the long-term sustainability of natural CaPs. In fact, despite natural materials are generally  
96 perceived as safer respect to synthetic ingredients in cosmetics, sustainability claims need to be  
97 supported by data and by in depth analysis of related environmental impact using a cradle-to-grave  
98 approach (Ögmundarson et al., 2020). In this light, it is important to carry out a life cycle assessment  
99 (LCA) to quantify the impacts and assess the potential environmental benefits that could be  
100 achieved by replacing chemicals with CaPs recovered from fish by-products by thermal treatments.  
101 Indeed, the importance of LCA of emerging nanotechnologies has been well recognized and LCA has  
102 been widely used over the years to evaluate the eco-friendliness of NPs production and to compare  
103 different nanomanufacturing processes. One first LCA of TiO<sub>2</sub> microparticles was performed as early  
104 as in 1999 on two mature processes (Reck and Richards 1999). Later, Grubb and Bakshi (2010)  
105 applied LCA to compare a mature and new method for TiO<sub>2</sub> NPs synthesis (sulphate process vs  
106 hydrochloride process). In 2015, the environmental performance of the hydrolytic sol–gel synthesis  
107 of anatase TiO<sub>2</sub> NPs was evaluated using the LCA methodology (Pini et al., 2015) and more recently,  
108 LCA was also applied to methods for ZnO NPs production (Stieberova et al., 2017 and 2019). And  
109 the list is far from being exhaustive.

110 Our work reports on the toxic effect of natural CaPs extracted from fish bones (CaP-N) on a battery  
111 of four species belonging to different trophic levels (primary producers and consumers), including  
112 algae (*Dunaliella tertiolecta*), copepoda (*Tigriopus fulvus*), and crustaceans amphipoda (*Corophium*  
113 *insidiosum* and *Gammarus aequicauda*). These species were selected because they are established  
114 model species in standardized toxicity tests, ecotoxicological studies and in ecological risk  
115 assessment (Annicchiarico et al., 2007; Faraponova et al., 2016; Manzo et al., 2013; Narracci et al.,

116 2009; Prato and Biandolino, 2006). Moreover, to quantify the environmental burdens linked to the  
117 virtual substitution or reduction of UV-filters by natural CaPs, a LCA was carried out comparing CaPs  
118 extracted from fish bones with ZnO as a proxy of ZnO NPs which are nowadays considered as the  
119 safest UV-filter and, unlike TiO<sub>2</sub> NPs, do not need photo-passivation to be employed in in sun care  
120 products (Labille et al. 2020).

121

## 122 **Materials**

123 Where not otherwise specified, all the chemicals were analytical grade (Sigma-Aldrich, St. Luis, MO,  
124 USA), and were used without further purification.

### 125 **CaPs production and characterization**

#### 126 *CaPs extraction from fish bones*

127 A by-product from fish meal production mainly made by dried fish bones obtained from the  
128 processing of salmon (*Salmo salar*), was taken from a local factory (Italy) producing fish meal and  
129 pet food, and had the following composition: proteins 47.0 wt.%, fats 7.5 wt.%, ashes 40.0 wt.%,  
130 and water 5.5 wt.%. 25 kg of fish meal were placed in an incinerator (Gem IEB 16 conform to E.C.  
131 reg. 1069/09, Gem Matthews, Udine, Italy) with a combustion chamber with a volume of 1.0 m<sup>3</sup> at  
132 the temperature of 850 °C. After complete incineration, the resulting material was removed from  
133 the chamber and cooled at room temperature, and then grinded with a high energy planetary ball  
134 mill (MMS S.r.l. and Nannetti, Faenza, Italy) as follows. Briefly, 1 kg of calcined materials are placed  
135 in a jar with 1.5 L of Millipore water and 1.5 kg of alumina grinding balls with a  $\varnothing$  between 1.0 cm  
136 and 1.5 cm, and then the planetary ball mill is actioned for 30 minutes. Grinding balls are removed  
137 and the solution is poured in a 5.0 L beaker and left still until powder sedimentation. After removing  
138 the water on top, the powder is placed in a ventilated drier at 40°C overnight, to be finally sieved  
139 through a stainless-steel sieve with a mesh of 450 (<32.0  $\mu$ m). The so obtained material is

140 henceforward named CaP-N and was used for physical-chemical characterization and toxicological  
141 tests.

142

#### 143 *CaPs physical-chemical characterization*

144 X-ray diffraction (XRD) patterns were recorded with a D8 Advance Diffractometer (Bruker –  
145 Massachusetts, US), equipped with a Lynx-eye position sensitive detector, with a  $\text{CuK}\alpha$  radiation ( $\lambda$   
146 = 1.54178 Å), at 40 kV and 40 mA. XRD profiles were acquired in the 10 – 60 ° ( $2\theta$ ) at step size of  
147 0.02° and a scanning speed of 0.5 s. Phase identification was performed through Rietveld  
148 refinement with the software TOPAS5. The weight composition of the phases was refined  
149 considering a multiphase system, using tabulated atomic coordinates of hydroxyapatite (ASTM Card  
150 file No. 09-0432), and  $\beta$ -TCP (ASTM Card file No. 09-0169).

151 The elemental analysis of CaP-N was carried out by inductively coupled plasma atomic emission  
152 (ICP-OES) spectrometer (Liberty 200; Varian, Palo Alto, CA, USA). 20 mg of samples were added to  
153 15 mL of  $\text{HNO}_3$  solution at 9.0 wt.% and placed in an ultrasonic bath sonicator at 37 °C until complete  
154 dissolution. Solutions were then cooled at room temperature and added with water up to 100.0 mL  
155 before ICP-OES analysis. Ca and P concentrations were measured by their atomic emission at the  
156 following wavelengths: 422.673 nm for Ca and 213.618 nm for P. Moreover, the occurrence of  
157 relevant toxic and ecotoxic elements such as As, Ba, Cr, Pb and Zn and was screened. Finally, field  
158 emission gun scanning electron (FEG-SEM) microscopy employing a Sigma NTS GmbH (Carl Zeiss,  
159 Oberkochen, Germany) was used to evaluate the morphology and the dimension of the samples.  
160 Powders were mounted on aluminium stubs using carbon tape and sputter coated with gold in a  
161 Sputter Coater E5100 (Polaron Equipment – Watford, Hertfordshire, UK) under argon at  $10^{-3}$  mbar  
162 for 4 minutes with a sputtering current of 30 mA. Energy dispersive X-ray spectroscopy data (EDS,  
163 spectra, and maps) were collected using an accelerating voltage of 15.0 kV and a working distance



164 of 10 mm by an INCA Energy 300 (Oxford instruments, Oxford, UK) coupled to the FEG-SEM  
 165 microscope.

166

167 **Toxicity testing**

168 The ecotoxicological screening performed with a battery of selected species, allowed to evaluate  
 169 the toxicity of CaP-N and ZnO NPs. Test conditions and tested species are summarized in **Table 1**.

170 **Table 1.** Summary of the test conditions utilized for the four selected testing species.

<i>Species</i> →	<i>D. tertiolecta</i>	<i>T. fulvus</i>	<i>C. insidiosum</i>	<i>G. aequicauda</i>
Test type	Static	Static	Static	Static
Stage of development	Agal cell	Naupilii (I-II)	Juveniles (2-4 mm)	Juveniles (2-4 mm)
Test chambers	6-well plates	12-well plates	Glass beakeres	Glass beakers
Luminosity	6000-10000 lx cool light	500-1200 lx cool light	500-1200 lx cool light	500-1200 lx cool light
Light/dark photoperiod	24h	16h:8h	16h:8h	16h:8h
Dilution water	Filtered sea water (0.45 μm)	Filtered sea water (0.22 μm)	Filtered sea water (0.45 μm)	Filtered sea water (0.45 μm)
Salinity %	36 ± 2	38 ± 2	36 ± 2	36 ± 2
Temperature	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C
pH	8.0 ± 0.2	8.0 ± 0.2	8.0 ± 0.2	8.0 ± 0.2
Aeration	Absent	Absent	Present	Present
Reference toxicant	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	CuSO <sub>4</sub> · 5H <sub>2</sub> O	Cd(NO <sub>3</sub> ) <sub>2</sub>	Cd(NO <sub>3</sub> ) <sub>2</sub>

Concentration of Ref. Toxicant	2.5-5-10-20-40 (Cr mg L <sup>-1</sup> )	0.03-0.06-0.12-0.25-0.5 (Cu mg L <sup>-1</sup> )	0.2-0.4-0.8-1.6-3.2 (Cd mg L <sup>-1</sup> )	0.2-0.4-0.8-1.6-3.2 (Cd mg L <sup>-1</sup> )
Testing volume	10 mL	3 mL	700 mL	700 mL
Bioassay duration	72h	96h	96h	96h
N° organisms/replicate	10000 L <sup>-1</sup>	10	20	20
N° replicates	3	3	3	3
N° run	3	3	3	3
End-point EC50/LC50	Inhibition/stimulation gowht	Mortality rate	Mortality rate	Mortality rate
Validity critria	Density cell >16	Control mortality <10%	Control mortality <15%	Control mortality <15%
Reference Protocols	(APHA, 2005); (ISO, 2006a)	(Faraponova et al., 2016); (ISO, 1999)	(ASTM, 1992); (US EPA, 1994)	(ASTM, 1992); (US EPA, 1994)

171

172

173 *Preparation of testing media*

174 Stock solutions of CaP-N were prepared by dispersing 1.0 g of the dry powders into 1.0 L of 0.22- $\mu$ m  
 175 filtered natural seawater (FNSW) plus Tween 20 (diluted at 10%) surfactant in order to obtain a good  
 176 sample dispersion. The surfactant was added for *D. tertiolecta* and *T. fulvus*'s test in a low

177 concentration ( $10 \mu\text{l L}^{-1}$ ) that did not affect algal cell growth and nauplii (Beiras et al., 2018; Oliviero  
178 et al., 2019).

179 The so obtained stock solutions were diluted to carry out the toxicological assays in concentrations  
180 ranged between 0.01 and 200.0  $\text{mg L}^{-1}$ .

181 Water-dispersed ZnO NPs (20.0 wt.%, purity of 99.95%) were purchased from US Research  
182 Nanomaterials, Inc. (Houston, USA) with a nominal particle size of 30–40 nm. A stock suspension  
183 ( $1000 \text{ mg L}^{-1}$ ) of ZnO NPs was prepared in 0.22- $\mu\text{m}$  filtered ultrapure water and sonicated for 15 min  
184 in a ultrasonic water bath (305 W, 50–60 Hz; Soltec Ultrasonic Baths) and stored in the dark at 4 °C  
185 for 15 min. Final testing suspensions were prepared in filtered natural seawater (FNSW) (GF/C  
186 Whatman, 0.22  $\mu\text{m}$ ) collected from the Mar Grande of Taranto (Ionian Sea, Italy; 40° 25' 0.1" N, 17°  
187 14' 24" E; pH  $8.0 \pm 0.1$ ).

188

#### 189 *Dunaliella tertiolecta*

190 The potential of CaP-N to inhibit/stimulate algal growth was evaluated using the green alga *D.*  
191 *tertiolecta*. Algae were obtained from culture collection of CNR IRSA section of Taranto (Italy).

192 The algal growth assay was performed according to ISO 10253 standard procedures (ISO, 2006a),  
193 modified by using multi-well plates instead of glass flasks (Lukavský et al., 2011). Algal cells were  
194 cultured in artificial seawater Instant Ocean® with complete F2 culture medium (Guillard and  
195 Ryther, 1962) at  $20.0 \pm 0.5 \text{ C}$  with a 12:12 h light:dark cycle and light intensity of 6000-10,000 lux  
196 (Sbrilli et al., 1997). An algal suspension sampled during the phase of exponential growth was  
197 prepared. Initial cellular concentration of  $1.5 \times 10^4$  was adopted for growth inhibition assay. The  
198 test was carried out in triplicate, with 5 serial dilutions: 0.01-0.1-1-10-100  $\text{mg L}^{-1}$ . Sterile polystyrene  
199 multi-well plates with 6 wells each with a capacity of 10.0 ml were used as support. Filtered artificial  
200 seawater (0.45- $\mu\text{m}$  Millipore® membrane) was used in toxicity assays and for dilution of standard

201 samples of reference toxicant. In parallel, a test was performed with a control (6 replicates)  
202 consisting of the growth medium only and a positive control using potassium dichromate in five  
203 scalar concentrations, namely 2.5, 5, 10, 20, and 40 mg Cr L<sup>-1</sup>.

204 The wells plates were kept in a thermostatic chamber (20 ± 2°C), continuously illuminated by a cool  
205 white light source in the 7000–8000 lux range for 72 h.

206 At the end of bioassay, cultures were stopped using a drop of Lugol's solution and the cell density  
207 of each replicate was measured using a Burker's counting cell. ~~Then~~ The inhibition/stimulation of  
208 growth of the algal biomass was calculated using comparisons of growth rates of the algal cultures  
209 in tested solutions and the control. During all assays, the initial and final pH values were registered  
210 in all the samples (Crison, MicropH 2001, electrode Hamilton, Slimtrode). For all bioassays, the pH  
211 drift was less than 0.5 pH unit over 72 h.

212

### 213 *Tigriopus fulvus*

214 *T. fulvus* specimens were obtained from laboratory massive cultures of adult brood stock at CNR  
215 IRSA (Taranto, Italy). Toxicity tests were conducted on newborn offspring (nauplii) originating from  
216 synchronized cultures (24–48 h). The nauplii were released by ovigerous females selected 24 h prior  
217 the test, transferred on an 80.0 µm mesh plankton net fixed to a Plexiglas tube and fed with a mix  
218 of *Tetraselmis suecica* and *Isochrysis galbana* algae cultures at 1.5 × 10<sup>8</sup> and 3.0 × 10<sup>8</sup> cells L<sup>-1</sup>  
219 density, respectively.

220 Briefly, ten organisms were placed into each well of multi-well plates (5 mL per well), containing 3.0  
221 mL of different concentrations of CaP-N and ZnO NPs (Table 1) and incubated, for 96 h, at 20.0 ± 0.5  
222 °C in dark conditions. CuSO<sub>4</sub> × 5 H<sub>2</sub>O were selected as reference toxicants for copepods nauplii to  
223 ensure the validity of test. All tests were performed in triplicates. After 96 h, lethality was the  
224 endpoint assessed. The number of dead organisms was counted under a stereomicroscope: larvae

225 that were completely motionless were counted as dead organisms. Copepods were considered dead  
226 if any movement of external appendages was registered for about 20 seconds in response to  
227 mechanical stimulation and the percentage of mortality was calculated compared to control.  
228 Oxygen content and pH of the test media were measured at the beginning and at the end of each  
229 test in the replicates of both control and testing solutions.

230

### 231 *Gammarus aequicauda* and *Corophium insidiosum*

232 Amphipods specimens were collected from an unpolluted area located in the Taranto Gulf (Ionian  
233 Sea, Southern Italy; 40° 49' 63" N, 17° 32' 35" E), using a 0.5 mm sieve. In the laboratory, the  
234 organisms were placed in aerated glass containers with their native sediment and algae and  
235 maintained for about 7-10 days prior the test, at  $18 \pm 2$  °C with a 16:8 h light:dark cycle. During the  
236 acclimation period, the amphipods were fed two times a week with *Chaetomorpha linum*, *Ulva sp*  
237 and benthic microalgae *Phaeodactylum tricornutum* to integrate the organic matter present in the  
238 native sediment.

239 The general design of toxicity tests was based on the standard guides for conducting acute sediment  
240 toxicity tests with marine–estuarine amphipods, with some modifications (ASTM 1993; SETAC  
241 1993). Juveniles (2-4 mm) of *C. insidiosum* and *G. aequicauda* were exposed for 96 h to six different  
242 concentrations of CaP-N and five of ZnO NPs (Table 1). For both species, three replicates were  
243 prepared per treatment and twenty individuals per concentration were randomly added to 1 L  
244 beakers containing 700 ml of test solution. During test exposure, beakers were maintained into a  
245 test chamber at  $18 \pm 2$  °C with a 12:12 h light:dark cycle. At the end of the test, the content of each  
246 beaker was sieved, and the survivors were counted. Apparently dead individuals were considered  
247 living if movement was exhibited after gentle stimulation. In order to have a reference measure for  
248 the acute toxicity assessment, a negative control test to determine the response of the animals in

249 the absence of contaminant, and a positive control test with cadmium nitrate ( $\text{Cd}(\text{NO}_3)_2$ ) as  
250 reference toxicant in a range of concentrations within 0.4 to 3.2 mg L<sup>-1</sup>, were performed.

251

#### 252 *Statistical analysis*

253 Data are presented as means  $\pm$  standard deviation (S.D., n = 3). Acute tests were considered valid if  
254 the mortality in the negative control was  $\leq 10\%$  for *T. fulvus*,  $\leq 15\%$  for *C. insidiosum* and *G.*  
255 *aequicauda*, and if the growth factor was  $> 16$  for *D. tertiolecta*. The 96-h LC50 values and their 95%  
256 confidence intervals (CI) were calculated using the Lichtfield-Wilcoxon method (Lichtfield and  
257 Wilcoxon 1949). A smooth function was used to fit dose-response curves with software GraphPad  
258 Prism 8.4.2.6.

259

#### 260 **Life cycle assessment**

261 LCA study was carried out according to ISO 14040 and 14044 guidelines (ISO, 2006b; ISO, 2006c),  
262 and comprises four steps: goal and scope definition, inventory analysis, life cycle impact assessment,  
263 interpretation. Gabi 10.0 modeling program was used for carrying out the LCA study.

264

#### 265 *Goal and scope definition*

266 The objective of the LCA study of this work was to evaluate the environmental sustainability of CaP-  
267 N and to compare its impacts with those of ZnO NPs assumed as representative of the synthetic  
268 inorganic UV-filters for sun care products.

269

270 Given that the raw material for CaP-N is a residual product of fish meal production destined for final  
271 disposal, the residues of fish meal production phases were not taken into account, according to the  
272 zero-burden approach. On the contrary, environmental credits were considered for the avoided

273 incineration of this residual product. Four main processes of CaP-N production were included in the  
274 assessment: (i) calcination of fish meal, (ii) heat exchanging, (iii) fume abatement and (iv) grinding.  
275 Landfilling of retained dust via fume abatement was added. **Figure 1** shows the flow diagram of the  
276 whole process.

277 On the industrial scale, calcination was assumed to take place in an incineration plant similar to the  
278 one already described for CaP-N production, with the only difference of the designed system  
279 consisting of a continuous type of incinerator furnace with a rotating drum. In the hypothesized  
280 system, a residual product from fish meal production mainly made by dried fish bones having an ash  
281 content around 40 wt.% is calcinated at a temperature of 850 °C for 1 hours in the incineration  
282 chamber. Afterwards, the fumes generated from the incineration are conveyed in an oxidation  
283 chamber to be heated at 850-1100 °C for complete oxidation. The energy demand of the process is  
284 mainly provided by thermal energy as each chamber is equipped with a burner operating with  
285 methane, with a very low input of electrical energy. Technical data report that fume coming out  
286 from the post combustor are characterized by a concentration of particulate matter (PM) that varies  
287 between 100 and 150 mg Nm<sup>-3</sup>. Therefore, before being expelled into the atmosphere, it has been  
288 assumed that fumes have to be purified. An air/water heat exchanger is then hypothesized to cool  
289 the fumes before conveying them to the purification process. As usual, a fraction of thermal energy  
290 is recovered through the air/water heat exchanger and sent to calcination process. Fumes are  
291 filtered through a water scrubber to reduce their pollutant content to levels compatible to  
292 regulation on air pollution. The fumes are then expelled into the atmosphere through a chimney  
293 while the PM retained by the scrubber is disposed to landfill as solid waste, considering that the  
294 CaPs is an inert and non-hazardous material. Finally, the CaPs calcinated are subsequently ground  
295 with a ball mill for 0.5-1.0 hour to bring the diameter of the particles below 100 μm.

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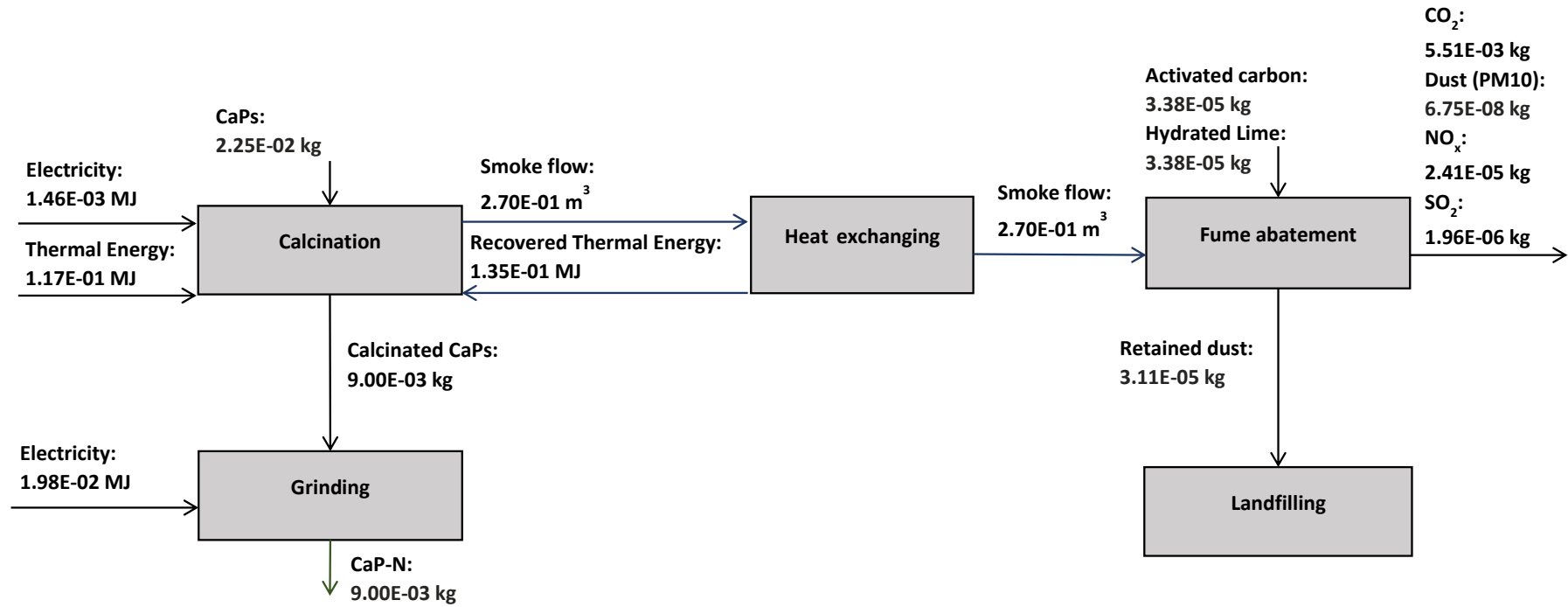


Figure 1. Flow diagram of the CaP-N production process.

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All the impacts related to the construction, maintenance and decommissioning of the machinery that make up the plant were considered negligible for the purposes of the study. At the same time, post-production phases for both CaP-N and ZnO NPs, such as use, transport and waste management at the end of their life were not considered because they were assumed to be equal in the two products. It should be noted, however, that this is a conservative assumption as CaPs is less toxic and ecotoxic than ZnO NPs.

The functional unit (FU) considered in this study is defined as the amount of UV-filters necessary to obtain 100.0 g of sunscreen characterized by an SPF of 20. This FU can be translated into a reference flow of material and energy inputs required to produce 9.0 g of natural CaPs, which has been compared to the same amount of ZnO NPs, the performance of the two materials in increasing the SPF of sunscreens being similar (Rastrelli et al., 2013). All process flows included within the system boundaries are consistent with the FU considered.

*Inventory analysis*

For what concerns CaP-N production, the study was based on primary data obtained directly from the designers of the experimental plant (pilot scale) and from tailored technical computations and on secondary data returned from GaBi professional database and from Ecoinvent 3.8 database. The model was created using both process datasets already existing within the software and process datasets created ad hoc. In order to considered that exploitation of fishing industry residues avoids their disposal and prevents environmental impacts, CaPs enter the system with credits calculated assuming the avoided incineration of an equal amount of organic waste. The dataset "EU-28: incineration of domestic waste" of GaBi professional database was used for this purpose. The Italian grid mix contained in the GaBi professional database was selected for the electrical input, while

25 regarding the thermal energy consumed by the process, it was assumed that it is produced totally  
 26 from natural gas. Air emissions have been estimated considering the composition of the incinerated  
 27 fish meal and the best available technology used for fumes abatement (Pinasseau et al., 2018).  
 28 Ecoinvent v.3.8 dataset that describes the ZnO production was used as proxy for modelling the ZnO  
 29 NPs production; this choice was due to the fact that an LCI dataset of ZnO NPs production is not  
 30 available and where data exist, they are incomplete and concerning very innovative processes non  
 31 present at a commercial scale (Stieberova et al., 2017; Muhammad et al., 2021). The Ecoinvent ZnO  
 32 dataset represents the production of zinc oxide out of secondary zinc materials by means of the  
 33 indirect (or French) way (ecoinvent, 2007).  
 34 The inventory of the natural CaPs is listed in Table 2.

36 **Table 2** Life Cycle Inventory of CaP-N production (referred to the FU)

Phase	Input/Output	Flow	Unit	Amount	Dataset
<b>Calcination</b>	I	CaPs	kg	2.25E-02	
	I	Electricity	MJ	1.46E-03	<i>IT: Electricity grid mix (Sphera)</i>
	I	Thermal Energy (natural gas)	MJ	1.17E-01	<i>IT: Thermal energy from natural gas (Sphera)</i>
	I	Thermal Energy (from hot smoke)	MJ	1.35E-01	<i>IT: Thermal energy from natural gas (Sphera)</i>
	O	Calcinated CaPs	kg	9.00E-03	
	O	Smoke flow	m <sup>3</sup>	2.70E-01	
<b>Heat exchanging</b>	I	Hot smoke flow	m <sup>3</sup>	2.70E-01	<i>Primary data</i>
	O	Thermal Energy (from hot smoke)	MJ	1.35E-01	<i>IT: Thermal energy from natural gas (Sphera)</i>
	O	Cold smoke flow	m <sup>3</sup>	2.70E-01	

<b>Fume abatement</b>	I	Cold smoke flow	m <sup>3</sup>	2.70E-01	<i>Primary data</i>
	I	Activated carbon	Kg	3.38E-05	<i>RoW: activated carbon production. granular from hard coal (Ecoinvent 3.5)</i>
	I	Hydrated Lime	kg	3.38E-05	<i>EU-27: Hydrated Lime EuLa (Sphera)</i>
	O	Filtered retained dust	kg	3.11E-05	
	O	Carbon dioxide (fossil)	kg	5.51E-03	
	O	Dust (PM10)	kg	6.75E-08	
	O	Nitrogen oxides	kg	2.41E-05	
	O	Sulphur dioxide	kg	1.96E-06	
<b>Grinding</b>	I	Calcinated CaPs	kg	9.00E-03	<i>Primary data</i>
	I	Electricity	MJ	1.98E-02	<i>IT: Electricity grid mix (Sphera)</i>
	O	CaP-N	kg	9.00E-03	
<b>Landfill solid waste</b>	I	Filtered retained dust	kg	3.11E-05	<i>RoW: treatment of municipal solid waste. sanitary landfill (Ecoinvent 3.7.1)</i>

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38

### 39 *Life cycle impact assessment*

40 The Environmental Footprint (EF 3.0) impact assessment method is used for this study, which lists  
41 16 impact categories inherent to all the most relevant aspects in the environmental field. More  
42 specifically, they are: Acidification (Ac), Climate change (CC), Ecotoxicity freshwater (EcoF),  
43 Eutrophication freshwater (EuF), Eutrophication marine (EuM), Eutrophication terrestrial (EuT),  
44 Human toxicity (cancer and non-cancer, HTc and HTnc), Ionizing radiation (Ir), Land use (Lu), Ozone  
45 depletion (OD), Particulate matter (PM), Photochemical ozone formation (POF), Resource use

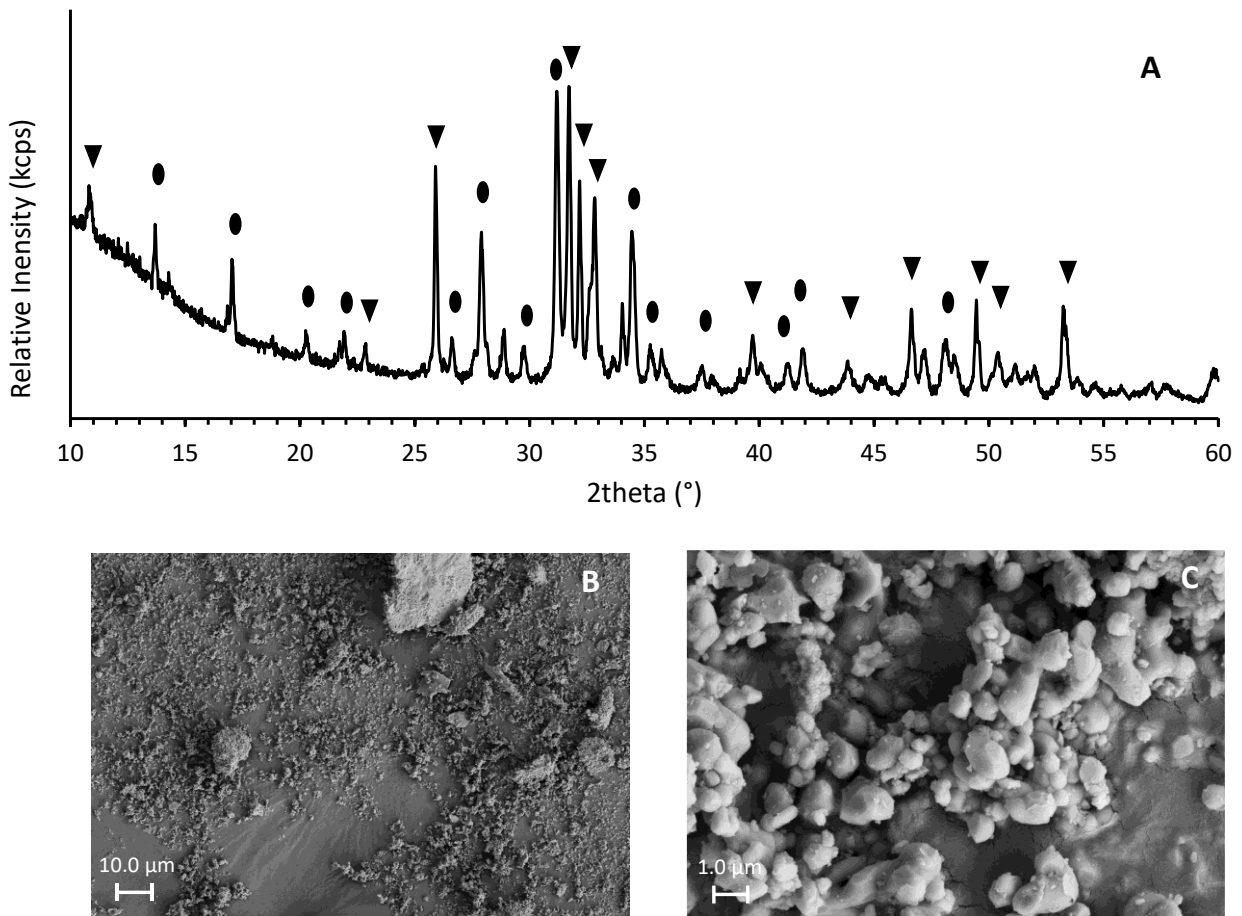
46 (fossils and minerals and metals, ResFos and ResMet) and Water use (Wu). In order to consider also  
47 the energy consumption, the impact category Primary energy demand from renewable and non-  
48 renewable resources, gross calorific value (PED) is added to environmental profile. Impact scores of  
49 the 16 EF impact categories are normalized and weighted using the EF 3.0 factors. Although PED  
50 consumptions are included in the environmental profile, it has not been possible to normalize and  
51 weight them due to the lack of EF 3.0 factors.

52

## 53 **Results and Discussion**

### 54 *Physical-chemical characterization of CaP-N*

55 A comprehensive physical-chemical characterization of CaP-N produced by thermal treatment of  
56 fish meal by-product in an electric furnace has been already reported elsewhere (Adamiano et al.,  
57 2021). Thus, as in the present work the material was produced in a pilot scale incinerator, we report  
58 the XRD profile and SEM micrographs in **Figure 2**, and the chemical composition in **Table 3** to give  
59 an overview of its main physical-chemical characteristics. The XRD analysis indicates that the  
60 material consists of a bi-phasic mixture of  $\beta$ -tricalcium phosphate ( $\beta$ -TCP,  $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>) and  
61 hydroxyapatite (HA, Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>) in the ratio  $\beta$ -TCP:HA of 53:47, as determined by Rietveld  
62 refinement. In the diffraction pattern in Figure 1, peaks matching the reference pattern of HA (ASTM  
63 Card file No. 09-0432), and  $\beta$ -TCP (ASTM Card file No. 09-0169) have been marked with black solid  
64 circles and inverted triangles, respectively. The powder presented a rather uniform morphology,  
65 with round particles having diameter in the range of a few microns. This is different respect to what  
66 typically reported in the literature for biphasic mixtures of HA and  $\beta$ -TCP, where HA is typically in  
67 the form of small rods with a major axis smaller than 1.0  $\mu$ m, and  $\beta$ -TCP in the form of round shaped  
68 microparticles with a diameter in the microns range. This peculiar shape of the materials is likely to  
69 be due to grinding by high-energy ball milling.



71

72 **Figure 2.** XRD pattern (A) and SEM pictures at increasing magnification (B, C) of CaP-N. Black solid  
 73 circles and inverted triangles in inset (A) indicates HA and  $\beta$ -TCP diffraction peaks, respectively. The  
 74 micrograph in inset (B) was collected at 2,500x magnifications, while the one in inset (C) at 25,000x.  
 75  
 76 The chemical composition of CaP-N is reported in Table 3. The Ca/P ratio is 1.40, a value much closer  
 77 to the ratio of stoichiometric  $\beta$ -TCP (1.5) respect to that of stoichiometric HA (1.67). In this case, the  
 78 recorded ratio was even lower than stoichiometric  $\beta$ -TCP because CaPs of biological origin are  
 79 typically non stoichiometric calcium-deficient materials, presenting many ionic substitutions, such  
 80 as  $\text{Na}^+$  and  $\text{Mg}^{2+}$  in place of  $\text{Ca}^{2+}$ . This is confirmed by the relative high amount of Mg detected. The  
 81 concentration of As and Pb, which are indeed among the most toxic elements for aquatic  
 82 ecosystems and human health, are below the limit of detection (LOD) of the employed analytical

83 method (LOD = 1 ppm). On the other hand, Ba and Cr have been detected in low concentrations. As  
 84 the present study envision the use of CaP-N in cosmetic products, it is worthy to note that there is  
 85 not a concentration limit for Ba and Cr in cosmetics, as barium sulfate and chromium (III) hydroxide  
 86 are both permitted for use as opacifying agent and color additives in cosmetics for skin applications,  
 87 respectively (Fuziwara et al., 2004; Kang et al., 2006).

88 **Table 3.** Chemical composition of CaP-N.

Sample	Al (wt%)	As (ppm)	Ba (ppm)	Ca (wt%)	Cr (ppm)	Fe (ppm)	Mg (wt%)	P (wt%)	Pb (ppm)	Sr (wt%)	Zn (wt%)
CaP-N	0.08	<1	19	32.13	6	6	0.65	17.63	<1	0.07	0.02
	±		±	±	±	±	±	±		±	±
	0.01		2	0.77	2	2	0.02	0.25		0.01	0.01

89

90

91 *Toxicity test of CaP-N*

92 The toxicity of CaP-N was screened against 4 different aquatic species, namely *T. fulvus*, *C.*  
 93 *insidiosum*, *G. aequicauda* and *D. tertiolecta*. The validity of the test was assessed by means of a  
 94 negative control using seawater only for each test. In the negative controls, all the tested species  
 95 displayed values of mean percentage of growth or mortality within the indicated limits (see **Table**  
 96 1). To ensure a correct evaluation of the effects recorded in the assays, positive control tests for  
 97 each species were carried out to test the organism's sensitivity to known reference toxicants.

98 For the three crustacean species, the reference toxicant LC50 values were 0.16 mg L<sup>-1</sup> for *T. fulvus*,  
 99 and 1.89 mg L<sup>-1</sup> and 0.82 mg L<sup>-1</sup> for *C. insidiosum* and *G. aequicauda*, respectively. These values fall  
 100 within a range of values already observed in previous studies (Annicchiarico et al., 2007; Narracci et  
 101 al., 2009; Prato and Biandolino, 2006). These three species were chosen as they are considered of

102 high interest in nanoecotoxicology for different reasons. *G. aequicauda* is a bottom filter feeder and  
103 deposit feeder; therefore, it is potentially at high risk of exposure in nature, due to the tendency of  
104 NPs to aggregate in seawater settling down and accumulating in the bottom (Garner and Keller,  
105 2014). *C. insidiosum* is a tube-building species that feeds both on sediment and suspended  
106 particulate matter; *T. fulvus*, on the other hand, lives in the water column and it may be exposed to  
107 NPs contamination and it feeds on microalgae.

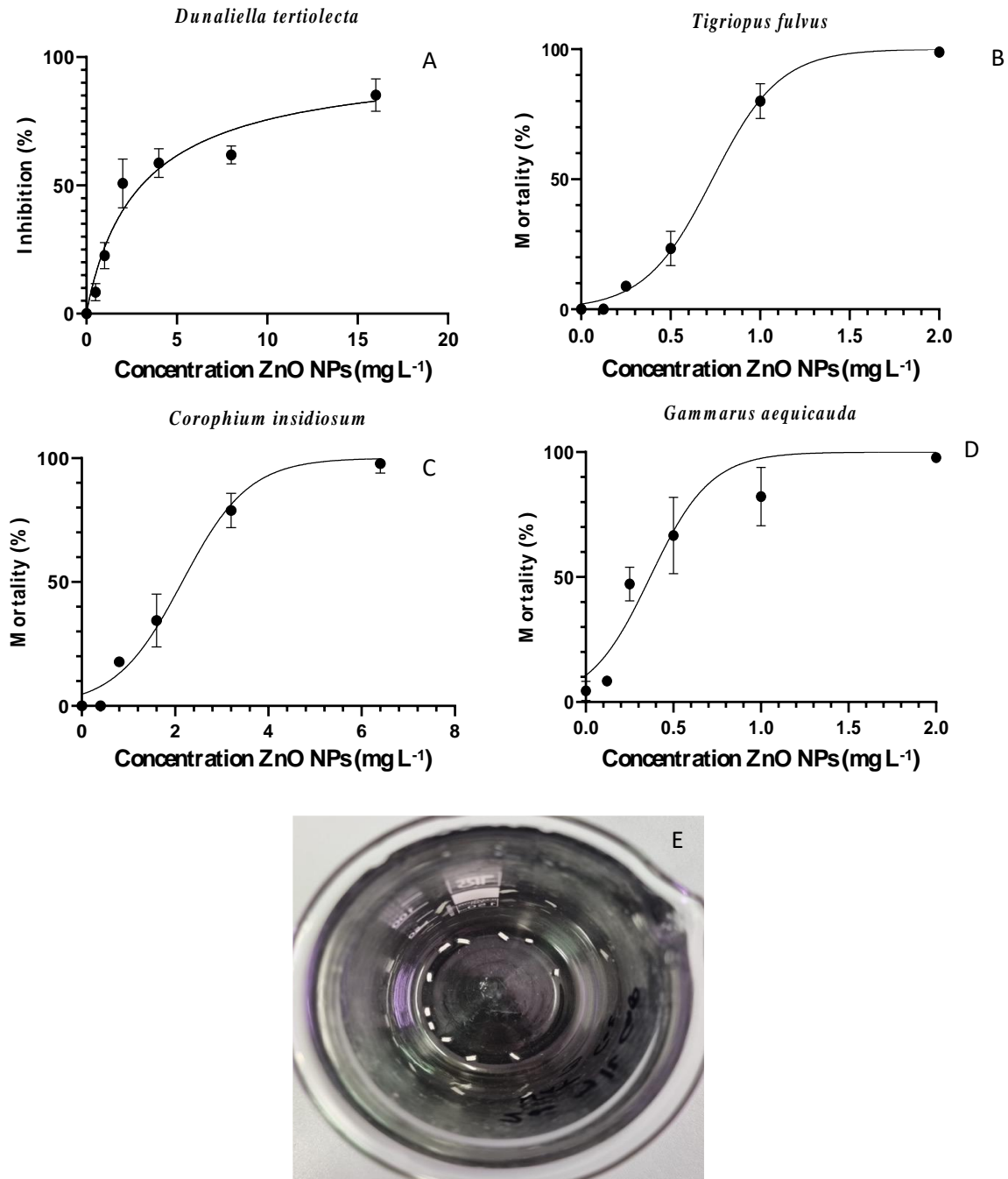
108 The last tested specie is *D. tertiolecta*, a green flagellated microalga which was chosen as it is an  
109 excellent toxicity test organism and valuable biomarker of stress induced by different kind of NPs,  
110 such as Ag NPs (Oukarroum et al., 2012). The results showed that the EC50 value to the reference  
111 toxicant for this specie was  $8.43 \pm 0.85 \text{ mg L}^{-1}$ , indicating a slight increase of sensibility respect to  
112 literature data (from 10.40 to 11.28  $\text{mg L}^{-1}$ ) (Prato et al., 2015).

113 The toxicity tests revealed that CaP-N does not produce any adverse effect in any of the tested  
114 species, all of them showing EC/LC50 values higher than  $100 \text{ mg L}^{-1}$  when tested against this  
115 material.

116

117 According to the EC-Directive 93/67/EEC (European Commission 1993), substances with EC/LC50  
118 values higher than  $100 \text{ mg L}^{-1}$  are not considered as harmful to aquatic organisms, so it possible to  
119 affirm that the CaP-N used in this study is not toxic to the marine species utilized.

120 In view of the possible substitution of ZnO NPs by CaPs in cosmetic products envisioned in this work,  
121 the dose-response curves obtained by exposing the tested species to commercial ZnO NPs have  
122 been recorded and are reported in **Figure 3(A-D)**. On the other hand, during the 96 h acute toxicity  
123 test on *C. insidiosum*, which is a tube-building species, as visible in **Figure 3E**, specimens built their  
124 tubes with the available CaP-N, further attesting its non-toxicity.



125

126 **Figure 3.** Dose-response curves obtained by exposing *D. tertiolecta* (A), *T. fulvus* (B), *C. insidiosum* (C)

127 and *G. aequicauda* (D) at increasing concentrations of ZnO NPs, and a picture of tubes of natural

128 CaPs built with CaP-N by the amphipod *Corophium insidiosum* (E).

129

130 The results of the acute toxicity tests for both ZnO NPs and CaP-N are summarized in **Table 4**. The

131 growth of the algal population was clearly affected by the presence of ZnO NPs, showing a EC50



132 similar to the one reported by Manzo et al. who found a EC50 value of 2.42 mg L<sup>-1</sup> for the same  
133 material (Manzo et al., 2013). Also for *T. fulvus*, the LC50 values recorded after 96 h of exposure to  
134 ZnO NPs are similar to those reported for the same species in an 48-h acute test (1.27 mg L<sup>-1</sup>) (Prato  
135 et al., 2020). On the other hand, Wong et al. (2010) and Park et al. (2014) indicated for *Tigriopus*  
136 *japonicus* a 96-h LC50 of 0.85 and 2.44 mg L<sup>-1</sup>, respectively (Park et al., 2014; Wong et al., 2010).  
137 Moreover, it is worthy to note that the chronic exposure (28 days) of *T. fulvus* to ZnO NPs cause  
138 negative effects on the reproductive traits, i.e. brood duration, brood size and brood number per  
139 female at much lower concentrations ( $\geq 100 \mu\text{g L}^{-1}$ ).

140 ZnO NPs elicited high toxicity to *C. insidiosum* and *G. aequicauda*, with very low LC50 concentrations.  
141 A previous study reported for *C. insidiosum* exposed to ZnO NP, an LC50 concentration within a  
142 range of 1.42 - 2.18 mg L<sup>-1</sup>, which is higher than the one recorded in this study (Vimercati et al.,  
143 2020). On the other hand, a similar LC50 value to the one here reported was found by Prato et al.  
144 for *G. aequicauda* (Prato et al., 2021).

145 Overall, the sensitivity to ZnO NPs of the examined species is as follows: *G. aequicauda* > *T. fulvus* >  
146 *C. insidiosum* > *D. tertiolecta*, where the lower sensitivity of *D. tertiolecta* to ZnO is probably due to  
147 the tendency of NPs to aggregate in seawater and deposit on the bottom of the flask as an effect of  
148 the very high concentration of NaCl, reducing their availability.

149 Finally, this study highlights the potential environmental benefit that can be achieved by using CaPs  
150 instead of ZnO NPs in cosmetic products, as the occurrence of this latter NPs in marine environments  
151 can have adverse effect on several organisms, even at low concentrations.

152

153

154 **Table 4. Acute toxicity test.** Results are expressed as median lethal concentration (EC50/LC50) in  
 155 mg L<sup>-1</sup> of the tested organisms exposed to ZnO NPs and natural and CaP-N, respectively. LL lower  
 156 limit and UP upper limit for a confidence interval of 0.95.

Aquatic species	Material	EC50/LC50	LL	UP
<i>D. tertiolecta</i>	ZnO NPs	3.04 ± 0.20	2.46	3.78
<i>T. fulvus</i>	ZnO NPs	0.68 ± 0.10	0.64	0.73
<i>C. insidiosum</i>	ZnO NPs	1.93 ± 0.05	1.74	2.13
<i>G. aequicauda</i>	ZnO NPs	0.32 ± 0.16	0.27	0.73
<i>D. tertiolecta</i>	CaPs	>100	-	-
<i>T. fulvus</i>	CaPs	>100	-	-
<i>C. insidiosum</i>	CaPs	>100	-	-
<i>G. aequicauda</i>	CaPs	>100	-	-

157

#### 158 *Environmental performance evaluation through LCA*

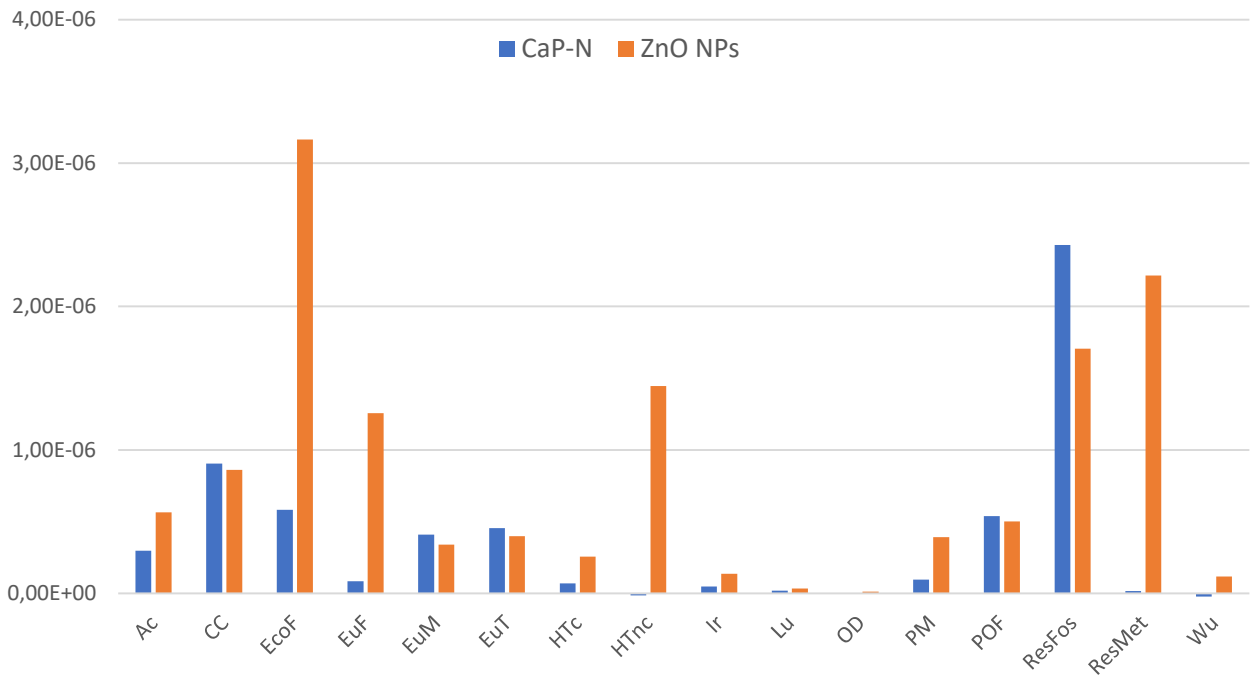
159 LCA results of CaP-N and ZnO NPs on the 16 impact categories before and after normalization are  
 160 shown in **Table 5** and **Figure 4**, respectively. Note that Table 5 lists also PED consumptions. Impact  
 161 scores of the two products are of the same order of magnitude for about half of the impact  
 162 categories except Eutrophication, freshwater; Human toxicity, cancer; Human toxicity, non-cancer;  
 163 Ionising radiation; Ozone depletion; Resource use, mineral and metals; and Water use. In these  
 164 cases, the impact of CaP-N is lower than that of ZnO NPs by at least an order of magnitude. On the  
 165 other side, the use of fossils resources represents the only impact category in which CaP-N has a  
 166 significantly higher impact than ZnO NPs (+142%).

167

168

**Table 5** Impact scores of CaP-N and ZnO NPs production resulting from LCA.

Impact categories	Units	CaP-N	ZnO NPs
Acidification	mol H <sup>+</sup> eq	1.65E-05	3.14E-05
Climate Change	kg CO <sub>2</sub> eq	7.33E-03	6.98E-03
Ecotoxicity, freshwater	CTUe	2.49E-02	1.35E-01
Eutrophication, freshwater	kg P eq	1.37E-07	2.02E-06
Eutrophication, marine water	kg N eq	8,01E-06	6.65E-06
Eutrophication, terrestrial	kg N eq	8.06E-05	7.06E-05
Human toxicity, cancer	CFUh	1.18E-12	4.32E-12
Human toxicity, non-cancer	CFUh	-3.18E-12	3.32E-10
Ionising radiation	kBq U235 eq	2.02E-04	5.80E-04
Land Use	Pt	1.62E-02	2.75E-02
Ozone depletion	kg CFC11 eq	5.38E-12	6.44E-10
Particulate matter	Disease incidences	5.77E-11	2.33E-10
Photochemical ozone formation	kg NMVOC eq	2.19E-05	2.04E-05
Resource use, fossils	MJ	1.58E-01	1.11E-01
Resource use, mineral and metals	kg Sb eq	1.00E-09	1.41E-07
Water use	m <sup>3</sup> world eq	1.02E-03	1.34E-03
Primary energy demand	MJ	2.15E-01	1.27E-01



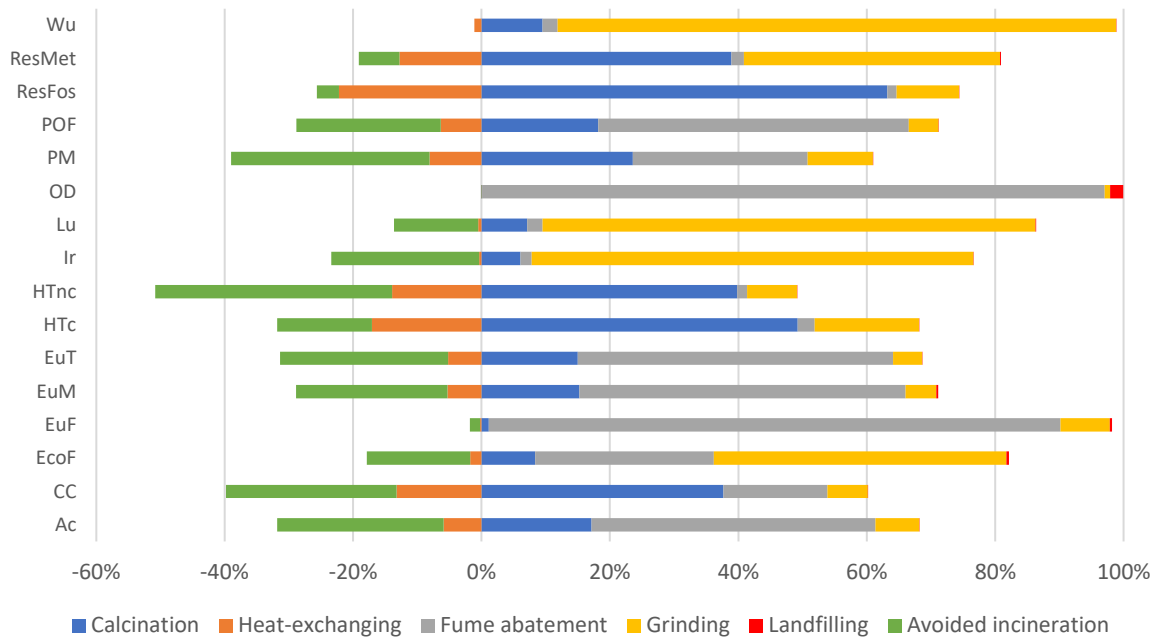
**Figure 4.** Normalized LCA results of CaP-N and ZnO NPs production.

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175 Figure 5 shows the relative contributions to the 16 selected impact categories of the four CaP-N  
 176 production steps, dust landfilling and avoided incineration. As it is possible to observe, calcination  
 177 step is responsible for the major contribution to the impact categories Climate Change, Human  
 178 toxicity and Resource use (fossils). Grinding has high impacts on Ecotoxicity, Ionising radiation, Land  
 179 Use, Resource use (mineral and metals) and Water Use. Finally, smoke abatement step contributes  
 180 predominately to Acidification, Eutrophication (freshwater, marine and terrestrial), Ozone  
 181 depletion, Particulate matter, and Photochemical ozone formation. On the other hand, the avoided  
 182 disposal of bone waste generates significant environmental credits (higher than 30%) for the impact  
 183 categories Acidification, Climate change, Eutrophication marine and terrestrial, Human toxicity,  
 184 non-cancer and Particulate matter. Relevant are also credits in several impact categories due to  
 185 heat recovery (Climate Change, Human Toxicity, Resource Use). Landfilling contribution is  
 186 observable only on Ozone Depletion.



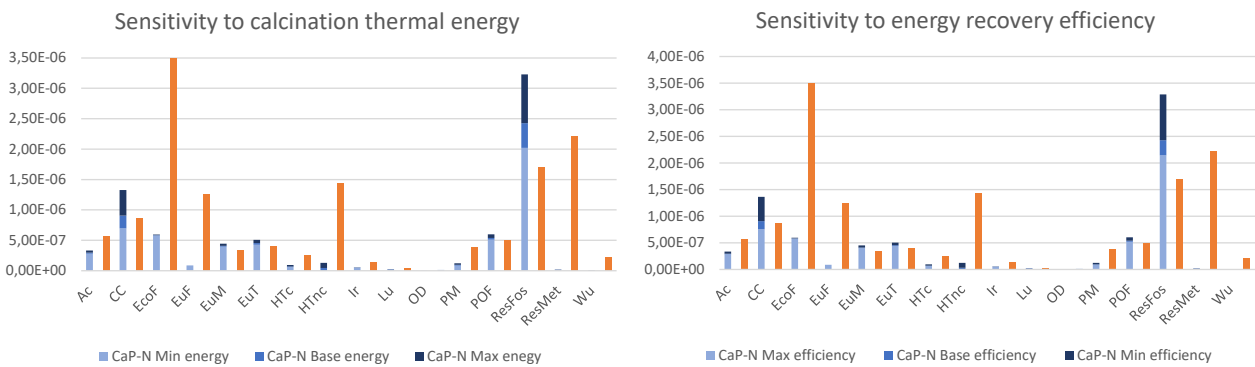
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189 **Figure 5.** Percentage contributions of the CaP-N production steps to the impact categories of the  
 190 LCA study.

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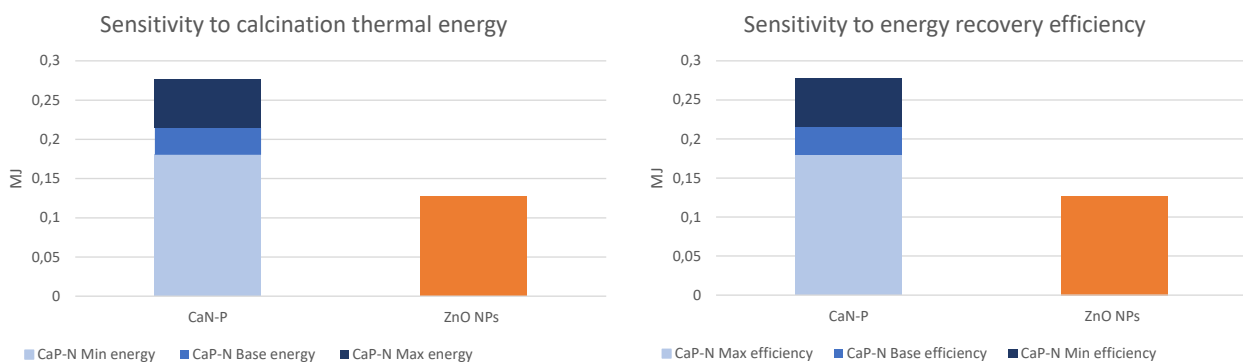
192 A sensitivity analysis was done on the two parameters of LCA model affected by the major  
 193 uncertainty: the thermal energy needed for calcination and the thermal energy recovered by the  
 194 heat-exchanger. In the case of the thermal energy necessary to calcinate the fish meal,  $28 \text{ MJ/kg} \pm$   
 195  $20\%$  was considered for the sensitivity analysis, on the base of experimental tests. For the heat-  
 196 exchanger, a minimum and a maximum recovery of  $40\%$  and  $60\%$ , respectively, were assumed with  
 197 respect to the base-value of  $55\%$  (due to the combination of exchanger and economizer). In this  
 198 case, the minimum value was set considering only exchanger without economizer while the  
 199 maximum value was set hypothesizing an improving in the heat exchanger. The possibility of  
 200 recovering heat through flue gas condensation has not been investigated but it could lead to a  $90\%$   
 201 of heat recovery. Figure 6 shows the results of the sensitivity analysis on the 16 EF impact categories,  
 202 results of ZnO NPs are also reported (all impact scores are normalized). As shown in Figure 6, Climate

203 change and Use of Fossils Resources are the two categories most affected by the parameters  
 204 analyzed. Figure 7 shows the results of the sensitivity analysis on PED required by CaN-P production  
 205 process, the comparison with ZnO-NPs is maintained. In this case, sensitivity analysis results have  
 206 been shown on not normalized impact scores.



207  
 208 **Figure 6.** Results of the sensitivity analysis of the CaN-P model to thermal energy consumption for  
 209 calcination (on the left) and to the efficiency of the heat-exchanger (on the right): light blue  
 210 represents the minimum energy consumption; blue represents the energy consumption of the base  
 211 scenario and dark blue represents maximum energy consumption. For a better comparison,  
 212 environmental profile of ZnO NPs after normalization is shown too (in orange).

213



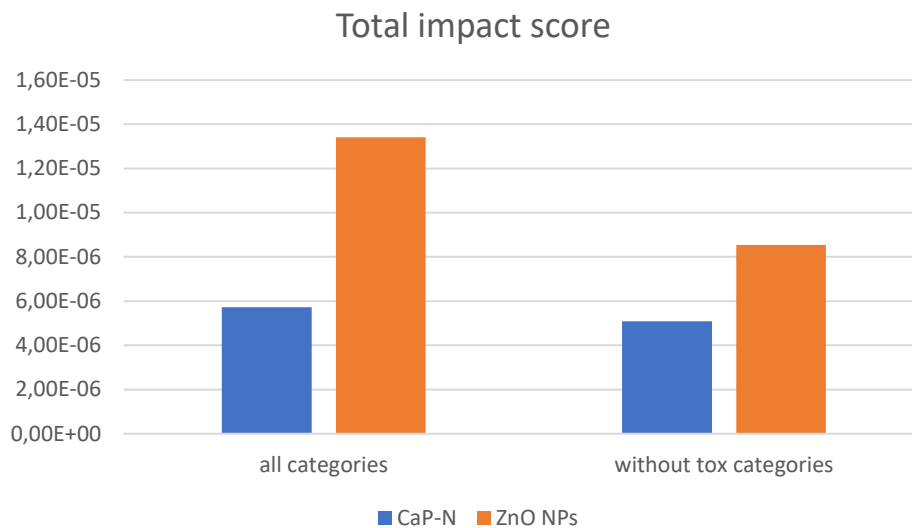
214  
 215 **Figure 7.** Results of the sensitivity analysis of PED of CaP-N production to thermal energy  
 216 consumption for calcination (on the left) and to the efficiency of the heat-exchanger (on the right):  
 217 light blue represents the minimum energy consumption; blue represents the energy consumption

218 of the base scenario and dark blue represents maximum energy consumption. For a better  
219 comparison, PED of ZnO NPs is shown too (in orange).

220

221

222 Finally, we have weighted the normalized impact scores of the environmental impacts of CaP-N and  
223 ZnO NPs production in order to facilitate the comparison. As it is possible to observe from Figure 8,  
224 CaP-N shows a better performance than ZnO NPs both when all impact categories are considered  
225 and in the case that toxicity categories are excluded (as recommended by PEF guidance; EC, 2018).



226

227 **Figure 8.** Comparison between the environmental impact of CaP-N and ZnO NPs production after  
228 normalization and weighting.

229

230 LCA results demonstrate that the environmental performance of CaP-N is better or comparable than  
231 that of ZnO NPs for the majority of the impact categories analyzed in this study. In the majority of  
232 cases, even when CaP-N has a higher impact than ZnO NPs the difference is almost negligible, except  
233 for Resources Use and Primary energy demand. Another essential element worth highlighting is the  
234 fact that primary inventory data for CaP-N production comes from a pilot plant. This means that its

235 environmental impacts, especially for Climate Change, could be further improved on the industrial  
236 scale by means of process and material efficiency enhancement.

237 As explained in “Inventory analysis” section, in this work, CaP-N has been compared with a proxy of  
238 ZnO NPs. In order to make the work as much complete as possible, a comparison among the  
239 environmental performances of CaP-N and ZnO NPs obtained by other studies has been attempted.

240 It is noteworthy that this comparison is made difficult by the use of different impact assessment  
241 methods that only made possible the comparison among the impact score concerning climate  
242 change, always assessed as kg of CO<sub>2</sub> eq emissions. Considering 1 kg of product, the impact score  
243 on global warming obtained in this study is 0.81 and 0.78 kg of CO<sub>2</sub> eq for CaP-N and ZnO,  
244 respectively. These values are much lower than the ones (33 and 21 kg of CO<sub>2</sub>eq/kg, respectively)  
245 reported for two ZnO production processes by Stieberova et al. (2019) and also much lower than  
246 the 25 kg of CO<sub>2</sub> eq/kg reported by Stieberova et al. (2017). These outcomes lead to two conclusions:  
247 (i) CaP-N from by-products obtained by calcination and grinding appears environmental interesting  
248 as substitute of ZnO NP, and (ii) the use of ZnO as proxy of ZnO NPs is conservative.

249 Overall, CaP-N represents a promising and more sustainable alternative to ZnO NPs in cosmetic  
250 products. Moreover, its conception as value-added product obtained from waste is perfectly in line  
251 with the circular economy and waste reduction concepts encouraged by EU legislation.

252

## 253 **Conclusions**

254 Natural calcium phosphates produced from the thermal extraction of fish bones (CaP-N) have been  
255 obtained by using a pilot-scale incineration plant. The material consists of a biphasic mixture 53:47  
256 of hydroxyapatite:tricalcium phosphate and is made of round particles having diameter in the range  
257 of a few microns. Differently from ZnO nanoparticles (NPs) commonly used in cosmetic products,  
258 CaP-N was found to be nor toxic nor ecotoxic as it was highly tolerated by 4 different aquatic species,



259 namely *T. fulvus*, *C. insidiosum*, *G. aequicauda* and *D. tertiolecta*. Life cyclic assessment (LCA) of CaP-  
260 N production from fish meal incineration was carried out and the outcomes were compared to that  
261 coming from LCA of ZnO, which was used as a proxy of ZnO NPs. LCA results demonstrate that the  
262 environmental performance of CaP-N is better than that of ZnO NPs for the majority of the 16 impact  
263 categories analyzed in this study, . The use of fossil resources represents the only impact category  
264 in which CaP-N has a significantly higher impact than ZnO NPs (+140%). It is noteworthy that  
265 normalization and weighting show that CaP-N production appears less impactful than ZnO NPs.  
266 However, as these data refers to a pilot-scale production, we envision that the process could be  
267 further improved at the industrial production scale to have a lower environmental impact. Finally,  
268 in this work we proved that CaP-N could actually increase the sustainability of cosmetic by increasing  
269 their safety for both human health and the environment, and by decreasing their impact on the  
270 environment.

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