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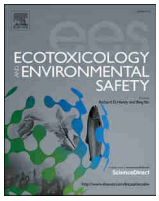

Preliminary assessment of environmental safety (ecosafety) of dextrin-based nanosponges for environmental applications

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ABSTRACT

The ability to employ waste products, such as vegetable scraps, as raw materials for the synthesis of new promising adsorbing materials is at the base of the circular economy and end of waste concepts. Dextrin-based nanosponges (D_NS), both cyclodextrin (CD) and maltodextrin (MD), have shown remarkable adsorption abilities in the removal of toxic compounds from water and wastewater, thus representing a bio-based low-cost solution which is establishing itself in the market. Nevertheless, their environmental safety for either aquatic or terrestrial organisms has been overlooked, raising concern in terms of potential hazards to natural ecosystems. Here, the environmental safety (ecosafety) of six newly synthesized batches of D_NS was determined along with their full characterization by means of dynamic light scattering (DLS), thermogravimetric analysis (TGA), Fourier transformed infrared spectroscopy with attenuated total reflection (FTIR-ATR) and transmission electron microscopy (SEM). Ecotoxicity evaluation was performed using a battery of model organisms and ecotoxicity assays, such as the microalgae growth inhibition test using the freshwater *Raphidocelis subcapitata* and the marine diatom *Dunaliella tertiolecta*, regeneration assay using the freshwater cnidarian *Hydra vulgaris* and immobilization assay with the marine brine shrimp *Artemia franciscana.* Impact on seedling germination of a terrestrial plant of commercial interest, *Cucurbita pepo* was also investigated. Ecotoxicity data showed mild to low toxicity of the six batches, up to 1 mg/mL, in the following order: *R. subcapitata* > *H. vulgaris* > *D. tertiolecta* > *A. franciscana* > *C. pepo.* The only exception was represented by one batch (NS-Q+_BDE_(GLU2) which resulted highly toxic for both freshwater species, *R. subcapitata* and *H. vulgaris*. Those criticalities were solved with the synthesis of a fresh new batch and were hence attributed to the single synthesis and not to the specific D_NS formulation. No effect on germination of pumpkin but rather more a stimulative effect was observed. To our knowledge this is the first evaluation of the environmental safety of D_ NS. As such we emphasize that current formulations and exposure levels in the range of mg/mL do not harm aquatic and terrestrial species thus representing an ecosafe solution also for environmental applications.

1. Introduction

Bio-based polymers are considered a promising building block material for a wide range of applications since they combine the sustainability of the production process with the potential safety of the derived products (Barhoum et al., 2020). Starch represents the most abundant carbohydrate polymer in nature, with some important advantages such as water solubility which allow its derivatization and application in various sectors including water treatment (Egharevba, 2019; Cecone et al., 2021). With an increasing global market of approx. 160 million metric tons in 2026 (http://www.prnewswire.com/news-releases/global-starch-market-to-reach-160-3-million-metric-tons-by-2026–301298834.html), starch derivatives as cyclodextrins (CD) have emerged as promising stable sorbent materials, characterized by slightly hydrophobic cavities (nanocavities in the range of 7–9 -Å) and external strong hydrophilic properties (Krabicová et al., 2020; Yousaf et al., 2022; Noor et al., 2023). Based on their unique properties of forming a host–guest type inclusion complex, CD have been employed in a wide range of biomedical applications (*i.e.,* pharmaceuticals, drug delivery systems), personal care products (cosmetics) but also in food and nutrition, textile

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and chemical industry (Sharma and Baldi, 2016; Poulson et al., 2021). The environmental application of cross-linked dextrin-based materials, known as CD and maltodextrin (MD) nanosponges (NS), includes water and wastewater treatment as adsorbents materials for the removal of toxic chemicals (either inorganic and organic) with also regenerative abilities (Morin-Crini et al., 2018; Sikder et al., 2019; Chaudhuri et al., 2020; Cova et al., 2021; Yadav et al., 2022).

Based on such wide range of applications, the global market of dextrin-based materials is thus expected to grow from USD 270.50 Million in 2021 to USD 365.47 Million by 2030 with an estimated production of 150 tons per year (https://www.databridgemarketresearch.com/reports/global-cyclodextrins-in-pharma-market). As the majority of other consumer and industrial products, they inevitably will end up in the natural environment either indirectly by their release into wastewater effluents or directly by application in the remediation sector for pollutants removal.

Being such promising materials, they are reaching a substantial stage of usage and applications, including environmental remediation, posing the need to unravel any potential impact on natural ecosystems and living beings. The use of natural biopolymer sources as building blocks for new materials does not necessarily guarantee a high safety standard, hence an ecosafety risk assessment framework must be applied (Zimmermann et al., 2020; Corsi et al., 2023; Wang et al., 2023; Rusconi et al., 2024).

D_NS were confirmed to be not toxic either upon acute or chronic exposure on rats; pyromellitic D_NS were well tolerated up to 2000 mg/Kg and repeated administration for 28 days did not show any relevant changes in hematological and biochemical parameters (Shende et al., 2015). Furthermore, oral administration of β-CD_NS in rats showed no toxic effects or changes in hematological parameters (Khalid et al., 2021).

In humans, the majority of studies investigating the suitability of CDs as drug carriers show no adverse effects on target organs such as the kidney and others (Stella and He, 2008; Arima et al., 2011), while also showing the absence of accumulation and damage to the gastrointestinal tract (Varan et al., 2020). A recent re-evaluation of β-CD (E 459) as a food additive by EFSA panel on Food Additives and Nutrient Sources added to Food (ANS) conclude that it has a low acute oral toxicity thus confirming the Admissible Daily Intake (ADI) of 5 mg/kg bw per day (EFSA 2016).

Although considered moderately safe for pharmacological applications, very few studies have addressed environmental safety which address transformation that the material may undergo upon contact with natural waters and/or organisms and the potential release of smaller fragments and/or chemicals associated (*i.e.,* leachates) (Esposito et al., 2023; Rusconi et al., 2024). Even in a scenario of possible criticality linked to a specific formulation affecting their behavior and ecotoxicity, a re-design of bio-based NS has made them safer thanks to the ecotoxicological evaluation, in accordance with the principle of eco-design (Fiorati et al., 2020; Corsi et al., 2023; Esposito et al., 2023).

Low but not absence of hazard has been associated with β-CD leachate exposure on freshwater microalga R. subcapitata and microcrustacean *Daphnia magna* (Sánchez-Trujillo et al., 2014).

Furthermore, the few available studies usually concern the safety of the CD themselves, but none of them investigate complex materials such as branched and cross-linked dextrin-based NS as those more widely used for environmental applications (Utzeri et al., 2022; Corsi et al., 2023).

In the effort of following the green chemistry approach and the newly safety and sustainability by design approach for chemicals and materials promoted by the European Commission (Caldeira et al., 2022), several attempts have been made to synthesize D_NS by replacing organic solvents with only water (*i.e.,* natural eutectic solvents) to obtain both branched and cross-linked dextrin-based polymers (Cecone et al., 2020; Pedrazzo et al., 2020).

In the present study, we therefore assessed the ecotoxicity of six batches of CD_NS and MD_NS on a battery of aquatic and terrestrial model species, using standardized ecotoxicity assays. Since no predicted environmental concentrations (PEC) are available for D_NS, the experiments were conducted using a concentration equal to 1 mg/mL, a condition considered potentially realistic based on the effective mass/volume ratio needed for the removal of pollutants from contaminated soils and waters, and based on known industrial and commercial applications from food, biomedical and pharmaceutical and personal care products (Sharma and Baldi, 2016; Poulson et al., 2021). Six batches were tested made by 3 CD and 3 MD made slightly differing in their surface charges in order to test how differences in formulation and charges might affect bio-interactions and ultimate environmental safety. Our study aimed to address whether water suspensions of CD_NS and MD_NS would affect the growth of freshwater (*Raphidocelis subcapitata*) and marine (*Dunaliella tertiolecta*) microalgae, the survival and regeneration of the freshwater cnidarian *Hydra vulgaris*, and the survival of the brine shrimp *Artemia franciscana*, in short-term exposure scenarios, and seed germination of the commercial plant *Cucurbita pepo*. Three assays were performed by directly testing the batches in the exposure media (microcrustacean, hydroid and courgette seeds) while in the case of the microalgae, previously conditioned waters were tested to avoid indirect interactions due to contact between the D_NS and the unicellular organism. Growth inhibition, regenerative capacity, predation and immobilization in the three model aquatic species and the germination rate in *C. pepo* were evaluated as endpoints. At the same time, the behavior of D_NS in the exposure media used in the ecotoxicity tests was characterized.

2. Materials and methods

2.1. CD_NS and MD_NS synthesis

In a typical procedure, the synthesis of the polymer was carried out by dissolving either alpha-cyclodextrin (α-CD) and/or beta-cyclodextrin (β-CD) and/or gamma-cyclodextrin (γ-CD) and/or a maltodextrin, Glucidex 2® (GLU2), in 20 mL of 0.2 M sodium hydroxide (NaOH) distilled water solution, using a round-bottom flask (Table 1). Thereafter, while continuously stirring the solution, 1,4-diazabicyclo[2.2.2]octane (DABCO) and/or 1,4-butanediol diglycidyl ether (BDE) were added, and the temperature was increased to either 70° or 90°C using a hotplate stirrer equipped with thermoregulation and a metal hemispheric bowl to obtain homogeneous heating of the flask. The reaction was then allowed to occur for 90 minutes, ultimately obtaining a monolith block as the

Table 1
Elemental analysis of the polymer batches and ζ-potential (mV) as measured in milliQ water and natural seawater (NSW), at 1 mg/mL and 25°C.

Batch number	Batch name	Element (% wt.)				ζ-potential (mV)	
		N	C	H	S	MilliQ water	NSW
1	NS-Q$^+$ BDE (βCD)	0.62	47.02	7.24	0.00	+20.9 ± 2.28	-3.23 ± 5.95
2	NS-Q$^+$ BDE (αβγCD)	0.61	47.49	7.32	0.00	+15.7 ± 1.37	-10.4 ± 1.42
3A	NS-Q$^+$ BDE (GLU2)	0.51	44.36	7.18	0.00	+25.6 ± 2.33	-10.1 ± 2.41
3B	NS-Q$^+$ BDE (GLU2)	0.51	44.36	7.18	0.00	+30.4 ± 1.1	—
3(x2)	NS-Q$^+$ BDE (GLU2*x2*)	0.44	45.75	7.17	0.00	+30.6 ± 1.6	—
4	NS BDE (βCD)	0.00	49.46	7.75	0.00	-14 ± 0.7	-8.24 ± 4.6
5	NS BDE (βCD/GLU2)	0.00	45.62	7.01	0.00	-12.8 ± 2.26	-6.52 ± 4.01
6	NS BDE (GLU2)	0.00	42.87	6.70	0.00	-19 ± 0.3	-3.84 ± 1.16

product. Subsequently, the product was recovered from the flask by crushing with a spatula and purified with distilled water, to remove any non-reacted reagents. At the end of the purification step, the product was dried in an oven at 70°C up to constant weight and ground with a mortar, obtaining a powder.

2.2. CD_NS and MD_NS characterization

Six batches of cross-linked polymers were used in this study: 1) NS-Q^+_BDE_(βCD); 2) NS-Q^+_BDE_(αβγCD); 3) NS-Q^+_BDE_(GLU2); 4) NS_BDE_(βCD); 5) NS_BDE_(βCD/GLU2); 6) NS_BDE_(GLU2) (Table S1 and Fig. S1, supplementary material). Based on ecotoxicity results on batch number 3, two new syntheses based on the same formulation were performed, namely, 3B and 3(x2). The D_NS were characterized for their surface charge by means of DLS (Malvern Panalytical, Zetasizer software) in both milliQ water and filtered (0.22 μm) natural sea water (NSW), at 1 mg/mL and 25°C. All D_NS were also analysed for their potential to change water pH and salinity in both milliQ water and NSW after 24 h incubation. Thermogravimetric analysis (TGA) was carried out using a TA Instruments Q500 TGA (New Castle, DE, USA), from 50 °C to 700 °C, under nitrogen flow, with a heating rate of 10 °C/min. A Perkin Elmer Spectrum 100 FT-IR Spectrometer (Waltham, MA, USA) equipped with a Universal ATR Sampling Accessory was used for FTIR-ATR (Attenuated Total Reflection) characterization. All spectra were collected in the wavenumber range of 650–4000 cm^{-1}, at room temperature, with a resolution of 4 cm^{-1} and 8 scans/spectrum. The sample chemical composition was studied using a Thermo Fisher FlashEA 1112 Series elemental analyser (Waltham, MA, USA). The morphology of the samples was studied using scanning electron microscopy (SEM). The images were acquired with a Tescan VEGA 3 (Brno, Czech Republic) using secondary electrons and 8 kV accelerating voltage. Prior to SEM characterization, the samples were ion-coated with 12 nm of gold using a Vac Coat DSR1 sputter coater (London, United Kingdom).

2.3. Environmental safety: ecotoxicity assessment

The six batches of D_NS were tested at the concentration of 1 mg/mL. Microalgal growth inhibition tests were performed on MilliQ water and NSW suspensions after removing D_NS to avoid mechanical interactions with cell membranes; all the other ecotoxicity assays were conducted without removing the D_NS from water suspensions. Table 2 reports a detailed summary of performed ecotoxicological assays.

2.3.1. Microalgal growth inhibition test

Algal growth inhibition assays were performed with the freshwater microalga *Raphidocelis subcapitata* following OECD201 protocol (OECD201, 2011) and with the marine microalga *Dunaliella tertiolecta* following ISO10253 protocol (ISO/10253, 2006). The assays were carried out using water, either milliQ or NSW, incubated with the D_NS for 24 h. The obtained solutions were then filtered (0.45 μm), algal medium nutrients (TG201 for *R. subcapitata* and F/2 for *D. tertiolecta*) were added, and the solutions were filtered (0.22 μm) again following our protocol previously reported in Fiorati et al. (2020). Both algae were maintained in axenic exponential growth conditions at 18 ± 1 °C with a 16/8 h light-dark cycle photoperiod in a growth chamber. Algae from a stock culture were inoculated 72 h prior to run the assay and maintained at 21 ± 1 °C and continuous illumination at 4500 lux. Tests were carried out in polystyrene single-use sterile multiwell with 2 mL capacity for each well and an initial algal concentration of 1×10^4 cells/mL. Multiwell plates were placed over an orbital shaker at 50 rpm to reduce algal settling and enabling gas exchanges. Three replicates with no added D_NS were set for each alga as controls. Three replicates for each D_NS batch were set and every test was repeated three times. After 72 h, algae were fixed with a 1:1 lugol/ethanol solution and cell density was estimated with an automated cell counter (LUNA II, Logos Biosystems). The number of cells/mL, growth rate (μ) and inhibition of growth rate (Iμi) compared to control were determined, following the protocol guidelines (OECD201, ISO10253).

2.3.2. Artemia franciscana immobilization assay

Brine shrimp *A. franciscana* short-term acute toxicity test (48 h) was conducted according to the standardized protocol APAT IRSA CNR 8060 (2003) and lately reported in ISO/TS20787 (2017). Dehydrated cysts were purchased from ECOTOX (LDS) and kept in the dark at 4°C until use. Cyst were allowed to hatch in a Petri dish filled with filtered (0.22 μm) NSW for 24 h at 25 °C under continuous illumination of 5000 lux. Toxicity tests were performed in filtered (0.22 μm) NSW aerated for 24 h before use with dissolved oxygen (DO) levels always at >80% of saturation level.

Newly hatched larvae < 24 h old (instar I nauplius stage) were placed in polystyrene single-use sterile multiwell with 2 mL capacity for each well (10 animals x well) and exposed to D_NS solutions at 1 mg/mL, with no prior filtration. Three replicates for each D_NS batch were set. Three replicates with no added D_NS were set as controls. Multiwells were placed at 25°C in the dark for 48 h and animals were not fed during tests. After 48 h mortality was assessed by recording the number of nauplii not showing any movement for at least 10 seconds. The test was considered acceptable if controls displayed an average mortality ≤ 20%. Every test was repeated three times.

2.3.3. Hydra vulgaris regeneration assay

Hydra vulgaris regeneration assay was carried out with polyps belonging to a nurtured population originally obtained from the Science Department of the Roma Tre University and kept in controlled conditions at the University of Siena. Specimens were cultured in hydra medium prepared with milliQ water with added salts ($CaCl_2 \cdot 2H_2O$ 1 M and $NaHCO_3$ 1 M), kept at 18 ± 1 ° C with a 16/8 h light/dark photoperiod and a light intensity of 400 lux. Twice a week, hydra specimens were fed ad libitum newly hatched brine shrimp *A. franciscana* nauplii, previously rinsed with deionized water., and transferred in new hydra medium 24 h

Table 2
Summary of performed ecotoxicological assays.

Test	Protocol	Test conditions	Number of organisms per replicate	Replicates/Test repetition	Toxicity Endpoint
Algal growth inhibition test (*R. subcapitata* and *D. tertiolecta*)	OECD201 (2011); ISO/10253 (2006)	21°C, continuous white light, 4500 lux, 72 h	$1*10^4$ cell/mL	3 for each D_NS/ 3 times	Inhibition of growth compared to control
Immobilization assay (*A. franciscana*)	ISO/TS20787 (2017)	25°C, no light, 48 h	10	3 for each D_NS/ 3 times	Mortality
Regeneration assay (*H. vulgaris*)	Wilby (1988)	18°C, 16/8 h light/dark photoperiod, 900 lux, 96 h–7 days	2	6 for each D_NS/ 3 times	Regeneration and predation
Germination assay (*C. pepo*)	OECD208 (2006)	25°C, no light, 6 days	3	2 for each D_NS/ 3 times	Germination, Shoot length, Primary root length, secondary roots number, root hair distribution

after feeding. The regeneration assay was carried out in single-use sterile multiwell filled with 3 mL of either hydra medium or D_NS solutions at 1 mg/mL in hydra medium, with no prior filtration. Specimens with no buds were selected for the assay and a cut was performed with a scalpel with a surgical blade to separate the hypostome (mouthparts, head and tentacles) from the column (remaining portion of the body). Right after decapitation, two columns were placed in each well and exposed to D_NS solutions. Six replicates for each D_NS batch were set, while six replicates with no added D_NS were set as controls (total of 12 animals for treatment). The multiwells were kept at 18 ± 1 ° C with a 16/8 h light/dark photoperiod and a light intensity of 900 lux and regeneration was determined after 96 h and 7 days using Wilby (1988) scoring system. The test was repeated three times. After 7 days of exposure, following the determination of the regenerative capacity, six animals for each treatment were placed, singularly, in 6-well plates filled with hydra medium, together with 10 *A. franciscana* nauplii. After 45 minutes, *H. vulgaris* specimens were removed from the wells and the number of ingested nauplii was determined (initial n. of nauplii (10) – n. of nauplii left in each well = n. of ingested nauplii) in order to assess the ability to capture and ingest prey.

2.3.4. *Cucurbita pepo germination assay*

C. pepo L. (Romanesco variety) seeds were exposed to D_NS according to OECD 208 guidelines for testing chemicals on terrestrial plants (OECD208, 2006) to assess germination capacity. The experiment has been performed in duplicate and repeated at least three times. For each batch of D_NS, solutions with Milli-Q water were prepared in order to have a final concentration of 1 mg/mL. 42 seeds of similar size and shape were selected and weighed (0.19 ± 0.04 g). 20 seeds were placed in a Petri dish with 20 mL of a 3.5% solution of H_2O_2 in Milli-Q water at 24 ° C in order to sterilize them. Seeds were gently immersed in the solution using tweezers, petri dishes were closed with aluminum foil and placed in an incubator at 24 ° C for 10 minutes. Seeds were then moved to a glass Petri dish containing 35 mL of Milli-Q water at 24 ° C and washed twice, changing Milli-Q water each time in order to remove H_2O_2 residues. The germination assay was performed in Petri dishes with 8 cm diameter filter paper disc and 3 seeds at a regular distance from each other in 10 mL of milliQ water with D_NS (1 mg/mL). Control was set up in milli-Q water without D_NS. Each Petri dish was then wrapped in aluminum foil. Petri dishes were shaken for 5 minutes with a low-speed tilting shaker and placed in an incubator at 25 ± 3 °C (in the dark) for the duration of germination. After 6 days, Petri dishes were opened, and the individual seeds were measured to calculate the following parameters: germinated and non-germinated seeds; measurement of shoot length (from the base to the cotyledons); measurement of primary root length; presence and distribution of root hairs; number of secondary roots. The following indices (Tiquia and Tam, 1998) were also calculated: Germination percentage = n. of germinated seeds/n. of total seeds; Germinative index (GI) percentage = (n. of treated germinated seeds x average length of treated primary roots/ n. of control germinated seeds x average length of control primary roots) x 100; Relative seed germination = (n. of treated germinated seeds/ n. of control germinated seeds) x 100; Relative root elongation = (average length of treated primary roots/average length of control primary roots) x 100; Seed vigor index (whole shoot) = germination percentage x (primary root length + shoot length); Seed vigor index (hypocotyl) = germination percentage x (primary root length + hypocotyl length).

Exposure waters (exudates) after the germination were analysed for the protein content according to the colorimetric method of (Bradford, 1976). 220 μL of water from the germination assay were placed in a 1 mL eppendorf, 20 μL of 0.02% Triton was added and gently stirred. Bio-Rad dye was diluted in Milli-Q water in a ratio of 1 mL Bio-Rad:5 mL total solution (1 mL Bio-Rad + 4 mL Milli-Q water) and 780 μL of diluted Bio-Rad and 220 μL of eppendorf solution were placed in 1.5 mL cuvette. The solution was mixed and left to stand for 5 minutes and then the blank solution containing 1 mL of Bio-Rad was prepared. After the spectrophotometer was set up, each sample was read in triplicate. A standard calibration curve of the spectrophotometer was made using BSA (bovine serum albumine). Total protein quantity was expressed in mg/mL. The percentage of total proteins *vs* control group was then calculated for each experimental group as follows: ((total protein experimental group)/(total protein control group)) x 100.

2.3.5. *Statistical analysis*

The statistical analysis of data was carried out by STATISTICA™ software using one-way analysis of variance (ANOVA) and Bonferroni post-test to detect differences between experimental groups. For all data, the level of statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. *D_NS characterization*

Morphological characterization of the six D_NS batches revealed dimensionally polydisperse polymer granules (Fig. 1) whose size was roughly between 10 and 150 μm. In addition, the occurrence of amine-mediated reactions resulted in a yellowing phenomenon of the products. Eventually, the SEM characterization revealed smooth external surfaces and the absence of macro-porosity. Thermal stability of the polymers was investigated via TGA and reported in Fig. S2. All the TGA were characterized by two main weight loss phenomena: the initial one, occurring approximately between 50°C and 150°C, was related to the volatilization of the water adsorbed on the surface of the samples and ranged approximately between 4% and 7% of the initial weight. Subsequently, a second weight loss phenomenon characterized by a one-step profile was observed between 250°C and 450°C which was related to the pyrolysis of the system. The T_{onset} observed from the thermogravimetric curves ranged between 285°C, associated with NS_BDE_(GLU2), and 315°C related to NS-Q^+_BDE_(βCD). A carbon residue between 6% and 12% of the initial weight, which was found to be stable up to 700°C, was observed as a result of the thermal degradation of the polymer.

The FTIR-ATR spectra acquired on the polymer products are reported in Fig. S3. As a confirmation of the proposed polymer structures, the FTIR-ATR spectra are mainly characterized by the signals in the 800–1200 cm^{-1} region related to the glucosidic bonds displayed by both CDs and MDs, the ether bridges formed during the ring-opening reaction of 1,4 butanediol diglycidyl ether's epoxy rings, and the C–O of CH_2OH bending signals. Moreover, the spectra are characterized by intense bands at 3300–3500 cm^{-1} due to O-H stretching vibration, while the vibration of the -CH and -CH_2- groups appear in the 2800–3000 cm^{-1} region.

Furthermore, the polymers composition was studied via elemental analysis and reported in Table 2. As previously reported (Cecone et al., 2021), the amine-mediated ring-opening reaction of diglycidyl ether was exploited to impart cationic features to dextrin-based polymers, where the presence of nitrogen atoms composing the polymer products further demonstrated the hypothesized reaction mechanism. On this basis, the synthetic batches involving amines to obtain positively charged product, displayed the presence of nitrogen atoms composing the polymer structure, as a confirmation.

The D_NS did not alter pH or salinity of either milliQ water or NSW (data not reported). Measure of Z-potential revealed a difference in D_NS surface charge in milliQ water (Table 2), with batches number 1–3 bearing a positive surface charge while batches 4–6 a negative one. Surface charge was positive also for batches 3B and 3(x2), whose values were slightly (+ ~5 mV) higher compared to batch 3 A. In NSW all D_NS displayed a mild negative surface charge, as a result of the shielding effect of seawater ions as previously reported for colloidal materials (Corsi et al., 2021).

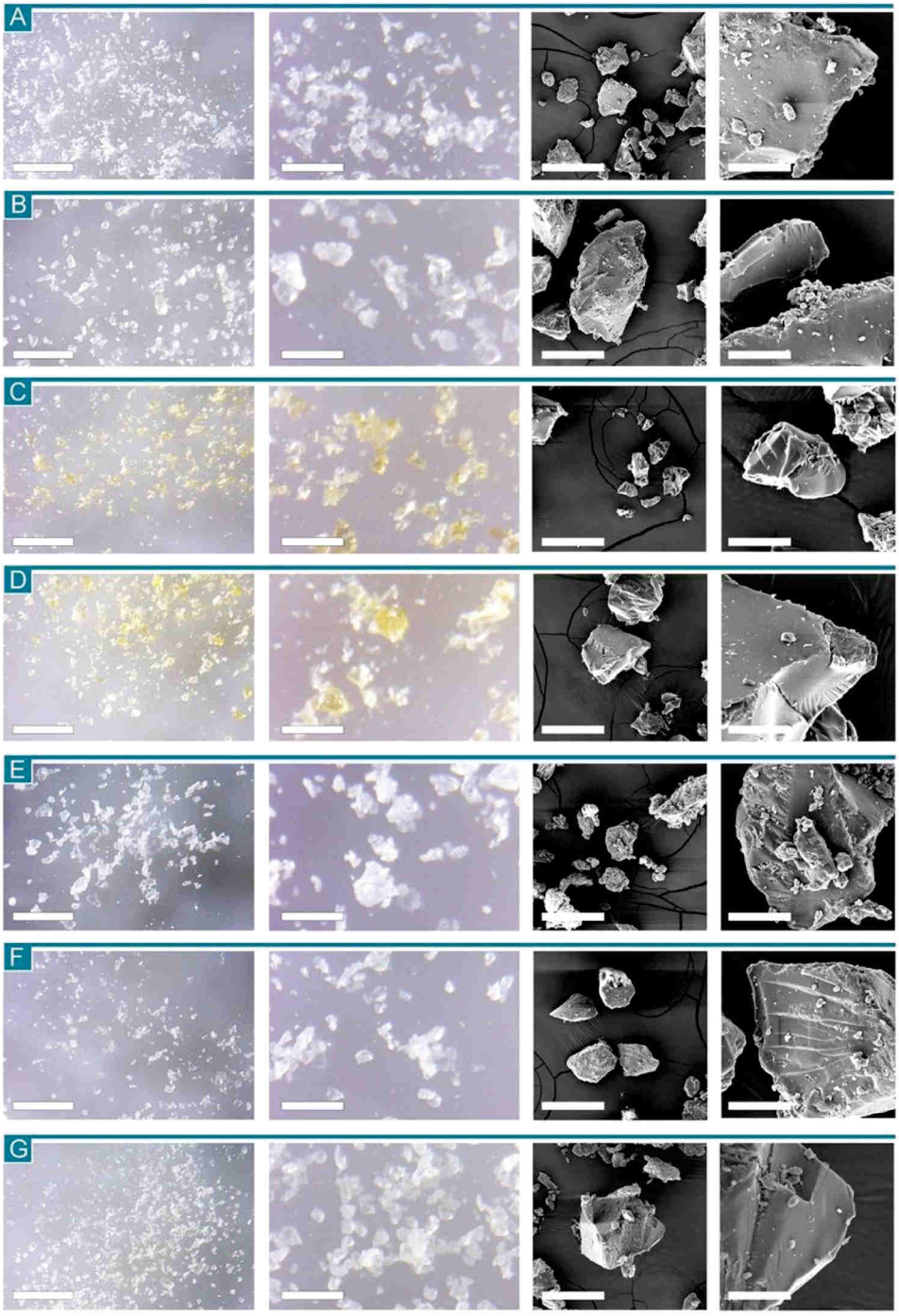

Fig. 1. Polymer granules and SEM images of (A) NS-Q$^+$ BDE (βCD), (B) NS-Q$^+$ BDE (αβγCD), (C) NS-Q$^+$ BDE (GLU2), (D) NS-Q$^+$ BDE (GLU2*x2*), (E) NS BDE (βCD), (F) NS BDE (βCD/GLU2), (G) NS BDE (GLU2). Scale bars: 1 mm (first column); 250 mm (second column); 100 μm (third column); 20 μm (fourth column).

3.2. D_NS environmental safety assessment

The overall data on ecotoxicity tests conducted on the six batches of D_NS revealed a general low hazard for the majority of the tested organisms (Figs. 2, 4 and Table S2), with the only exception of batch 3A (Fig. 3) which showed 100% inhibition of growth in the freshwater species, *R. subcapitata* and 100% inhibition of regeneration in the *H. vulgaris*. *R. subcapitata* also suffered a mild (~ 20%) inhibition of growth when exposed to the other D_NS batches (Fig. 2A), while a mild impairment of regeneration of *H. vulgaris* was observed for batches 1 and 2 (Fig. 2C).

The D_NS had no effect on marine species (*D. tertiolecta* and *A. franciscana*) (Fig. 2B, D), while some batches seemed to have a stimulating effect on germination of *C. pepo* seeds (Table S2).

Based on the data obtained for batch 3, ecotoxicological assays with *R. subcapitata* and *H. vulgaris* were repeated using two newly synthetized batches, 3B and 3(x2), together with batch 3 A. Results (Fig. 3) showed that the new synthesis (3B) and the new formulation (3(x2)) successfully reduced the toxicity of batch number 3 towards freshwater species, compared to batch 3 A. This was confirmed also by the predation assay (Fig. S4) performed with regenerated *H. vulgaris*, showing a total impairment of the predation capacity upon exposure to batch 3 A and a complete recovery after exposure to batches 3B and 3(x2).

The negligible toxicity observed for the majority of D_NS batches

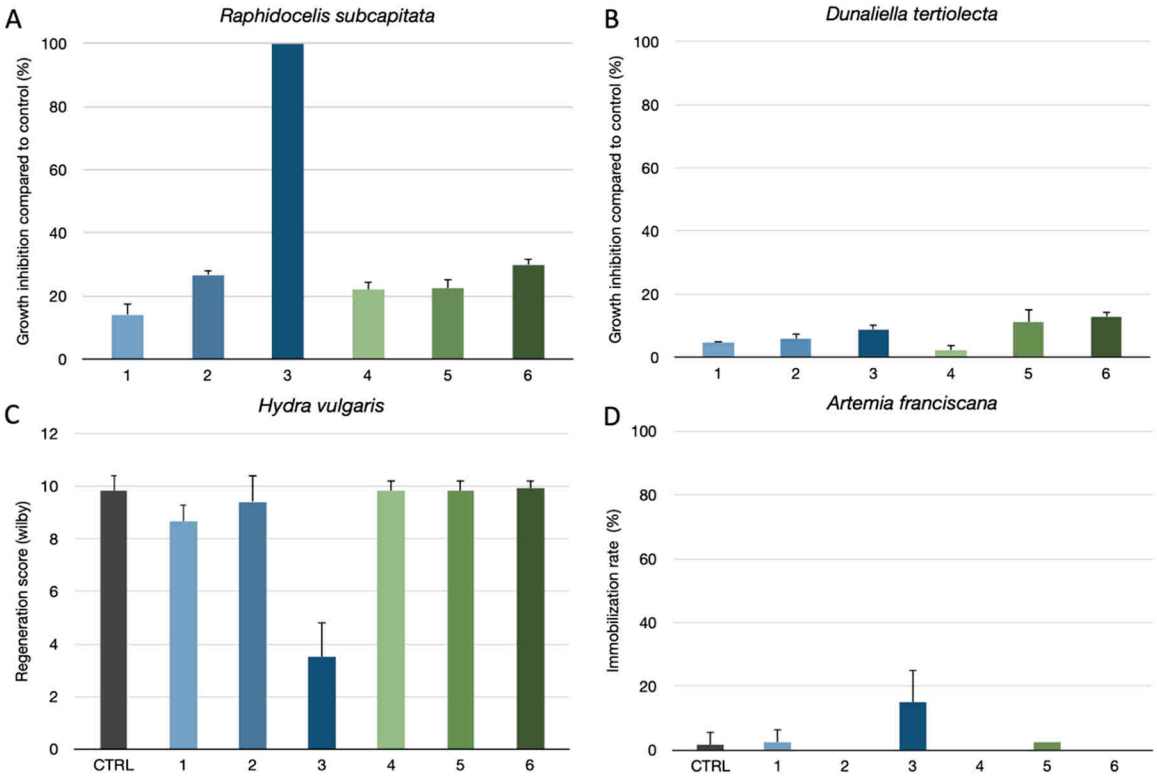

Fig. 2. Effects of D_NS batches 1–6 on: a) *Raphidocelis subcapitata*, b) *Dunaliella tertiolecta*, c) *Hydra vulgaris* and d) *Artemia franciscana* larvae. The effects are expressed as: a), b) growth inhibition compared to control, c) regeneration score according to Wilby (1988) and d) immobilization rate percentage. All results are expressed as mean ± standard deviation.

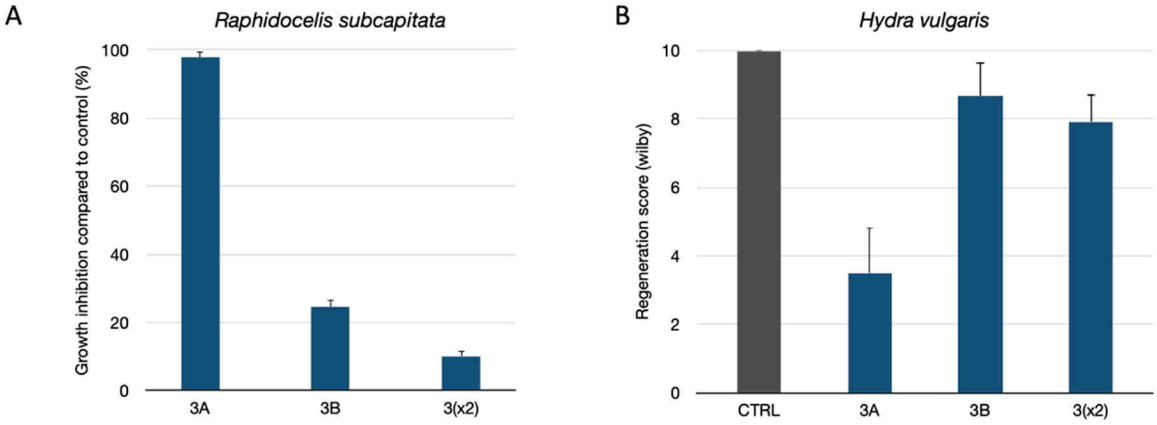

Fig. 3. Effects of D_NS batches 3A, 3B and 3(x2) on: (A) *Raphidocelis subcapitata* and (B) *Hydra vulgaris*. The effects are expressed as growth inhibition compared to control for *R. subcapitata* and regeneration score according to Wilby (1988) for *H. vulgaris*. All results are expressed as mean ± standard deviation.

agrees with what reported by Krawczyk et al. (2022), who tested the effects of a β-CD polymer (β-CDP) on *R. subcapitata* and reported a negligible inhibition of growth and photosynthetic efficiency, at a lower concentration (0.12 mg/mL). While, Fai et al. (2009) observed that β-CD were significantly inhibiting *R. subcapitata* growth only at concentrations of 30 mM (~34 mg/mL) and above.

The scarcity of ecotoxicological studies and the differences in formulations and synthesis of D_NS batches pose difficulties in the comparison with literature data. In this study, the positive surface charge of batches 1–3 could be considered the main trigger for the effects observed towards *R. subcapitata* and *H. vulgaris*. As the surface charge of aquatic organisms is usually known to be negative, positively charged materials often result to be more toxic than negative ones (Bergami et al., 2016; Spielman-Sun et al., 2019). However, relevant detrimental effects were observed only following exposure to batch 3 A and were almost completely solved when the synthesis was repeated (3B). Hence, the cause of the toxicity of batch 3 A is more likely to be found in its synthesis than in its formulation. Since the D_NS are cross-linked polymers, we may hypothesize the occurrence of an un-linked colloidal fraction in batch 3 A, able to interfere with cells and organisms. This hypothesis might find support in the fact that toxicity occurs in freshwater exposures exclusively, in which the aggregation of colloids usually plays a minor role compared to high ionic strength media such as NSW (Klaine et al., 2008; Lead et al., 2018). Moreover, the shielding of positive surface charges by seawater ions, together with the enhanced aggregation usually observed for colloids in seawater, could explain the total lack of effects observed for marine water species (*D. tertiolecta* and *A. franciscana*).

Results of *C. pepo* germination assay show no negative effect of D_NS while, on the opposite, all 6 batches stimulated the development of all morphological parameters (primary and secondary roots, shoot and hypocotyl) of the seedlings (Fig. 4, Table S2). This is particularly evident for seeds exposed to positively charged D_NS, for which a significant increase in indices *vs* control ones was observed ($p < 0.05$). Conversely, those negatively charged despite still stimulating seedling growth do not cause a similar effect by resembling more the controls.

Concerning indices, it is clear that seeds that showed higher average values *vs* control are those that were exposed to the positively charged D_NS batches. In particular, batch 1 seems to be the one that most favored the germination process. On the contrary, the negatively charged batches showed average values comparable to the control. The results related to the protein content reveals that proteins were released

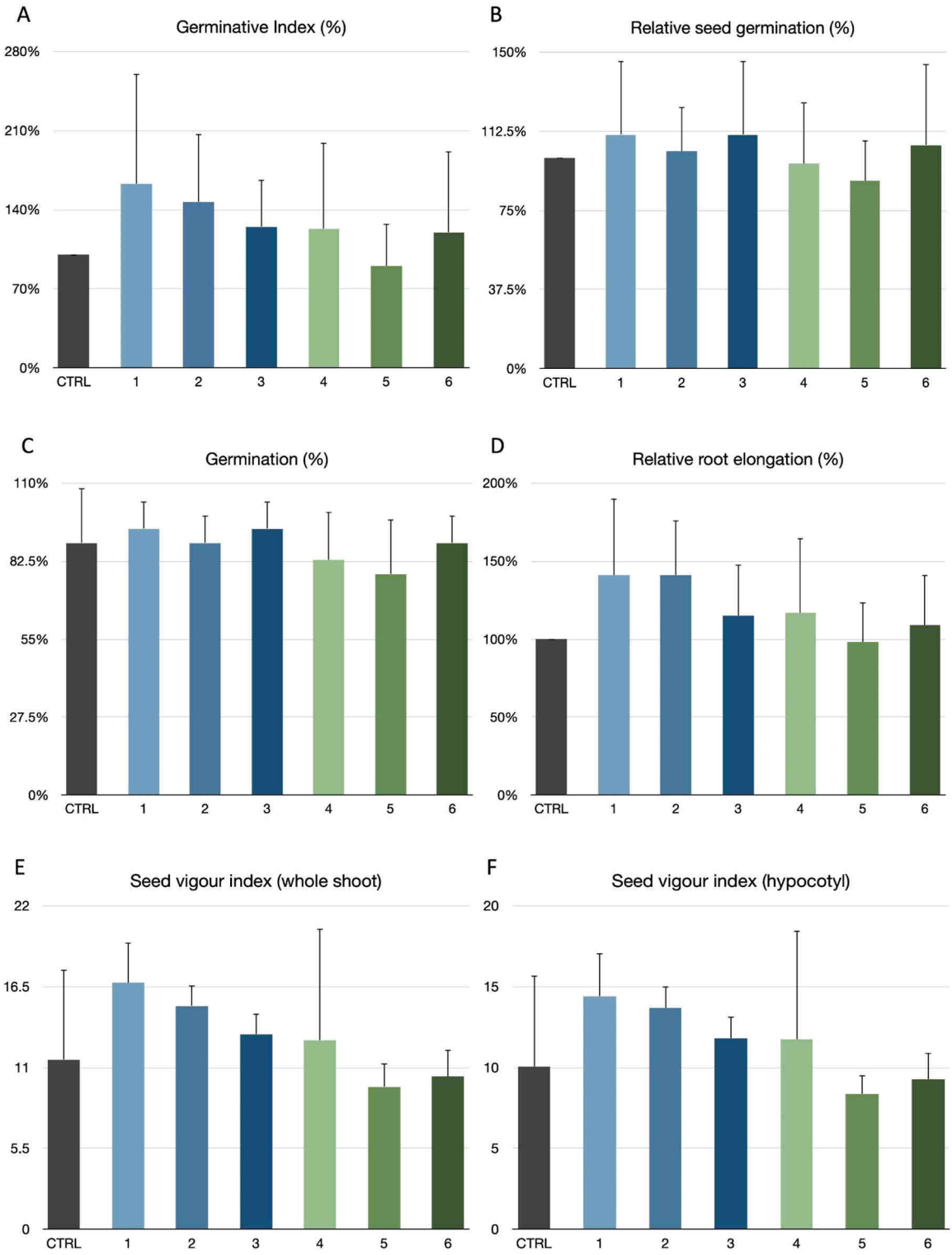

Fig. 4. Effects of D_NS batches 1–6 on *C. pepo*: (A) germinative index (%), (B) relative seed germination (%), (C) germination (%), (D) relative root elongation (%), (E)seed vigor index (whole shoot), (F) seed vigor index (hypocotyl). Data are shown as mean ± standard deviation.

in the exposure medium, with the production of root exudates. All D_NS batches stimulated protein production, especially batch 3 (positively charged) and batch 5 (negatively charged).

Based on such findings, it can be hypothesized that the surface charges of D_NS may be determinant for germination by stimulating growth responses. As a matter of fact, the surface charge, as well as the production of proteins and exudates, which are usually negatively charged, can influence the uptake and radical translocation of nanoscale materials in plants (Lv et al., 2019).

Dextrin-based materials have been extensively used in the food industry as GRAS (General Regarded as Safe) (Fenyvesi et al., 2016). Also, their pharmaceutical and cosmetic applications as antioxidants, stabilizers and carriers in drug delivery rely on being no hazardous for humans and the environment (Laza-Knoerr et al., 2010; Duchene and Bochot, 2016). Their use as encapsulating agents for plants bioactive compounds confirmed no negative effects on seeds germination and root length (Inoue et al., 2018).

A positive stimulating role of D_NS on plants in *in vitro* cultures (50 mM; 0.1 mg/mL) have been already documented by increasing the production of bioactive compounds as secondary metabolites and antioxidants, which are beneficial for the plant itself but also for human health (Pinho et al., 2014; Almagro and Pedreño, 2020). Being structurally similar to pectic oligosaccharides, β-CD act as elicitors in plant cell culture by promoting the production of bioactive compounds (*i.e.,* phenolic compounds, alkaloids, terpenes) having an important role in plant growth and development ad involved in plant defense reactions. They are also able to increase the ability of hydrophobic compounds to cross cell membranes by inclusion in their hydrophobic cavity and thus enhance solubility in aqueous media avoiding also their oxidation and favouring accumulation (Cardillo et al., 2021).

4. Conclusions

The growing application of dextrin-based nanosponges in various scientific and technological fields will inevitably lead to their release into the natural environment. Environmental safety has not yet been adequately studied although natural polymers used as building blocks have proven to be as toxic as those synthetics used in other applications. Our findings revealed that acute exposure to both aquatic organisms and plants does not cause harm even at a concentration probably far higher than those eventually found in the aquatic and terrestrial ecosystems.

Our results paved the way to environmentally safe applications of D_NS whether the formulation and physical chemical properties are similar and/or comparable to those investigated in the present study.

CRediT authorship contribution statement

Arianna Bellingeri: Writing – original draft, Supervision, Investigation. **Claudio Cecone:** Writing – original draft, Investigation. **Gian Marco Palmaccio:** Investigation. **Ilaria Corsi:** Writing – original draft, Supervision, Resources, Conceptualization. **Francesco Trotta:** Resources, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116120.

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