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Published Version:
Righi, S., Prato, E., Magnani, G., Lama, V., Biandolino, F., Parlapiano, I., et al. (2023). Calcium phosphates from fish bones in sunscreen: An LCA and toxicity study of an emerging material for circular economy. SCIENCE OF THE TOTAL ENVIRONMENT, 862, 1-12 [10.1016/j.scitotenv.2022.160751].

Availability:
This version is available at: https://hdl.handle.net/11585/963006 since: 2025-01-13

Published:
DOI: http://doi.org/10.1016/j.scitotenv.2022.160751

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

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Abstract

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

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

Introduction

During the last 30 years, new green and sustainable chemicals capable to replace dangerous or brown ones have been sought by researchers worldwide. Among the plethora of alternative materials, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$ – HA) and more in general calcium phosphates (CaPs) have gained high interest due to their wide range of applications and to their intrinsic bio and eco-compatibility (Yoon et al., 2020). CaPs are a family of materials that are ubiquitous in the biosphere, as they can be found in water bodies, soils, vegetables and animals, where they mainly constitute the mineral part of bones and teeth. They are widely recognized as biologically safe and are considered standard materials for biomedical applications (Dorozhkin, 2021); moreover, they are employed in many other fields (Degli Esposti et al., 2022) (Adamiano et al., 2019; Pérez-Álvarez et al., 2021; Ghosh et al., 2022) including cosmetic (Carella et al., 2021a). .

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

Several works have proposed a circular economy approach for the extraction of chemicals from several by-products deriving from macroalge, sea urchin and shrimps to be used in cosmetic as excipients, additives, preservatives, anti-aging and whitening agents, and photoactive ingredients (Lourenco-Lopes et al., 2020; Zilia et al. 2021; King et al. 2017). Among them, an increasing number of papers reports on the use of CaPs extracted from fish bones as photo-protective materials, where they are claimed to be more eco-compatible and safer respect to UV-filters commonly used in commercial products (Piccirillo et al., 2014) (Teixeira et al., 2017). CaPs have been also proposed as boosters of the Sun Protection Factor (SPF), i.e., as materials that do not hold intrinsic UV-filtering properties but have the ability to increase the SPF by scattering light and increase the adsorption by UV-filters occurring in the formulation. In this case, the advantage of using CaPs is reducing the concentrations of UV-filters as these chemicals have been in the spotlight for their controversial toxic and reef-damaging potential (Moeller et al., 2021).

Among UV-filters, the inorganic ones such as zinc and titanium oxides (ZnO and TiO_2) are generally considered safer respect to organic ones; nonetheless, their occurrence in cosmetic products is a matter of concern for consumers and the environment as they are typically used in the form of nanoparticles (NPs) (Musial et al., 2020). In fact, it has been reported that NPs can penetrate through the epidermis into the body in case of damaged or diseased skin (Monteiro-Riviere et al., 2011). Moreover, since increasing concentrations of TiO_2 and ZnO NPs derived from sunscreens in the environment have been detected, attention towards potential drawbacks for both aquatic and terrestrial ecosystem is increasing (Labille et al., 2020) (Gondikas et al., 2018) (Gomes et al., 2021). A number of studies showed the negative effects of ZnO and TiO_2 NPs used in sunscreen on stony corals in reefs across the world, with coral bleaching being significantly greater after ZnO NPs exposure compared with TiO_2 NPs, calling for further investigation of the potential harms of these NPs on marine ecosystems (Corinaldesi et al., 2018).

Overall, despite CaPs are widely renowned as highly biocompatible materials for humans, their potential toxicity on aquatic species, such as gastropods or crustaceans populating aquatic environments, has not been properly investigated. Moreover, whether the production and use of CaPs extracted from fish bones in cosmetic would be beneficial for the environment remains unclear as incineration, calcination or pyrolysis, which are the most employed methods for CaPs recovery from circular economy due to their low cost and large-scale applicability (Carella et al., 2021b), are also high energy consuming processes, and their environmental footprint may pose serious issues on the long-term sustainability of natural CaPs. In fact, despite natural materials are generally perceived as safer respect to synthetic ingredients in cosmetics, sustainability claims need to be supported by data and by in depth analysis of related environmental impact using a cradle-to-grave approach (Ögmundarson et al., 2020). In this light, it is important to carry out a life cycle assessment (LCA) to quantify the impacts and assess the potential environmental benefits that could be achieved by replacing chemicals with CaPs recovered from fish by-products by thermal treatments. Indeed, the importance of LCA of emerging nanotechnologies has been well recognized and LCA has been widely used over the years to evaluate the eco-friendliness of NPs production and to compare different nanomanufacturing processes. One first LCA of TiO_2 microparticles was performed as early as in 1999 on two mature processes (Reck and Richards 1999). Later, Grubb and Bakshi (2010) applied LCA to compare a mature and new method for TiO_2 NPs synthesis (sulphate process vs hydrochloride process). In 2015, the environmental performance of the hydrolytic sol–gel synthesis of anatase TiO_2 NPs was evaluated using the LCA methodology (Pini et al., 2015) and more recently, LCA was also applied to methods for ZnO NPs production (Stieberova et al., 2017 and 2019). And the list is far from being exhaustive.

Our work reports on the toxic effect of natural CaPs extracted from fish bones (CaP-N) on a battery of four species belonging to different trophic levels (primary producers and consumers), including algae (*Dunaliella tertiolecta*), copepoda (*Tigriopus fulvus*), and crustaceans amphipoda (*Corophium insidiosum and Gammarus aequicauda*). These species were selected because they are established model species in standardized toxicity tests, ecotoxicological studies and in ecological risk assessment (Annicchiarico et al., 2007; Faraponova et al., 2016; Manzo et al., 2013; Narracci et al., 2009; Prato and Biandolino, 2006). Moreover, to quantify the environmental burdens linked to the virtual substitution or reduction of UV-filters by natural CaPs, a LCA was carried out comparing CaPs extracted from fish bones with ZnO as a proxy of ZnO NPs which are nowadays considered as the safest UV-filter and, unlike TiO_2 NPs, do not need photo-passivation to be employed in in suncare products (Labille et al. 2020).

Materials

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

CaPs production and characterization

CaPs extraction from fish bones

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

CaPs physical-chemical characterization

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

The elemental analysis of CaP-N was carried out by inductively coupled plasma atomic emission (ICP-OES) spectrometer (Liberty 200; Varian, Palo Alto, CA, USA). 20 mg of samples were added to 15 mL of HNO_3 solution at 9.0 wt.% and placed in an ultrasonic bath sonicator at 37 °C until complete dissolution. Solutions were then cooled at room temperature and added with water up to 100.0 mL before ICP-OES analysis. Ca and P concentrations were measured by their atomic emission at the following wavelengths: 422.673 nm for Ca and 213.618 nm for P. Moreover, the occurrence of relevant toxic and ecotoxic elements such as As, Ba, Cr, Pb and Zn and was screened. Finally, field emission gun scanning electron (FEG-SEM) microscopy employing a Sigma NTS Gmbh (Carl Zeiss, Oberkochen, Germany) was used to evaluate the morphology and the dimension of the samples. Powders were mounted on aluminium stubs using carbon tape and sputter coated with gold in a Sputter Coater E5100 (Polaron Equipment – Watford, Hertfordshire, UK) under argon at 10^{-3} mbar for 4 minutes with a sputtering current of 30 mA. Energy dispersive X-ray spectroscopy data (EDS, spectra, and maps) were collected using an accelerating voltage of 15.0 kV and a working distance of 10 mm by an INCA Energy 300 (Oxford instruments, Oxford, UK) coupled to the FEG-SEM microscope.

Toxicity testing

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

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

Species →	***D. tertiolecta***	***T. fulvus***	***C. insidiosum***	***G. aequicauda***
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
Aereation	Absent	Absent	Present	Present
Reference toxicant	$K_2Cr_2O_7$	$CuSO_4 \cdot 5H_2O$	$Cd(NO_3)_2$	$Cd(NO_3)_2$

Concentration of Ref. Toxicant	2.5-5-10-20-40 (Cr mg L^{-1})	0.03-0.06-0.12-0.25-0.5 (Cu mg L^{-1})	0.2-0.4-0.8-1.6-3.2 (Cd mg L^{-1})	0.2-0.4-0.8-1.6-3.2 (Cd mg L^{-1})
Testing volume	10 mL	3 mL	700 mL	700 mL
Bioassay duration	72h	96h	96h	96h
N° organisms/replicate	10000 L^{-1}	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)

Preparation of testing media

Stock solutions of CaP-N were prepared by dispersing 1.0 g of the dry powders into 1.0 L of 0.22-µm filtered natural seawater (FNSW) plus Tween 20 (diluted at 10%) surfactant in order to obtain a good sample dispersion. The surfactant was added for *D. tertiolecta* and *T. fulvus*'s test in a low concentration (10 µl L^{-1}) that did not affect algal cell growth and nauplii (Beiras et al., 2018; Oliviero et al., 2019).

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

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

Dunaliella tertiolecta

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

The algal growth assay was performed according to ISO 10253 standard procedures (ISO, 2006a), modified by using multi-well plates instead of glass flasks (Lukavský et al., 2011). Algal cells were cultured in artificial seawater Instant Ocean® with complete F2 culture medium (Guillard and Ryther, 1962) at 20.0 ± 0.5 C with a 12:12 h light:dark cycle and light intensity of 6000-10,000 lux (Sbrilli et al., 1997). An algal suspension sampled during the phase of exponential growth was prepared. Initial cellular concentration of 1.5×10^4 was adopted for growth inhibition assay. The test was carried out in triplicate, with 5 serial dilutions: 0.01-0.1-1-10-100 mg L^{-1}. Sterile polystyrene multi-well plates with 6 wells each with a capacity of 10.0 ml were used as support. Filtered artificial seawater (0.45-µm Millipore® membrane) was used in toxicity assays and for dilution of standard samples of reference toxicant. In parallel, a test was performed with a control (6 replicates) consisting of the growth medium only and a positive control using potassium dichromate in five scalar concentrations, namely 2.5, 5, 10, 20, and 40 mg Cr L^{-1}.

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

At the end of bioassay, cultures were stopped using a drop of Lugol's solution and the cell density of each replicate was measured using a Burker's counting cell. ~~Then t~~ The inhibition/stimulation of growth of the algal biomass was calculated using comparisons of growth rates of the algal cultures in tested solutions and the control. During all assays, the initial and final pH values were registered in all the samples (Crison, MicropH 2001, electrode Hamilton, Slimtrode). For all bioassays, the pH drift was less than 0.5 pH unit over 72 h.

Tigriopus fulvus

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

Briefly, ten organisms were placed into each well of multi-well plates (5 mL per well), containing 3.0 mL of different concentrations of CaP-N and ZnO NPs (Table 1) and incubated, for 96 h, at 20.0 ± 0.5 °C in dark conditions. $CuSO_4 \times 5 H_20$ were selected as reference toxicants for copepods nauplii to ensure the validity of test. All tests were performed in triplicates. After 96 h, lethality was the endpoint assessed. The number of dead organisms was counted under a stereomicroscope: larvae that were completely motionless were counted as dead organisms. Copepods were considered dead if any movement of external appendages was registered for about 20 seconds in response to mechanical stimulation and the percentage of mortality was calculated compared to control.

Oxygen content and pH of the test media were measured at the beginning and at the end of each test in the replicates of both control and testing solutions.

Gammarus aequicauda and Corophium insidiosum

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

The general design of toxicity tests was based on the standard guides for conducting acute sediment toxicity tests with marine–estuarine amphipods, with some modifications (ASTM 1993; SETAC 1993). Juveniles (2-4 mm) of *C. insidiosum* and *G. aequicauda* were exposed for 96 h to six different concentrations of CaP-N and five of ZnO NPs (Table 1). For both species, three replicates were prepared per treatment and twenty individuals per concentration were randomly added to 1 L beakers containing 700 ml of test solution. During test exposure, beakers were maintained into a test chamber at 18 ± 2 °C with a 12:12 h light:dark cycle. At the end of the test, the content of each beaker was sieved, and the survivors were counted. Apparently dead individuals were considered living if movement was exhibited after gentle stimulation. In order to have a reference measure for the acute toxicity assessment, a negative control test to determine the response of the animals in the absence of contaminant, and a positive control test with cadmium nitrate (Cd $(NO_3)_2$ as reference toxicant in a range of concentrations within 0.4 to 3.2 mg L^{-1}, were performed.

Statistical analysis

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

Life cycle assessment

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

Goal and scope definition

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

Given that the raw material for CaP-N is a residual product of fish meal production destined for final disposal, the residues of fish meal production phases were not taken into account, according to the zero-burden approach. On the contrary, environmental credits were considered for the avoided incineration of this residual product. Four main processes of CaP-N production were included in the assessment: (i) calcination of fish meal, (ii) heat exchanging, (iii) fume abatement and (iv) grinding. Landfilling of retained dust via fume abetment was added. **Figure 1** shows the flow diagram of the whole process.

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

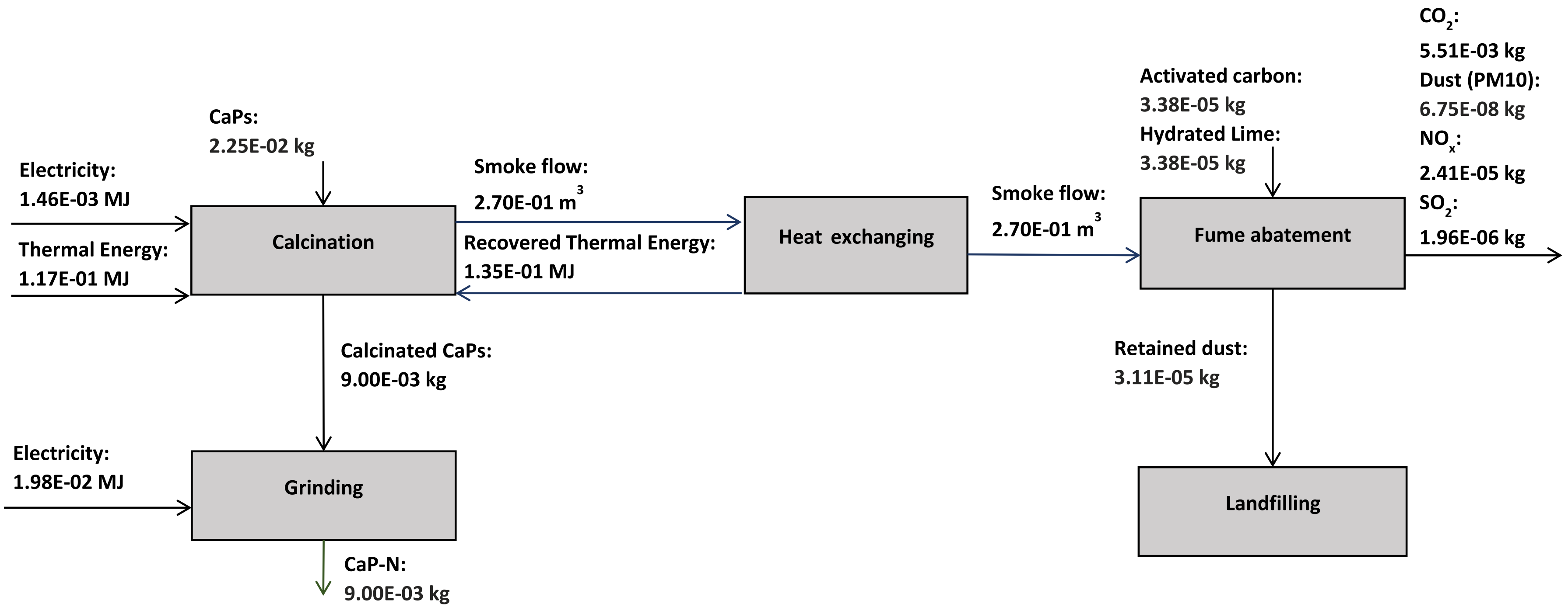

Figure 1. Flow diagram of the CaP-N production process.

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

The inventory of the natural CaPs is listed in Table 2.

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

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

Fume abatement	I	Cold smoke flow	m^3	2.70E-01	*Primary data*
	I	Activated carbon	Kg	3.38E-05	*RoW: activated carbon production. granular from hard coal (Ecoinvent 3.5)*
	I	Hydrated Lime	kg	3.38E-05	*EU-27: Hydrated Lime EuLa (Sphera)*
	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	
Grinding	I	Calcinated CaPs	kg	9.00E-03	*Primary data*
	I	Electricity	MJ	1.98E-02	*IT: Electricity grid mix (Sphera)*
	O	CaP-N	kg	9.00E-03	
Landfill solid waste	I	Filtered retained dust	kg	3.11E-05	*RoW: treatment of municipal solid waste. sanitary landfill (Ecoinvent 3.7.1)*

Life cycle impact assessment

The Environmental Footprint (EF 3.0) impact assessment method is used for this study, which lists 16 impact categories inherent to all the most relevant aspects in the environmental field. More specifically, they are: Acidification (Ac), Climate change (CC), Ecotoxicity freshwater (EcoF), Eutrophication freshwater (EuF), Eutrophication marine (EuM), Eutrophication terrestrial (EuT), Human toxicity (cancer and non-cancer, HTc and HTnc), Ionizing radiation (Ir), Land use (Lu), Ozone depletion (OD), Particulate matter (PM), Photochemical ozone formation (POF), Resource use (fossils and minerals and metals, ResFos and ResMet) and Water use (Wu). In order to consider also the energy consumption, the impact category Primary energy demand from renewable and non-renewable resources, gross calorific value (PED) is added to environmental profile. Impact scores of the 16 EF impact categories are normalized and weighted using the EF 3.0 factors. Although PED consumptions are included in the environmental profile, it has not been possible to normalize and weight them due to the lack of EF 3.0 factors.

Results and Discussion

Physical-chemical characterization of CaP-N

A comprehensive physical-chemical characterization of CaP-N produced by thermal treatment of fish meal by-product in an electric furnace has been already reported elsewhere (Adamiano et al., 2021). Thus, as in the present work the material was produced in a pilot scale incinerator, we report the XRD profile and SEM micrographs in **Figure 2**, and the chemical composition in **Table 3** to give an overview of its main physical-chemical characteristics. The XRD analysis indicates that the material consists of a bi-phasic mixture of β-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$) and hydroxyapatite (HA, $Ca_{10}(PO_4)_6$)) in the ratio β-TCP:HA of 53:47, as determined by Rietveld refinement. In the diffraction pattern in Figure 1, peaks matching the reference pattern of HA (ASTM Card file No. 09-0432), and β-TCP (ASTM Card file No. 09-0169) have been marked with black solid circles and inverted triangles, respectively. The powder presented a rather uniform morphology, with round particles having diameter in the range of a few microns. This is different respect to what typically reported in the literature for biphasic mixtures of HA and β-TCP, where HA is typically in the form of small rods with a major axis smaller than 1.0 µm, and β-TCP in the form of round shaped microparticles with a diameter in the microns range. This peculiar shape of the materials is likely to be due to grinding by high-energy ball milling.

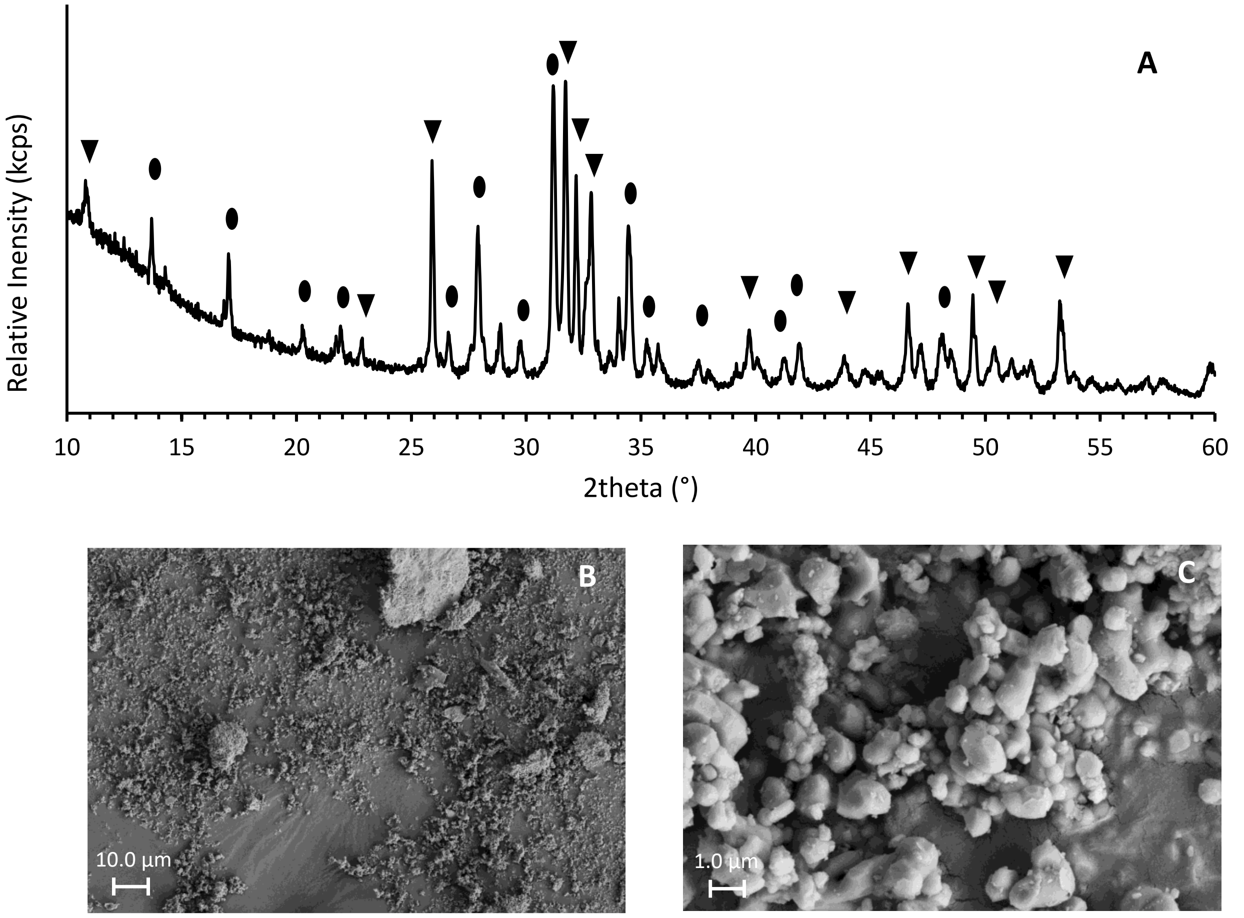

Figure 2. XRD pattern (A) and SEM pictures at increasing magnification (B, C) of CaP-N. Black solid circles and inverted triangles in inset (A) indicates HA and β-TCP diffraction peaks, respectively. The micrograph in inset (B) was collected at 2,500x magnifications, while the one in inset (C) at 25,000x.

The chemical composition of CaP-N is reported in Table 3. The Ca/P ratio is 1.40, a value much closer to the ratio of stoichiometric β-TCP (1.5) respect to that of stoichiometric HA (1.67). In this case, the recorded ratio was even lower than stoichiometric β-TCP because CaPs of biological origin are typically non stoichiometric calcium-deficient materials, presenting many ionic substitutions, such as Na^+ and Mg^{2+} in place of Ca^{2+}. This is confirmed by the relative high amount of Mg detected. The concentration of As and Pb, which are indeed among the most toxic elements for aquatic ecosystems and human health, are below the limit of detection (LOD) of the employed analytical method (LOD = 1 ppm). On the other hand, Ba and Cr have been detected in low concentrations. As the present study envision the use of CaP-N in cosmetic products, it is worthy to note that there is not a concentration limit for Ba and Cr in cosmetics, as barium sulfate and chromium (III) hydroxide are both permitted for use as opacifying agent and color additives in cosmetics for skin applications, respectively (Fuziwara et al., 2004; Kang et al., 2006).

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 ± 0.01	<1	19 ± 2	32.13 ± 0.77	6 ± 2	6 ± 2	0.65 ± 0.02	17.63 ± 0.25	<1	0.07 ± 0.01	0.02 ± 0.01

Toxicity test of CaP-N

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

For the three crustacean species, the reference toxicant LC50 values were 0.16 mg L^{-1} for *T. fulvus,* and 1.89 mg L^{-1} and 0.82 mg L^{-1} for *C. insidiosum* and *G. aequicauda*, respectively. These values fall within a range of values already observed in previous studies (Annicchiarico et al., 2007; Narracci et al., 2009; Prato and Biandolino, 2006). These three species were chosen as they are considered of high interest in nanoecotoxicology for different reasons. *G. aequicauda* is a bottom filter feeder and deposit feeder; therefore, it is potentially at high risk of exposure in nature, due to the tendency of NPs to aggregate in seawater settling down and accumulating in the bottom (Garner and Keller, 2014). *C. insidiosum* is a tube-building species that feeds both on sediment and suspended particulate matter; *T. fulvus, on the other hand,* lives in the water column and it may be exposed to NPs contamination and it feeds on microalgae.

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

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

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

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

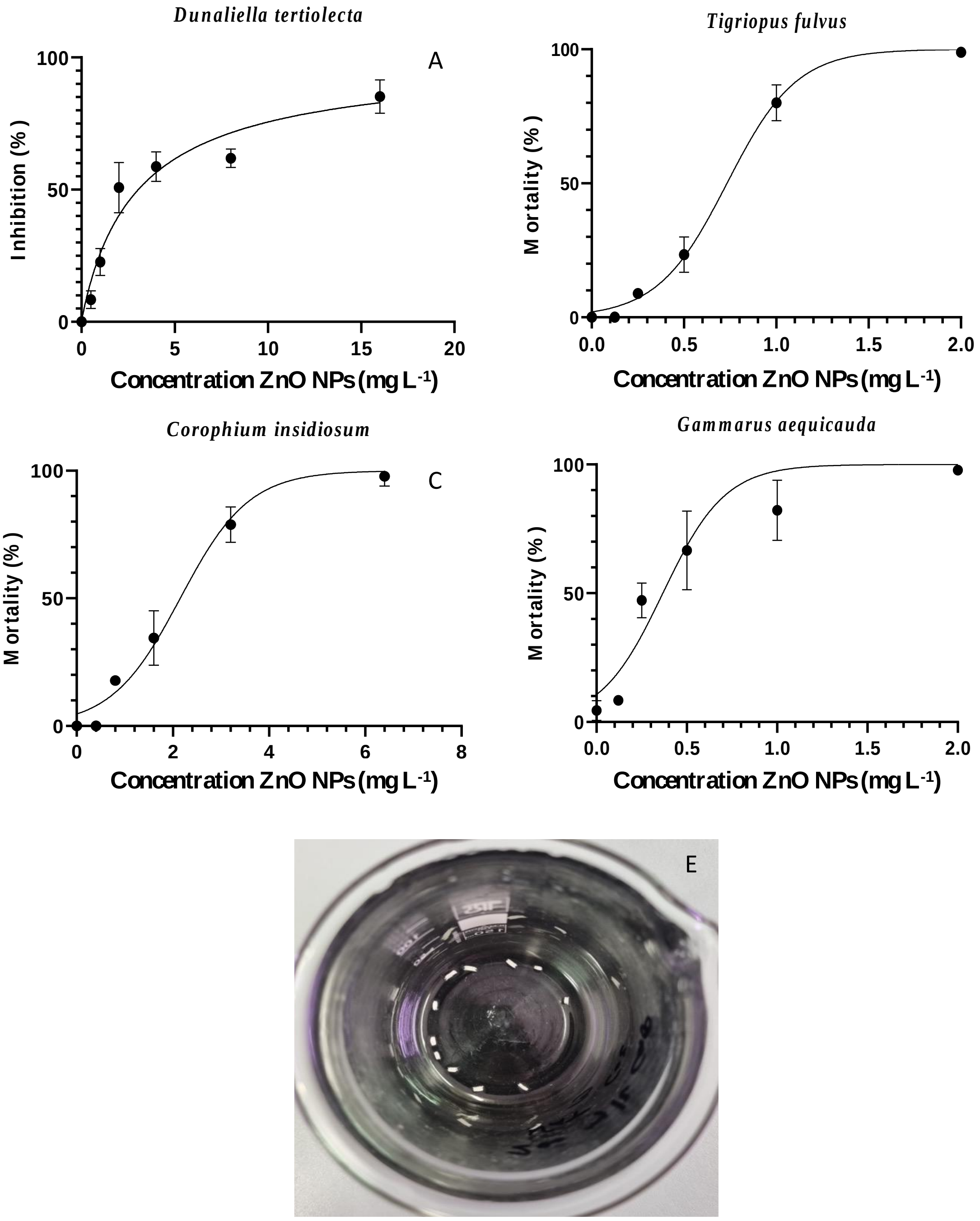

Figure 3. Dose-response curves obtained by exposing *D. tertilecta* (A), *T. fulvus* (B), *C. insidiosum* (C) and *G aequicauda* (D) at increasing concentrations of ZnO NPs, and a picture of tubes of natural CaPs built with CaP-N by the amphipod *Corophium insidiosum* (E).

The results of the acute toxicity tests for both ZnO NPs and CaP-N are summarized in **Table 4.** The growth of the algal population was clearly affected by the presence of ZnO NPs, showing a EC50 similar to the one reported by Manzo et al. who found a EC50 value of 2.42 mg L^{-1} for the same material (Manzo et al., 2013). Also for *T. fulvus*, the LC50 values recorded after 96 h of exposure to ZnO NPs are similar to those reported for the same species in an 48-h acute test (1.27 mg L^{-1}) (Prato et al., 2020). On the other hand, Wong et al. (2010) and Park et al. (2014) indicated for *Tigriopus japonicus* a 96-h LC50 of 0.85 and 2.44 mg L^{-1}, respectively (Park et al., 2014; Wong et al., 2010). Moreover, it is worthy to note that the chronic exposure (28 days) *of T. fulvus* to ZnO NPs cause negative effects on the reproductive traits, i.e. brood duration, brood size and brood number per female at much lower concentrations (≥ 100 µg L^{-1}).

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

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

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

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

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

Environmental performance evaluation through LCA

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

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

Impact categories	Units	CaP-N	ZnO NPs
Acidification	mol H$^+$ eq	1.65E-05	3.14E-05
Climate Change	kg CO$_2$ 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^3 world eq	1.02E-03	1.34E-03
Primary energy demand	MJ	2.15E-01	1.27E-01

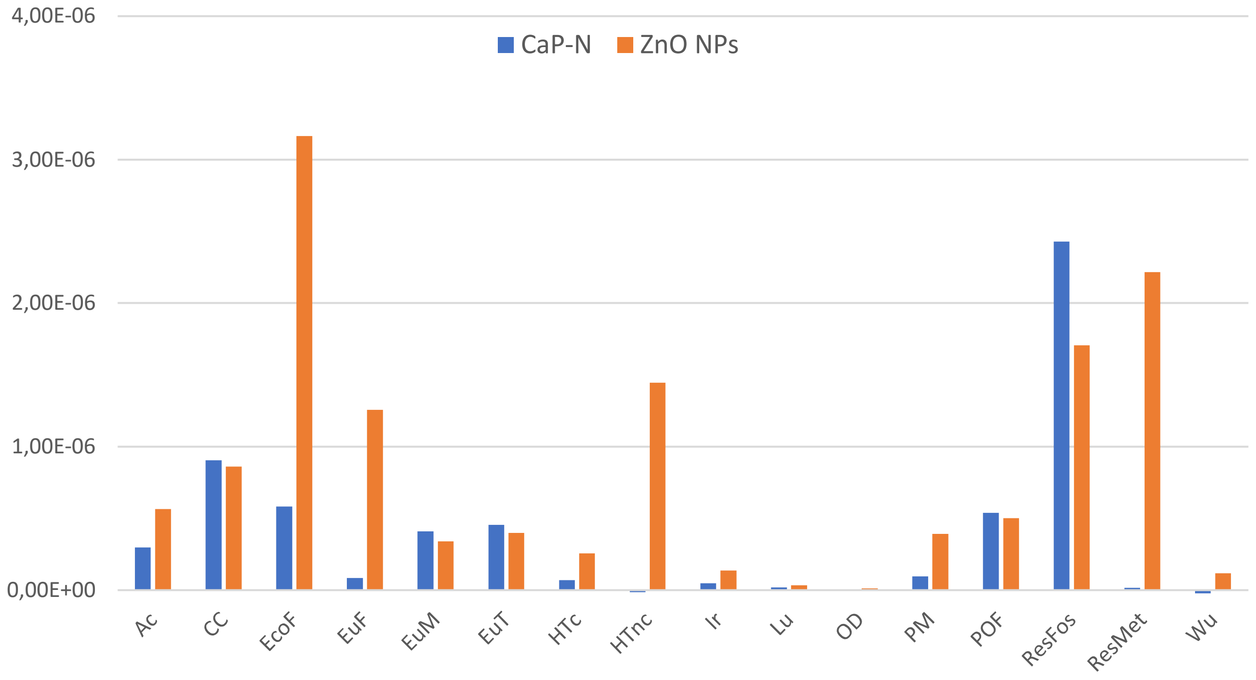

Figure 4. Normalized LCA results of CaP-N and ZnO NPs production.

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

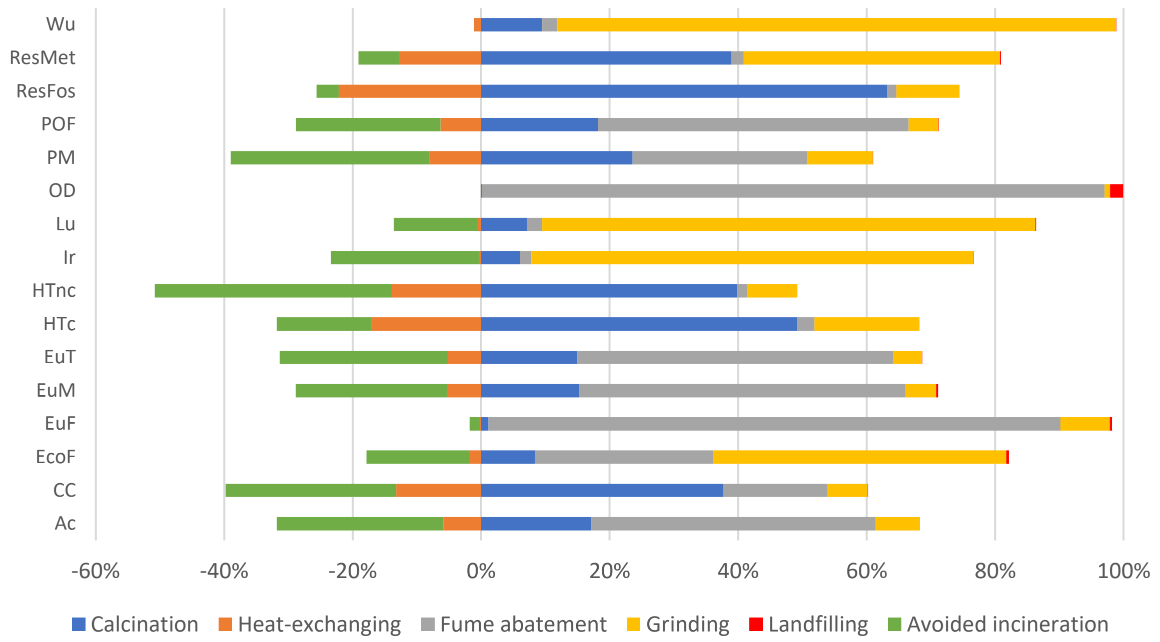

Figure 5. Percentage contributions of the CaP-N production steps to the impact categories of the LCA study.

A sensitivity analysis was done on the two parameters of LCA model affected by the major uncertainty: the thermal energy needed for calcination and the thermal energy recovered by the heat-exchanger. In the case of the thermal energy necessary to calcinate the fish meal, 28 MJ/kg ± 20% was considered for the sensitivity analysis, on the base of experimental tests. For the heat-exchanger, a minimum and a maximum recovery of 40% and 60%, respectively, were assumed with respect to the base-value of 55% (due to the combination of exchanger and economizer). In this case, the minimum value was set considering only exchanger without economizer while the maximum value was set hypothesizing an improving in the heat exchanger. The possibility of recovering heat through flue gas condensation has not been investigated but it could lead to a 90% of heat recovery. Figure 6 shows the results of the sensitivity analysis on the 16 EF impact categories, results of ZnO NPs are also reported (all impact scores are normalized). As shown in Figure 6, Climate change and Use of Fossils Resources are the two categories most affected by the parameters analyzed. Figure 7 shows the results of the sensitivity analysis on PED required by CaN-P production process, the comparison with ZnO-NPs is maintained. In this case, sensitivity analysis results have been shown on not normalized impact scores.

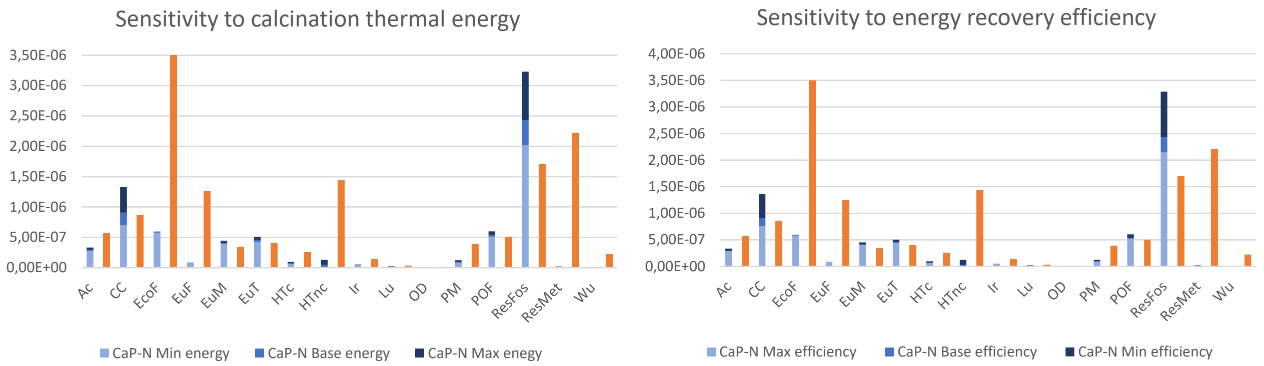

Figure 6. Results of the sensitivity analysis of the CaN-P model to thermal energy consumption for calcination (on the left) and to the efficiency of the heat-exchanger (on the right): light blue represents the minimum energy consumption; blue represents the energy consumption of the base scenario and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

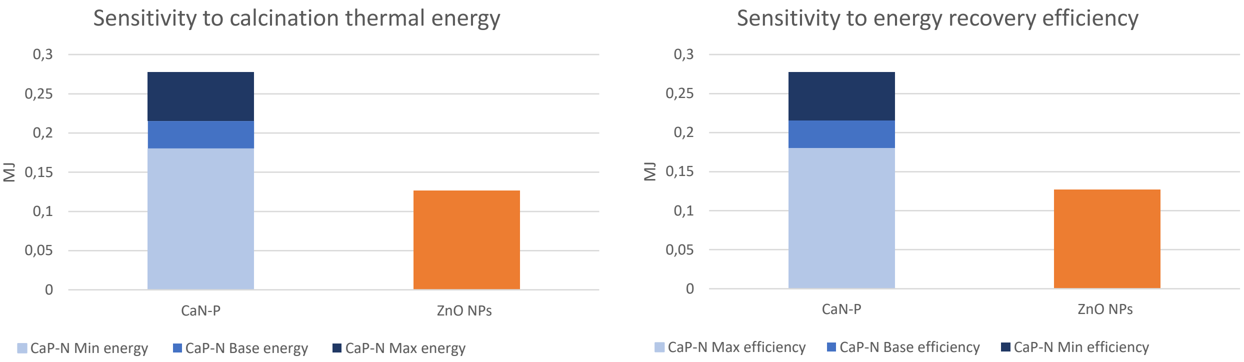

Figure 7. Results of the sensitivity analysis of PED of CaP-N production to thermal energy consumption for calcination (on the left) and to the efficiency of the heat-exchanger (on the right): light blue represents the minimum energy consumption; blue represents the energy consumption

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

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

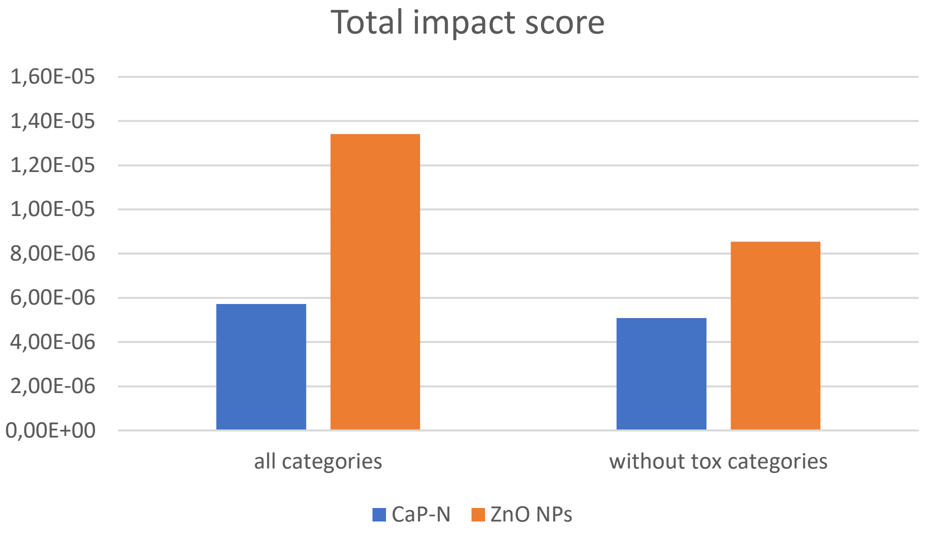

Figure 8. Comparison between the environmental impact of CaP-N and ZnO NPs production after normalization and weighting.

LCA results demonstrate that the environmental performance of CaP-N is better or comparable than that of ZnO NPs for the majority of the impact categories analyzed in this study. In the majority of cases, even when CaP-N has a higher impact than ZnO NPs the difference is almost negligible, except for Resources Use and Primary energy demand. Another essential element worth highlighting is the fact that primary inventory data for CaP-N production comes from a pilot plant. This means that its environmental impacts, especially for Climate Change, could be further improved on the industrial scale by means of process and material efficiency enhancement.

As explained in “Inventory analysis” section, in this work, CaP-N has been compared with a proxy of ZnO NPs. In order to make the work as much complete as possible, a comparison among the environmental performances of CaP-N and ZnO NPs obtained by other studies has been attempted. It is noteworthy that this comparison is made difficult by the use of different impact assessment methods that only made possible the comparison among the impact score concerning climate change, always assessed as kg of CO_2 eq emissions. Considering 1 kg of product, the impact score on global warming obtained in this study is 0.81 and 0.78 kg of CO_2 eq for CaP-N and ZnO, respectively. These values are much lower than the ones (33 and 21 kg of CO2eq/kg, respectively) reported for two ZnO production processes by Stieberova et al. (2019) and also much lower than the 25 kg of CO_2 eq/kg reported by Stieberova et al. (2017). These outcomes lead to two conclusions: (i) CaP-N from by-products obtained by calcination and grinding appears environmental interesting as substitute of ZnO NP, and (ii) the use of ZnO as proxy of ZnO NPs is conservative.

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

Conclusions

Natural calcium phosphates produced from the thermal extraction of fish bones (CaP-N) have been obtained by using a pilot-scale incineration plant. The material consists of a biphasic mixture 53:47 of hydroxyapatite:tricalcium phosphate and is made of round particles having diameter in the range of a few microns. Differently from ZnO nanoparticles (NPs) commonly used in cosmetic products, CaP-N was found to be nor toxic nor ecotoxic as it was highly tolerated by 4 different aquatic species, namely *T. fulvus*, *C. insidiosum*, *G. aequicauda* and *D. tertiolecta*. Life cyclic assessment (LCA) of CaP-N production from fish meal incineration was carried out and the outcomes were compared to that coming from LCA of ZnO, which was used as a proxy of ZnO NPs. LCA results demonstrate that the environmental performance of CaP-N is better than that of ZnO NPs for the majority of the 16 impact categories analyzed in this study, . The use of fossil resources represents the only impact category in which CaP-N has a significantly higher impact than ZnO NPs (+140%). It is noteworthy that normalization and weighting show that CaP-N production appears less impactful than ZnO NPs. However, as these data refers to a pilot-scale production, we envision that the process could be further improved at the industrial production scale to have a lower environmental impact. Finally, in this work we proved that CaP-N could actually increase the sustainability of cosmetic by increasing their safety for both human health and the environment, and by decreasing their impact on the environment.

Acknowledgment

The authors acknowledge the CNR research project “SEARCULAR” for providing financial support to the present research, and Dr. Clara Piccirillo for providing assistance and revising the original draft of the paper.

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