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# Additives in bioplastics: Chemical characterization, migration in water and effects on photosynthetic organisms

Alberto Crema<sup>a</sup>, Enrico Dinelli<sup>b,c</sup>, Elena Fabbri<sup>b,c</sup>, Paola Galletti<sup>a,c</sup>, Nicolas Greggio<sup>b,c</sup>,  
Valentina Lastella<sup>a</sup>, Adriano Parodi<sup>a</sup>, Andrea Pasteris<sup>b,c</sup>, Marta Pedrizzi<sup>b</sup>, Chiara Samori<sup>a,c,\*</sup>

<sup>a</sup> Department of Chemistry “Giacomo Ciamician”, University of Bologna, Via S. Alberto 163, 48123 Ravenna, Italy

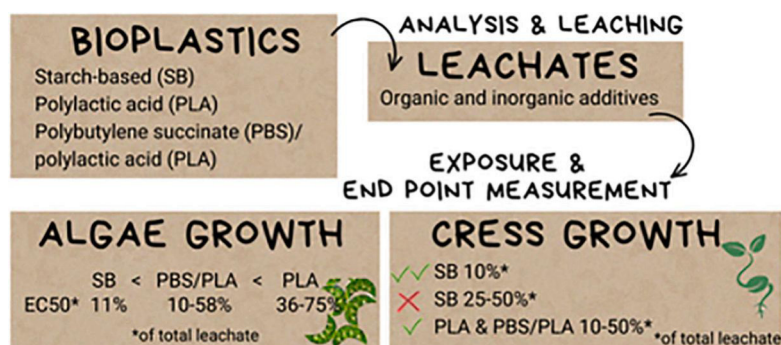
<sup>b</sup> Department of Biology, Geology and Environmental Science, University of Bologna, Via S. Alberto 163, 48123 Ravenna, Italy

<sup>c</sup> Interdepartmental Centre for Research in Environmental Sciences (CIRSA), Via S. Alberto 163, 48123 Ravenna, Italy

## HIGHLIGHTS

- The main organic additives of commercial bioplastics do not migrate in water.
- Na, Mg, K and Ca are found in all leachates in the 0.1–100 ppm concentration range.
- PBS/PLA- and SB-leachates at 0.5 % concentration inhibit *R. subcapitata* growth.
- Cress development is promoted by 10 % SB-leachates but inhibited at 25 and 50 %.
- PLA- and PBS/PLA-leachates do not affect cress germination and development.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The potential release in the environment and biological effects of chemicals like additives and non-intentionally added substances present in conventional plastics and bioplastics is an issue that could occur if these materials are not properly disposed of. Herein, seven leachates of biobased and biodegradable plastics made of polylactic acid (PLA), polybutylene succinate (PBS)/PLA blends, and starch-based blends (SB) were characterized and compared for the inorganic and organic additives present in the source materials. The main inorganic elements found in the leachates were Na, Mg, K, and Ca (0.1–100 mg L<sup>-1</sup>), corresponding to the main elements present in the bioplastics. Also trace elements such as Ba, Zn, Sr, B, Fe, Ti, Al, Mn, Cu, and Sn occurred in leachates with concentrations between 1 and 1000 µg L<sup>-1</sup>. In contrast, most of the organic additives found in the bioplastics did not migrate in water and the few organic compounds detected and identified were not of concern. The lowest tested concentration of PBS/PLA- and SB-leachates (0.5 % of the corresponding initial leachate) induced a significant algal growth inhibition (corresponding to bioplastic concentrations in water of 0.4 g L<sup>-1</sup>). Conversely, PLA-based materials were less toxic (LOEC corresponding to 10 % of the leachates or >75 %). No effect on seed germination nor the development of roots and shoots of cress was observed for any leachate prepared from PLA and PBS/PLA materials. Leachates prepared from SB bags inhibited the growth of roots and shoots at the

\* Corresponding author at: Department of Chemistry “Giacomo Ciamician”, University of Bologna, Via S. Alberto 163, 48123 Ravenna, Italy.  
E-mail address: [chiara.samori3@unibo.it](mailto:chiara.samori3@unibo.it) (C. Samori).

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concentrations of 25 and 50 %, while they induced hormesis at 10 % concentration promoting a growth higher than the control.

## 1. Introduction

From an environmental and economic standpoint pollution prevention should be the first choice in chemical and materials production, and the concept of “Benign by Design” that underpins a sustainable approach to chemical production should represent a guideline to face environmental challenges and prevent pollution (Zimmerman et al., 2020). Among the various sources of environmental pollution, plastics play a pivotal role: it is widely known that inadequately managed plastic waste affects aquatic and terrestrial organisms through various biological and ecological impairments, and possible effects on human health have also been reported (Li et al., 2020; Prata et al., 2020; Rillig and Lehmann, 2020; Wang et al., 2021; Xu et al., 2020). In particular, the deterioration of plastic waste into micro- and nanofragments (i.e. micro- and nanoplastics) once they enter the environment and the effects they can cause have widely been investigated and are the core of many lab and in-field studies. Microplastic debris i) may interact with organisms through ingestion causing obstruction or internal abrasions; ii) may translocate and impact a variety of physiological functions, from apical endpoints like locomotion, growth, and reproduction to molecular/cellular effects like cellular metabolisms, gene expression, and oxidative stress; iii) may enter the food chain; iv) may act as vectors for pathogens/alien species; v) may accumulate and release pollutants inside organisms (Franzellitti et al., 2019). Moreover, a further risk for humans and the environment is posed by the multitude of chemical additives contained in all plastic items available on the market, added for conferring specific properties, independently of the polymer type, biobased and biodegradable polymers included (Cuthbertson et al., 2024). While some polymers (man-made or natural) used to manufacture plastic items are almost inert and resistant to any transformation, the organic and inorganic additives used for transforming all the polymers into plastics are far from being inert, especially in terms of biological activity and variety of sub-lethal effects (Qadeer et al., 2024). Being not covalently bound, intentionally added chemicals like fillers, plasticizers, flame retardants, stabilizers, antioxidants, and pigments, but also non-intentionally added substances (NIAS) like side products, breakdown products, and contaminants, can easily migrate/leachate from the polymeric matrix to liquids, solids and gas which they are in contact with (Hahladakis et al., 2018). Since many of the additives are known to be hazardous, even at low concentrations causing various types of damage to organisms, they are indicated as one of the main drivers of plastic toxicity; some of these chemicals like bisphenol A (BPA) are well-established endocrine disruptors and they have already been banned or restricted in the manufacturing of specific plastic items (Canesi and Fabbri, 2025; EFSA, 2023). The EU Plastic Strategy for addressing the issues posed by plastics in their entire life cycle includes separate collection, recycling and management at the end-of-life, ban of single-use items, restriction of the use of microplastics intentionally added to products, and restriction of the use of specific chemicals like BPA in plastic infant feeding bottles, or phthalates identified as toxic to reproduction in children's toys (European Union, 2018). However, plastic additives also include many organic and inorganic compounds that are not classified as hazardous but can become of concern when released as a mixture. Recent ecotoxicological studies conducted on different targets like copepods, barnacles, oysters, mussels, urchins, lugworms, and fishes exposed to the additives present in both conventional plastics and bioplastics highlighted clear biological effects and non-negligible hazards of the additives present in plastics made of natural and biodegradable polymers, capable of inducing baseline toxicity, oxidative stress, and endocrine activity (Akoueson et al., 2023; Beiras et al., 2021; Capolupo et al., 2023; Chagas et al., 2021b, 2021a; de Oliveira et al., 2021; Klein et al., 2021; Malafaia et al.,

2021; Zimmermann et al., 2020). However, these effects are not easily attributable to specific additives and few studies deal with analyzing chemicals released in water by plastics or bioplastics and the associated biological responses (Akoueson et al., 2023; Capolupo et al., 2023; Zimmermann et al., 2020). Regarding photosynthetic organisms, various cyanobacteria and microalgae belonging to the classes of Mediophyceae and Chlorophyta were exposed to plastic leachates (Nam et al., 2022) but, to the best of our knowledge, leachates from commercially available bioplastic items have never been tested. On the other hand, cress seeds have already been used for evaluating the potential effects on germination and early development of terrestrial plants caused by plastic and bioplastic leachates, even if the majority of the studies in this field are focused on conventional plastics and their additives like phthalates, BPA, nonylphenols and polybrominated diphenyl ethers, known to induce phytotoxic effects, decreasing germination rates and inhibiting root development (Cao et al., 2023). The effects of leachates from starch-based and PLA-bioplastic on cress and other plants like allium, ryegrass, basil, tomato, and lettuce include a negligible inhibition of seed germination but a generally compromised plant development i.e., reduced growth, development abnormalities, low content of chlorophylls, low dry weight of roots and shoots (Balestri et al., 2019; Barbale et al., 2021; Celletti et al., 2023; Moraes Sinohara Souza et al., 2013; Wright et al., 2024).

The present study aimed to investigate and deepen the effects of organic and inorganic additives released in water by biodegradable and bio-based plastics on photosynthetic organisms. Seven samples representing the main commercially available bioplastic types in Italy for single-use applications were selected, three films and four rigid items: 1) food packaging films made of polylactic acid (PLA), 2) plates made of PLA, 3) glasses made of PLA, 4) plates made of polybutylene succinate (PBS)/PLA blends, 5) glasses made of PBS/PLA blends, 6) starch-based (SB) shopping bags, and 7) SB plastic mulch films. The organic additives profile and the inorganic content of the selected materials were determined, and each material was subjected to a leaching experiment for 14 days at the end of which the leachates were chemically characterized and then evaluated for their effects on seed germination and algal growth. To this purpose, two of the most used organisms to assess the toxicity of chemicals towards aquatic and terrestrial photosynthetic organisms, i.e., the microalga *Raphidocelis subcapitata* and cress (*Lepidium sativum* L.), were exposed to bioplastic leachates in acute toxicity tests.

## 2. Materials and methods

### 2.1. Chemicals and bioplastic materials

All reagents, solvents, and chemicals used in this work were of analytical grade and purchased from various commercial suppliers. Bioplastic samples were purchased from a local supermarket in Ravenna, Italy. Three items per bioplastic sample were cut into pieces of 1 × 1 cm size and “merged” to have an integrated sample for each bioplastic material on which all the analyses and leaching experiments were performed. Apart from the three bioplastic items made of PLA (food packaging, glasses, and plates) in which the polymeric composition was clearly stated on the label, and the SB shopping bag, known to be composed of starch, polybutylene adipate terephthalate (PBAT) and sorbitol as a plasticizer, the polymeric composition of the other items generally indicated as “biodegradable and compostable” was assessed in the following way (Parodi et al., 2023): the samples were extracted twice, first with methanol under reflux to remove the plasticizers, then in a closed-cap glass vial with ethyl acetate (90 °C for 20 min) or

dichloromethane (RT for 30 min) to solubilize the polymeric component. The extracted solutions were centrifuged at 1000g for 5 min, the supernatants were withdrawn, and the solvent was evaporated under vacuum. The extracted polymeric components were weighted to give the yield based on the material in input (wt%) and then characterized through  $^1\text{H}$  NMR spectroscopy (Fig. S1 in ESI). The residual component not soluble in organic solvents obtained after centrifugation was collected from the bottom of the glass vial and weighted to give the yield based on the material in input (wt%). This fraction includes polymers that are not soluble in organic solvents, like starch, and inorganic additives (see below for the quantitative and qualitative analysis of inorganic additives). The main composition of each material has been reported in Table S1 in ESI and accordingly, the following codes (polymer type-item) have been assigned to the bioplastic materials analyzed: 1) PLA-packaging, 2) PLA-plate, 3) PLA-glass, 4) PBS/PLA-plate, 5) PBS/PLA-glass, 6) SB-bag, and 7) SB-mulch.

## 2.2. Non-target analysis of the organic additives in the bioplastic materials

Each material (2 g) was charged in a flask and then extracted with methanol (20 mL) for 1 h under ultrasounds. The solvent was withdrawn while the material was extracted a second time with methanol (20 mL) for 1 h under ultrasounds. The solvent phases were then collected, filtered, and dried under vacuum. The extract was silylated for 60 min at 70 °C with 0.1 mL ethyl acetate, 0.08 mL bis(trimethylsilyl)trifluoroacetamide containing 1 % of trimethylchlorosilane, and 0.02 mL of pyridine, diluted with 0.4 mL of ethyl acetate and then analyzed by GC-MS. Compounds were identified by comparison with the NIST database; the unidentifiable compounds were indicated as “unknown”. A blank extract was prepared by following the procedure described above without any bioplastic material in the flask. All compounds found in the GC-MS analysis of the blank extract were not considered additives contained in the bioplastic materials. The analysis of the silylated extracts was performed with an Agilent HP 6850 gas chromatograph connected to an Agilent HP 5975 quadrupole mass spectrometer. Analytes were separated on an HP-5MS fused-silica capillary column (stationary phase poly[5 % diphenyl/95 % dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness), with helium as the carrier gas (at constant pressure, 36  $\text{cm s}^{-1}$  linear velocity at 200 °C). Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan  $\text{s}^{-1}$  within the 12–600  $m/z$  range. The injection port temperature was 250 °C. The column temperature was kept at 50 °C for 5 min, then increased from 50 to 325 °C at 10 °C  $\text{min}^{-1}$ .

## 2.3. Analysis of the inorganic compounds in the bioplastic materials

Each material (0.2 g) was rinsed with ultrapure water (Milli-Q), cut into smaller pieces of 5 × 5 mm size with a ceramic knife on a glass plate and then digested in a microwave (Milestone ETHOS UP) with nitric acid ( $\text{HNO}_3$ , super-pure grade, 67–70 %, Merck, Darmstadt, Germany), sulfuric acid ( $\text{H}_2\text{SO}_4$ , super-pure grade, 95–97 %, Merck, Darmstadt, Germany) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ , Suprapur, 30 %, Merck, Darmstadt, Germany) (Klößner et al., 2021). A mixture of  $\text{HNO}_3$  (6 mL) and  $\text{H}_2\text{O}_2$  (2 mL) was used to digest all the materials apart from the PLA-based ones for which 8 mL of  $\text{HNO}_3$  and 2 mL of  $\text{H}_2\text{SO}_4$  were used. The microwave program was set at 220 °C and a peak power of 1800 watts for 2 cycles of 20 min. After digestion, samples were filtered and diluted to a final volume of 50 mL with ultrapure water and stored for the ICP-MS analysis (see Table S2 in ESI for details). The inorganic elements analyzed were Na, Mg, K, Ca, Ag, Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Ti, Tl, V, Zn. A procedural blank was analyzed accordingly. Each analysis was performed in duplicate, and the concentrations were reported as the mean of the two replicates.

## 2.4. Total inorganic content analysis of the bioplastic materials

The total inorganic content of the materials was determined by calcination. Each material (0.5 g) was placed in a ceramic lab crucible, previously weighed. The samples were heated at 550 °C for 5 h, and then the crucibles were weighed again to quantify the amount of residual ashes, taken as a measure of the inorganic content of the materials. Each analysis was performed in duplicate, and the concentrations were reported as the mean of the two replicates.

## 2.5. Leachate preparation and characterization

Leachates were prepared as previously described by Capolupo et al. (2020). Briefly, each material was charged in a flask with 75 mL of distilled water, using a sample-water ratio of 80  $\text{g L}^{-1}$ . The sample was shaken in a rotating incubator (125 rpm) at rt. for 14 days in the dark, and then the leaching solution containing the organic and inorganic additives was separated by pipetting and filtered through a sterile filter (0.45  $\mu\text{m}$  Nalgene®). A control sample was prepared using the same procedure as above but without the addition of any bioplastic material to the flask. The analysis of the organic additives released from the bioplastic materials in water was performed by drying under  $\text{N}_2$  an aliquot of the solution (1 mL), followed by silylation and GC-MS analysis as described above. The analysis of the inorganic additives in the leachates was carried out as described above using ICP-MS. The control sample was analyzed accordingly. Each analysis was performed in duplicate, and the concentrations were reported as the mean of the two replicates.

## 2.6. Algal growth inhibition assay with *Raphidocelis subcapitata*

The algal growth inhibition test was conducted following the OECD 201 guideline (OECD, 2011), adapted to the use of 24-well plates as test vessels. The test organism was the freshwater unicellular green micro-alga *Raphidocelis subcapitata*, strain SAG 61.81 (formerly named *Pseudokirchneriella subcapitata*), originally purchased from EPSAG Göttingen University and cultivated in the laboratory. In each well, 1.5 mL of leachate, diluted with distilled water, was mixed with 0.5 mL of concentrated culture medium and inoculated with an aliquot of algae from an exponentially growing culture. By diluting the leachate, six concentrations arranged in a geometric progression from 0.5 to 75 % were prepared, each in three replicates. Three replicates of the control treatment were also prepared (1.5 mL of distilled water +0.5 mL of concentrated culture medium and algae). Algae were incubated for 72 hours at  $23 \pm 2$  °C on an orbital shaker at 100 rpm under continuous “cool white” fluorescent light (intensity of 6000 lx). The algal density (cell  $\text{mL}^{-1}$ ) in each well, at the beginning and the end of the incubation, was determined by counting the number of cells in a sample of known volume, under a microscope at 400×, with a Burkner hemocytometer. The specific growth rate  $\mu$  ( $\text{d}^{-1}$ ) of the algal population in each well was calculated, assuming exponential growth, according to the following equation:

$$\mu = \frac{\ln N_f - \ln N_i}{t}$$

where:  $N_i$  is the initial algal density,  $N_f$  is the final algal density after 72 hours, and  $t$  is the test duration. Differences in specific growth rates between the control and each concentration were evaluated using Dunnett's test and considered statistically significant if  $P < 0.05$ . The toxicity of the leachates was expressed as EC50, the concentration causing a 50 % reduction in the algal growth rate in comparison to the control. EC50 values were estimated by fitting a three-parameter log-logistic function to the experimental concentration-effect data, using the drc package in R (Ritz et al., 2015).

## 2.7. Filter paper contact germination test

Two germination tests on cress (*Lepidium sativum* L.) seeds were conducted according to the procedure described in UNI 11357:2010 (UNI, 2010): in the first one, the leachates from PLA and PBS/PLA-based bioplastics were tested, while in the second one, the leachates from SB-based materials and sorbitol were tested. Fifty seeds of cress were incubated with three dilutions of each leachate in distilled water (10, 25, and 50 %, 10 mL) and placed on sterilized cellulose filter paper (Whatman no. 1) in a Petri dish sealed with laboratory film to prevent water evaporation. Sorbitol was tested in parallel at a concentration of 0.4 and 2 g L<sup>-1</sup>: these two concentrations were selected by considering that the content of sorbitol in SB-bag is 5 % (Parodi et al., 2023) and hypothesizing that all this amount would migrate once the material is immersed in water, therefore giving a concentration of sorbitol of 4 g L<sup>-1</sup>; this concentration was diluted 10 times to simulate the dilution of 10 % of the leachate from SB-bag, and 2 times to simulate the dilution of 50 %. Each dilution was tested in triplicate, while nine replicates were tested for the control (seeds exposed to 10 mL of distilled water). The samples were incubated for 72 hours at 23 ± 2 °C in the dark. At the end of the incubation period, the number of germinated seeds in each dish was counted and the percentage of germination was calculated based on the controls (i.e. relative seed germination rate). Seeds were considered germinated when the length of the root was ≥ 2 mm; the percentage of germinated seeds with shoot and root length ≤ 4 mm was also calculated. Relative shoot length (% on the control), and relative root length (% on the control) were reported at the end of the test for the germinated seeds by measuring the lengths on squared paper; these values were used to calculate the root-to-shoot ratio. Differences in these parameters between the treatments were evaluated using Tuckey's test and considered statistically significant if *P* < 0.05.

## 3. Results and discussion

### 3.1. Characterization of the bioplastic materials: organic additives

The bioplastic materials selected for the study contained a variety of inorganic and organic additives according to their application and polymer composition (Fig. 1 and Table 1). All the GC-MS detectable compounds found in the methanolic extracts of the bioplastic materials through a non-target analysis are not reported of particular concern for environment and health. The main organic additives have been grouped into categories, highlighting some general trends and a few peculiarities (Fig. 1). As expected, lactic acid derivatives (i.e. lactic acid, the dimer of lactic acid, and lactide) are ubiquitous in all the PLA-based materials, deriving from unreacted reagents during the polymerization process or intentionally added as plasticizers to improve the flexible properties of PLA: the greatest relative percentages were found in the methanol extract from rigid PLA items (83 % of all the GC-MS detectable compounds found in the extract of PLA-glass, 41 % in the extract of PLA-plate) and PBS/PLA ones (14 % in glasses, 7 % in plates), while the contribution of this class in the flexible PLA-packaging extract is < 6 %. The extracts from the materials that contain PLA also contain fatty acid derivatives (monoglycerides and free fatty acids commonly used as lubricants/plasticizers or antistatic agents) in high percentages: plates (51 and 21 % in the extracts from PBS/PLA and PLA-plates, respectively) are richer than glasses (11 %), while, again, the contribution of this class in the flexible PLA-packaging extract is < 4 %. Polyols like glycerol and 1,4-butanediol occur in all the materials containing PLA but with a relevant contribution only in the extract from PLA-plate (19 %); in all the other extracts polyols are < 5 %. Other small oxygenated plasticizers like the dicarboxylic succinic, adipic, and azelaic acids are present only in the PBS/PLA materials (4–6 %) (De Bruyne et al., 2023). In addition to the previously mentioned classes of compounds, PBS/PLA-glass extract is also characterized by the peculiar presence of 1,6-dioxacyclododecane-7,12-dione (28 %, a cyclic adipate that was previously also found as a

NIAS in biodegradable polyesters) (Balestri et al., 2019; Canellas et al., 2015; Capolupo et al., 2023), long-chain amides (erucamide and oleamide, 26 %), and oleonitrile (6 %), bio-based lubricants/smoothing agents in the plastic industry (Savva et al., 2023). Also, PLA-packaging extract contains long-chain amides and nitriles (13 %), together with tributyl acetyl citrate (50 %), a non-toxic plasticizer commonly blended with PLA, and abietic acid derivatives (abietic acid, dehydroabietic acid, and oxo-dehydroabietic acid, 5 %), known to have good emulsifying and adhesive properties that favorably influence polymer preparation and characteristics (Savva et al., 2023). The extracts from the two SB films (bags and plastic mulch) present the same categories of compounds but with different relative distributions and single molecules: unsaturated and saturated fatty acids, in the free form or esterified, are present in the extract of SB-bag (16 %), together with long-chain wax esters (39 %), characterized by the molecular weight of 400, 420 and 440; on the other hand, the relative distribution of fatty acid derivatives in the extract from SB-mulch is 8 % and saturated compounds are mainly present. Both extracts present the same relative distribution of long-chain amides (14 %) and erucamide as the main compound, even if the SB-bag extract also contains eicosenamide and behenamide. Both extracts contain 1,6-dioxacyclododecane-7,12-dione and small carboxylic acids (nonanoic, adipic, terephthalic, and lactic acids) in low amounts (≤ 4 %); like PLA-packaging film, also the extract from SB-bag contains tributyl acetyl citrate (1 %) and abietic acid derivatives (1 %). The main difference between the two extracts obtained from SB film is the categories of sugars/polyols and small oxygenated compounds, all of them considered as intentionally added compounds for starch gelatinization/plasticization (Talja et al., 2007; Zhang and Han, 2006): if sorbitol dominates the extract of SB-bag (18 %), other polyols are the main compounds found in the extract of SB-mulch (55 %), together with small oxygenated compounds containing ether moieties (15 %).

### 3.2. Characterization of the bioplastic materials: inorganic compounds

All the PLA materials are characterized by a low inorganic content (< 0.5 % and 2 ± 0.3 % for rigid glasses and plates, respectively, and 4 ± 0.1 % for food packaging film), as well as the two SB films (5 ± 1.1 % for shopping bags and 0.3 ± 0.1 % for mulch film) (Table S1 in ESI). On the other hand, about one-third of the weight of PBS/PLA materials is composed of inorganics (35 ± 2 % and 31 ± 0.1 % in plates and glasses, respectively) (Table S1 in ESI). Accordingly, the mean inorganic element concentrations found in PLA materials were the lowest ones and followed an order of abundance consistent with the literature (Lombardi et al., 2024), apart from the concentrations of Mg and Al, which were one order of magnitude higher: Mg > Al > Sn > Ti > Ca > Na > Fe > Cu > Zn > K >> other elements (373, 166, 67, 66, 29, 25, 11, 6, 5, 4 mg kg<sup>-1</sup>, respectively) (Table S3 in ESI). As expected from the rigidity of the materials, PLA-plate and PLA-glass contained concentrations of Mg, Al, Ti, Ca, and Fe species that were one order of magnitude higher than the ones found in PLA packaging (Table 1); these elements can be associated with the presence of inorganic fillers like CaCO<sub>3</sub>, kaolin, talc, commonly used to improve processing, rigidity, and dimensional stability (Klößner et al., 2021), rather than the presence of pigments since the tested PLA-plate and PLA-glass were white and transparent, respectively. SB-films contained Ti >> Al > Na > Ca, > Mg > Sn > K > Fe > Cu > Ba > Zn > Sr (2563, 132, 117, 106, 87, 81, 79, 49, 37, 23, 7, 3 mg kg<sup>-1</sup>, respectively) (Table S3 in ESI). SB-mulch had the highest concentration of Na, Sn, Mg, Fe, K, Ti, Cr, and Mn (228, 165, 153, 133, 131, 127, 4 and 4 mg kg<sup>-1</sup>, respectively) (Table 1). The inorganic composition of colored and white pieces of SB-bag was similar, except for higher concentrations of Cu, Ba, and Sr species (presumably related to the use of specific inorganic pigments) in the colored ones and higher Ti concentrations in white ones (associated with TiO<sub>2</sub>) (Samori et al., 2021) (Table 1). PBS/PLA-plate and PBS/PLA-glass had the highest inorganic content with the following order of abundance: Mg >> Ti >> Al >> Fe >> Na > Ca > Sn > K > Mn > Zn > Ba > Sr (9385, 2574, 629, 260, 84,

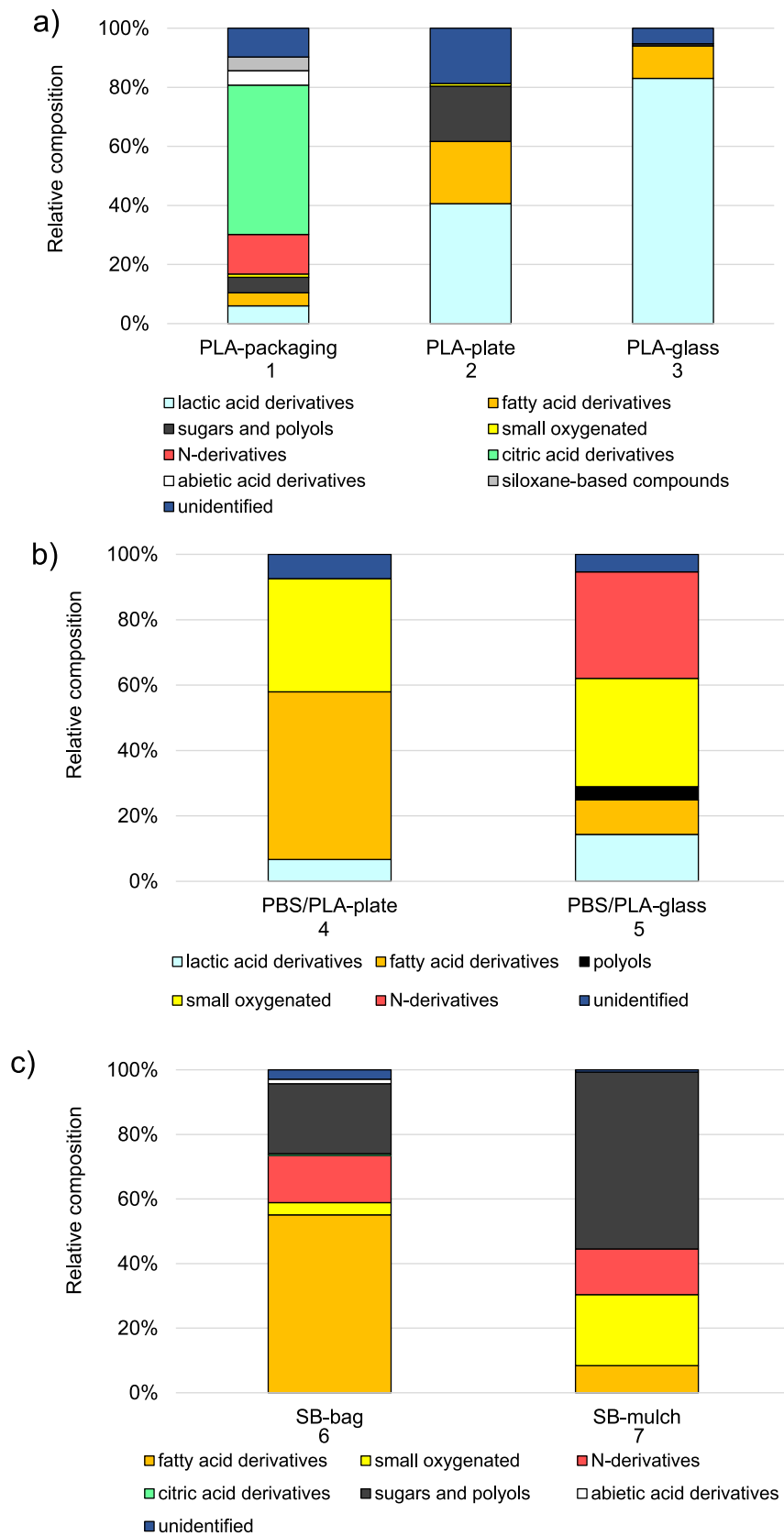


Fig. 1. Relative distribution of the GC-MS detectable compounds identified in the methanolic extracts of PLA- (a), PBS/PLA- (b), and SB-materials (c).

**Table 1**

Concentrations of the elements in the tested bioplastic materials (data are reported as the mean of two replicates, mg kg<sup>-1</sup>). Detection limit (d.l.): 0.005 mg kg<sup>-1</sup>. The elements are reported based on average abundance.

	PLA-packaging	PLA-plate	PLA-glass	PBS/PLA-plate	PBS/PLA-glass	SB-bag	SB-bag color	SB-mulch
Mg	17	628.42	475.32	8572.91	10,196.79	49.91	59.71	152.9
Ti	0	41.46	157.42	5086.93	61.4	7403.8	158.8	127.43
Al	10.81	103.74	383.47	887.87	369.63	136.66	128.87	131.8
Fe	2.98	12.92	16.36	322.72	197.87	6.26	7.79	133.53
Na	38.92	14.38	23.13	102.55	64.7	42.73	81.25	228.33
Ca	1.07	47.56	37.78	46.4	20.61	136.75	146.77	35.44
Sn	147.2	17.24	37.72	11.07	14.38	42.01	36.19	164.72
K	6.94	2.69	3.25	5.81	4.29	37.43	67.61	131.22
Cu	0.55	0.41	16.31	0	0.82	0.27	108.96	1.73
Ba	0.23	0.38	0.25	3.06	1.15	0.32	67.52	1.7
Zn	0.08	3.22	12.13	1.44	5.78	10.13	3.26	9.07
Sr	1	1.18	0.03	0.99	2.5	1.36	5.99	3.06
Mn	0.21	0.31	0.94	2.54	5.58	0.23	0.28	3.74
Cr	0.13	0.46	0.72	0.32	1.47	1.05	0.86	4.13
Ni	0.25	0.3	0.16	0.1	0.6	2.99	0.41	1.11
B	0.37	0.04	0.08	0.01	0.54	0.04	0.23	0.82
V	0.01	0.06	0.07	0.96	0.58	0.08	0.08	0.28
Pb	0.04	0.08	0.19	0.25	0.06	0.31	0.33	0.28
Cd	<0.005	0.01	0.33	0.82	0.2	0.06	0.06	0.01
Ag	<0.005	0.01	0.21	0.01	0.01	0.06	0.04	0.82
Mo	0.01	0.04	0.22	0.03	0.02	0.05	0.07	0.21
Co	<0.005	0.01	<0.005	0.1	0.23	0.04	0.04	0.11
As	0.01	0.02	0.14	0.14	0.05	0.04	0.02	0.06
Sb	0.06	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.18
Hg	<0.005	<0.005	0.04	<0.005	0.01	0.01	0.02	<0.005
Tl	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.01	0.02
Be	<0.005	<0.005	<0.005	0.02	0.01	<0.005	<0.005	<0.005

27, 33, 13, 5, 4, 4, 2, 2 mg kg<sup>-1</sup>, respectively) (Table S3 in ESI). As for PLA, also for PBS/PLA, the concentrations are similar among the two rigid materials except for Ti, more abundant in PBS/PLA plates than in glass (5087 and 61 mg kg<sup>-1</sup>, respectively) (Table 1). The majority of the concentrations here found in PBS/PLA items were lower than what was reported in the literature for the same polymer blend (Lombardi et al., 2024) (Table S3 in ESI).

### 3.3. Characterization of the leachates: organic additives

The non-target GC-MS analysis of the leachate solutions obtained after 14 days of incubation of the bioplastic materials in water at a concentration of 80 g L<sup>-1</sup> did not reveal the presence of hazardous organic compounds, and only a partial migration of the most polar additives present in the materials was observed (Fig. 2). This was in line with the organic compound composition reported in the literature for the leachates from SB-bag and PLA materials (Balestri et al., 2019; Capolupo et al., 2023). No N-derivatives, citric acid derivatives, or abietic acid derivatives were observed. In contrast, small oxygenated compounds like carboxylic acids and polyols like glycerol were identified in almost all the leachates: lactic acid and adipic acid were the main carboxylic acids found in the leachates from PLA materials and SB-bag, respectively, while lactic acid and succinic acid characterized the leachates from PBS/PLA materials. Sorbitol and various polyols were the most abundant compounds in SB-bag and SB-mulch leachates, respectively. Boric acid (H<sub>3</sub>BO<sub>3</sub>) was detected in the leachates prepared from PLA and PBS/PLA materials, presumably derived from boron compounds that can be used as flame-retardants for (bio)plastic materials (Avci et al., 2024; Hahladakis et al., 2018).

### 3.4. Characterization of the leachates: inorganic compounds

The ICP-MS results of the leachate solutions obtained after 14 days of incubation of the bioplastic materials in water at a concentration of 80 g L<sup>-1</sup> revealed that the main dissolved elements were Na, Mg, K, and Ca, with concentrations ranging from 0.1 to 100 mg L<sup>-1</sup>. Na is the most abundant element in all the leachates, particularly in those from PBS/

PLA bioplastics (101.81 and 18.51 mg L<sup>-1</sup> for glasses and plates, respectively), followed by the leachates from PLA-based materials (about 5 mg L<sup>-1</sup>). The Na, Mg, K, and Ca concentrations in the SB-bag leachate were one order of magnitude higher than those from SB-mulch, 15–12 mg L<sup>-1</sup> vs 1.9–1.3 mg L<sup>-1</sup> (Fig. 3). The same concentration of Mg was found in both the leachates from PBS/PLA bioplastics, while the leachates from PLA-based materials had the lowest concentrations of Mg, K, and Ca (0.1–1 mg L<sup>-1</sup>, see Table S4 in ESI).

The concentrations of the trace elements (Ba, Zn, Fe, Ti, Mn, Sn, Ni, As, Cr, Pb, Sb, V, Co, and Hg) in the leachates from SB-based materials were 2 orders of magnitude higher than those found in all the other leachates, with the highest values found in the leachates from SB-bag. Fe and Mn were the only elements more concentrated in the SB-mulch, reflecting their higher concentrations in the original material. Zn, Sr, B, Fe, and Ti have concentrations above 100 µg L<sup>-1</sup>, while Ba reaches 2.2 mg L<sup>-1</sup>, reflecting the abundance in the original composition of the analyzed materials (Table 1). The concentrations of Fe, Zn, As, and Ni found in these samples were 2–10 times higher than those reported in the literature for leachates in seawater (Capolupo et al., 2023), while the concentrations of Cu and Pb were similar (23 and 2 µg L<sup>-1</sup>, respectively). The concentrations of B and Al in the leachates from PLA-based items were around 25 µg L<sup>-1</sup>, followed by Sr, Zn, Ba, Fe, and Cu ranging from 1 to 10 µg L<sup>-1</sup>. Capolupo et al. (2023) analyzed leachates from PLA items, but only Cu was detected at a double concentration than the present study. The leachates from both PBS/PLA items contained B, Sr, Zn, Fe, Ba, Cu, Mn, and Sn. The concentrations of B, Sr, Fe, Zn, Mn, and Sn in the leachate from PBS/PLA-glass (81, 59, 4, 5, 2, and 0.5 µg L<sup>-1</sup>, respectively) were twice as high as those found in the leachate from PBS/PLA-plate.

### 3.5. Algal growth inhibition tests

The toxicity of nano-, micro-, and mesoplastics towards freshwater and marine microalgae has been investigated in the last years, given the crucial role that these organisms have in all aquatic ecosystems as primary producers (Capolupo et al., 2020; Chae et al., 2020; Luo et al., 2020, 2019; Rummel et al., 2022; Schiavo et al., 2021; Simon et al.,

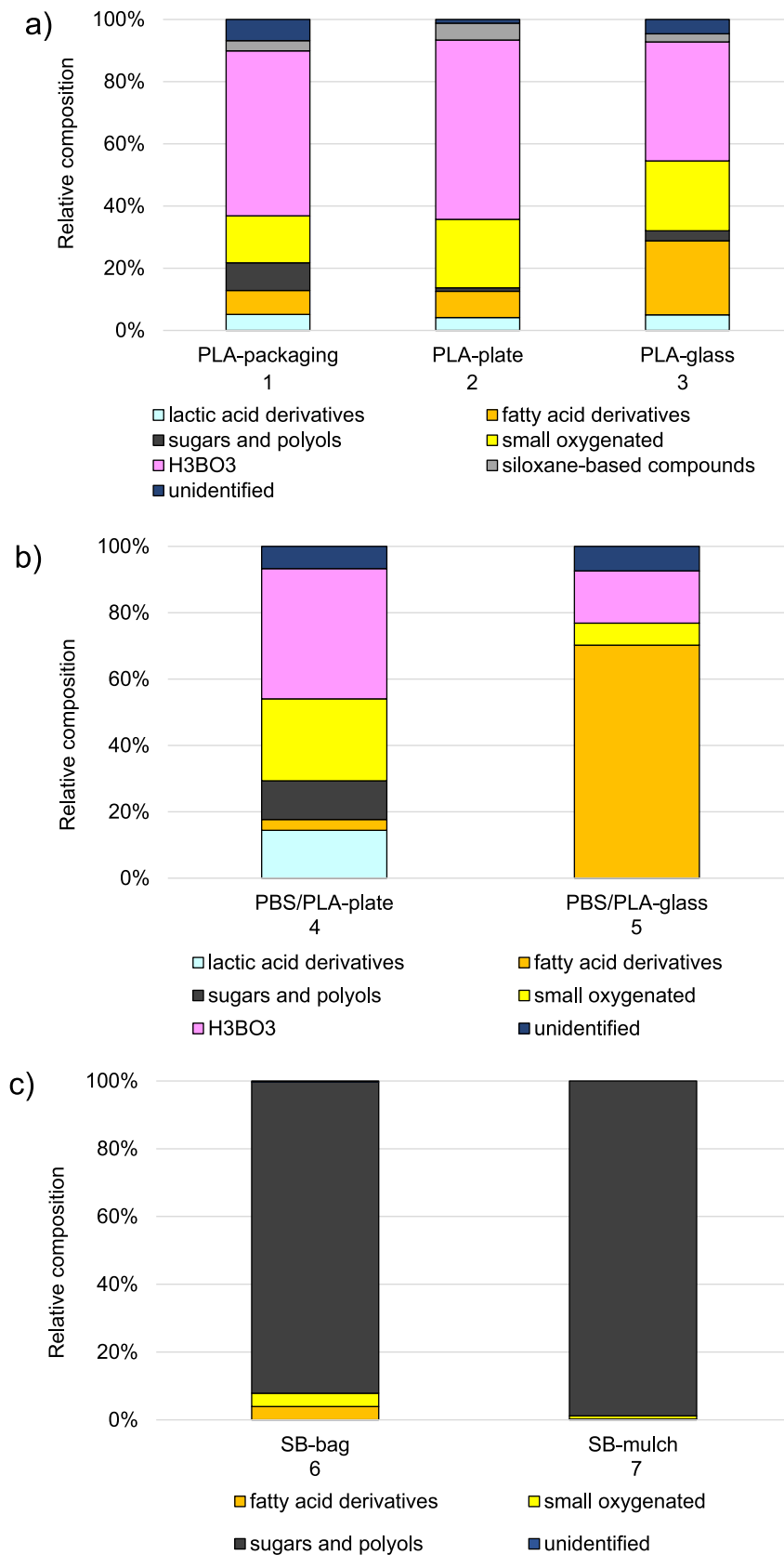


Fig. 2. Relative distribution of the GC-MS detectable compounds identified in the leachates obtained from PLA- (a), PBS/PLA- (b), and SB-materials (c).

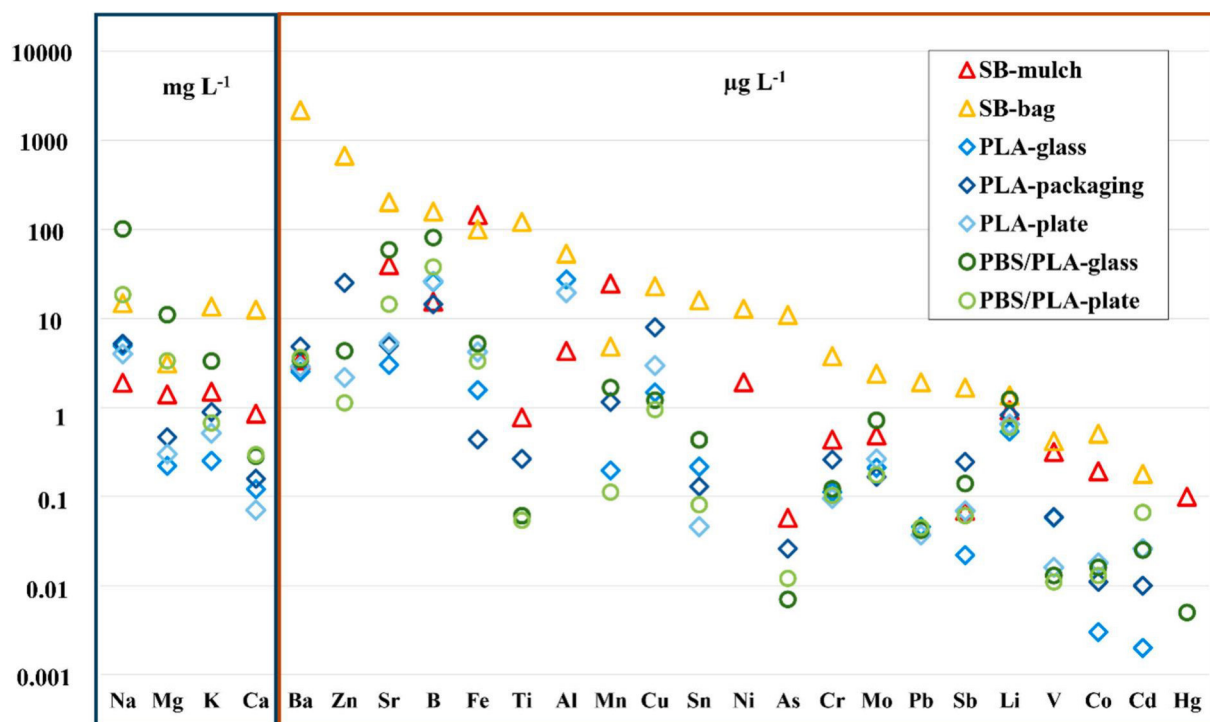


Fig. 3. Concentrations of inorganic elements in the leachates obtained from PLA-, PBS/PLA-, and SB-materials.

2021). The effects related to the physical absorption of plastics on the cells and the combined toxicity of plastics and pollutants (i.e. Trojan horse effects) have been described in the literature much more than the effects given by exposure to plastic additives (Amaneeh et al., 2023; Gunaalan et al., 2020; Nam et al., 2022). To the best of our knowledge, the effects of bioplastic leachates on *R. subcapitata* or other microalgae have never been reported before, while there are reports on the effects of leachates from expanded polystyrene (Chae et al., 2020), polyurethane sponge microplastics (Luo et al., 2019), and microplastics made of polyethylene, car tyre rubber, polypropylene, polyethylene terephthalate, polystyrene, and polyvinyl chloride (Capolupo et al., 2020; Luo et al., 2020). The leachates obtained from the bioplastic materials were diluted at 6 different concentrations from 75 to 0.5 % of total leachate and tested against the control in an algal growth inhibition test. The effect of the bioplastic leachates on the algal-specific growth rate was concentration-dependent and all the leachates apart from the one obtained from PLA plates caused an inhibition of the growth of about 70 % at the highest concentration tested (75 %, Fig. S2 in ESI). Contrary to the findings reported on the exposure to leachates from expanded polystyrene microplastics (Chae et al., 2020), the present study did not observe an increase in the photosynthetic activity of microalgae and the consequent enhanced cell growth. Here in particular, the leachates obtained from PLA-based materials were the least toxic among the tested ones: PLA-packaging and PLA-glass leachates did not show any inhibition at the three lowest tested concentrations (0.5–3.7 %). The LOEC, i.e. the lowest concentration that caused a statistically significant reduction in the specific growth rate, compared to the control was 10.1 % (Fig. S2 in ESI). The estimated EC50 values were 36 % (95 % confidence interval: 31–42 %) and 47 % (42–53 %) for PLA-packaging leachate and PLA-glass leachate, respectively, highlighting a similar algal response to the exposure of both (Table 2). The EC50 value for PLA-plate leachate was not determinable given the fact that none of the tested concentrations caused a significant growth inhibition. The lowest tested concentration (0.5 %) of the leachates obtained from PBS/PLA-based materials caused a significant growth inhibition. In the case of PBS/PLA-glass leachate, an inhibition of about 25 % of the growth rate was observed at the concentrations from 0.5 to 27.5 %, while in the case of PBS/PLA-

Table 2

Estimated toxicity values (EC50, % of total leachate; LOEC, Lowest-Observed Effect Concentration, % of total leachate) for algae growth inhibition. Values in parentheses represent 95 % confidence intervals.

Bioplastic	EC50 (% of the total leachate)	LOEC (% of the total leachate)
1 PLA-packaging	36 (31–42)	10.1
2 PLA-plate	>75	> 75
3 PLA-glass	47 (42–53)	10.1
4 PBS/PLA-plate	9.7 (6.2–15.1)	0.5
5 PBS/PLA-glass	58 (46–74)	0.5
6 SB-bag	10.9 (6.2–19.1)	0.5

plate a growth inhibition of about 50 % was observed already at the concentration of 10 % (Fig. S2 in ESI). The estimated EC50 values were 58 % (46–74 %) for PBS/PLA-glass and 9.7 % (6.2–15.1 %) for PBS/PLA-plate (Table 2). The lowest concentration (0.5 %) of the leachate obtained from SB bags caused a significant growth inhibition; however, the highest concentration tested (75 %) caused an effect lower than the other leachates tested at the same concentration (excluding the non-toxic leachate from PLA-plate) (Fig. S2 in ESI). The estimated EC50 value was 10.9 % (6.2–19.1 %), similar to what was found for PBS/PLA-plate (Table 2). PBS/PLA-plate, PBS/PLA-glass, and SB-bag leachates were characterized by the lowest LOEC values (0.5 %) and also by the highest concentrations of inorganic elements (23–117 mg L<sup>-1</sup>), while the leachate obtained from PLA-plate was the one with the highest LOEC (>75 %) and the lowest concentration of inorganic elements (5 mg L<sup>-1</sup>).

These results indicate that the release of biologically active substances in the aquatic environment from the tested bioplastic materials cannot be ruled out and that inhibition of the specific growth rate occurred above 10 % of leachate in the case of PLA-based materials, and already at 0.5 % of leachate for the PBS/PLA and SB-based materials tested. These were characterized by the highest values of Na, Mg, Sr, and B, and by a total amount of inorganic elements above 10 mg L<sup>-1</sup> (23 and



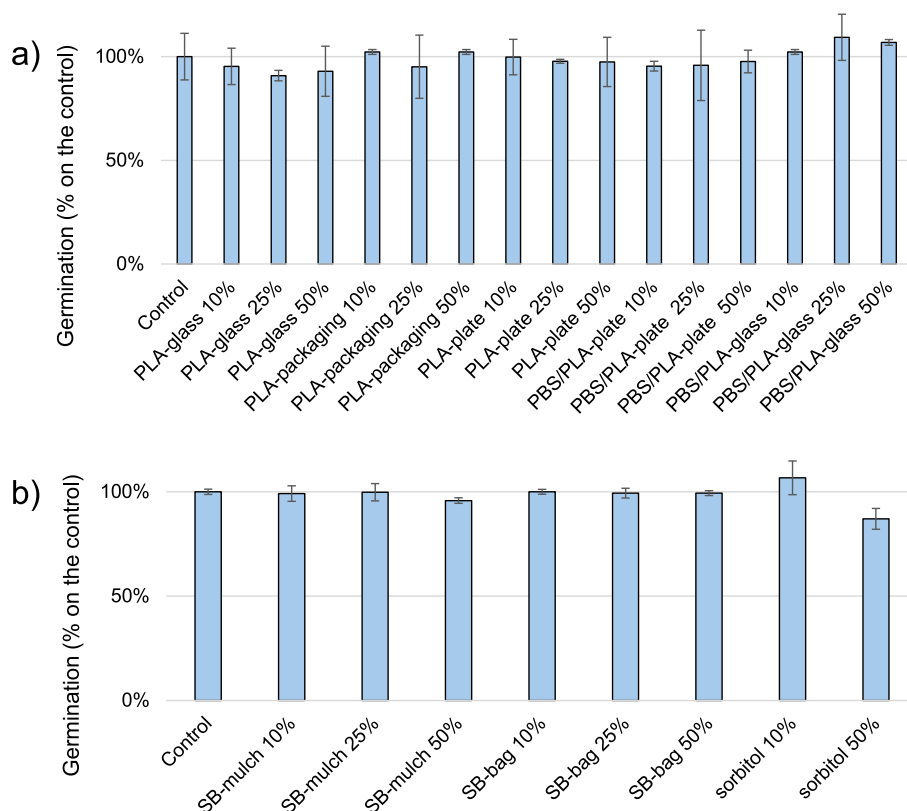
117 mg L<sup>-1</sup> for PBS/PLA plates and glasses, respectively, and 48 mg L<sup>-1</sup> for SB-bag leachate, Table S4 in ESI). The estimated toxicity values (EC50 values) were above the concentration of 75 % of the total leachate in the case of PLA-plate, between 30 and 40 % in the cases of PLA-glass, PLA-packaging, and PBS/PLA-glass, and around 10 % in the cases of PBS/PLA-plate and SB-bag. These values fall in the range of observations reported in the literature on the effects of some fossil-based plastic leachates (from 0.5 to 64 % of total leachate), prepared from an initial plastic concentration of 80 g L<sup>-1</sup>, on the same algal species. In particular, the EC50 values here found for leachates from PLA packaging and glasses, and PBS/PLA-glass (36, 47 and 58 % of total leachate, respectively) were close to the EC50 values reported for polypropylene leachates (64 %, Capolupo et al., 2020), while the EC50 values found for leachates from PBS/PLA plates and SB-bag (9.7 and 10.9 %, respectively) were in the same order of magnitude of polystyrene-leachates (23 %, Capolupo et al., 2020) but 10–20 times less toxic than leachates from car tyre rubber and polyvinyl chloride (0.5 and 1.6 %, respectively, Capolupo et al., 2020). On the other hand, leachate from PLA plates did not cause any algal growth inhibition at the highest tested concentration, similar to what is reported for polyethylene terephthalate leachate (Capolupo et al., 2020). These similarities highlight that the bio-based origin and the biodegradability of bioplastic materials do not ensure per se lower effects on freshwater microalgae than conventional plastics (Capolupo et al., 2020; Zimmermann et al., 2020) and that the toxicity is presumably ruled by the content of inorganic and organic additives released in water. On the other hand, it is important to underline that the sensitivity of the tested organisms can influence the response: an EC50 value corresponding to a concentration of plastic in water of 0.5 g L<sup>-1</sup> was found for the microalga *Rhodomonas salina* exposed to PLA-leachates (Laranjeiro et al., 2024), while here EC50 values above the PLA concentration of 30 g L<sup>-1</sup> were observed for all the

leachates from PLA items.

### 3.6. Seed germination tests

There are two routes through which plastics and bioplastics can enter soil ecosystems: the first one is the leakage of (bio)plastic items not used in agricultural applications and the second one is the leakage of items made of (bio)plastics used for agricultural activities that can be damaged, degraded or discarded (FAO, 2021). Although the majority of bioplastic items available on the market nowadays are classified as “compostable” and “biodegradable”, they are subjected to an unavoidable leakage into terrestrial and aquatic environments like all the other types of plastic, due to mismanaged waste (the projections suggest 44 Mt. of plastics enter the environment by 2060) (OECD, 2022). The impact of bioplastics and their leachates on terrestrial primary producers has been studied in recent years (Balestri et al., 2019; Celletti et al., 2023; Chah et al., 2022; Martin-Closas et al., 2014; Wright et al., 2024), especially for what concerns SB-bag leachates and residues, but the effects of bioplastic residues/leachates on the germination success of seeds are still not clear (Barbale et al., 2021; Boots et al., 2019; Li et al., 2021; Zarski et al., 2020). The phytotoxicity of SB-bag leachate on early seedling growth has already been reported, (Balestri et al., 2019), while inhibition of the growth of cress roots has been described after exposure to PLA microplastics even at low concentrations (0.02 w w<sub>soil</sub><sup>-1</sup>) (Liwarska-Bizukojc, 2022).

None of the tested leachates at any dilution (10, 25, and 50 %) inhibited the germination of cress seeds (*Lepidium sativum* L.) (Fig. 4), in agreement with the results obtained after the exposure of cress seeds to SB-bag leachates prepared at higher plastic-to-water ratios (100 and 200 g L<sup>-1</sup>, Balestri et al., 2019; Barbale et al., 2021). The number of normal seedlings with reduced growth was not significantly different



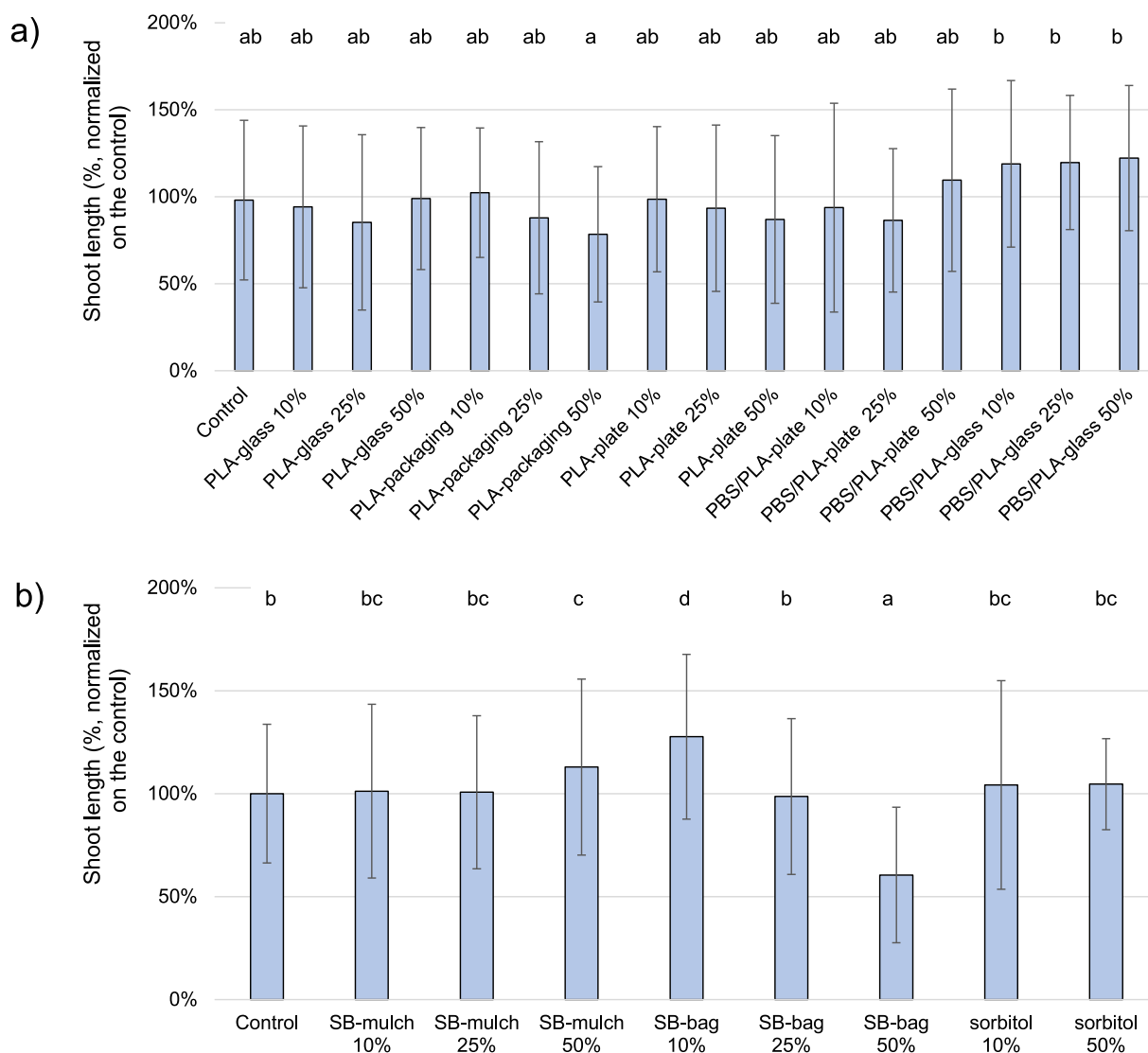
**Fig. 4.** Seed germination (% normalized on the control) after exposure to leachates obtained from a) PLA- or PBS/PLA-bioplastics, and b) SB-bioplastics, tested at three dilutions (10, 25 and 50 %) against the control. Sorbitol was tested at 0.4 and 2 g L<sup>-1</sup> (sorbitol 10 % and sorbitol 50 %, respectively) under the same conditions. No statistically significant differences were observed between the treatments and the control. Data are reported as the mean  $\pm$  standard deviation;  $n = 3$  for the treatments and  $n = 9$  for the control.

from the control regardless of the leachate and the concentration tested (Fig. S3 in ESI), in agreement with some previous studies (Barbale et al., 2021); however, other studies reported abnormal seedling development also when cress seeds were exposed to SB-bag leachates prepared by using a lower plastic-to-water ratio ( $2 \text{ g L}^{-1}$ , Balestri et al., 2019), analogously to what was observed with leachates prepared from polyethylene bags.

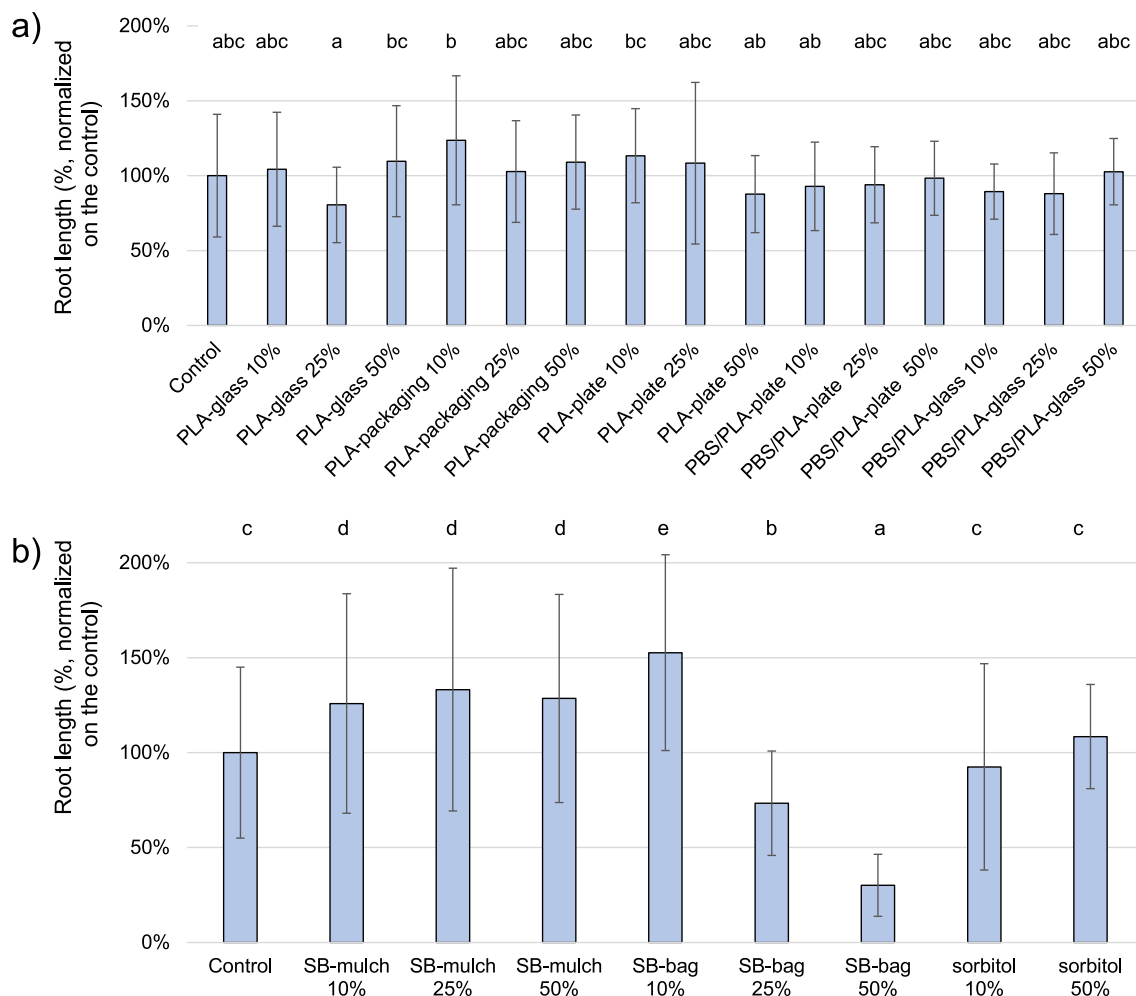
On the other hand, the effects presently observed on the shoot and root lengths were dependent on the original bioplastic and the tested concentration, confirming germination as a less sensitive endpoint than the plant's early development (Martin-Closas et al., 2014). Specifically, no leachate from PLA- and PBS/PLA-based bioplastics affected shoot and root elongation (Figs. 5 and 6), while SB-mulch leachates slightly promoted shoot and root elongation at all the tested dilutions. The leachate from the SB-bag was inhibitory at the 50 % dilution on both shoot and root (shoot length was  $60 \pm 33 \%$  of the control, while root length was  $30 \pm 16 \%$  of the control) but caused an elongation higher than the control at the 10 % dilution ( $128 \pm 40 \%$  in the case of shoot and  $153 \pm 52 \%$  in the case of root) (Figs. 5b and 6b). This result disagreed with the strong reduction of hypocotyl and radicle length reported in the literature after exposure to SB-bag leachates prepared from a plastic-to-water

ratio of  $2 \text{ g L}^{-1}$  (Balestri et al., 2019). Sorbitol tested at  $0.4$  and  $2 \text{ g L}^{-1}$  did not cause any effect, thus the hormesis observed after exposure to the leachate from the SB-bag was not attributable to this plasticizer. Enhanced growth of lettuce and tomato has been already observed in the literature after exposure to adipic acid at a concentration of  $5 \text{ mg L}^{-1}$ , lactic acid, and 1,4-butanediol (both at concentrations up to  $0.5 \text{ g L}^{-1}$ , Martin-Closas et al., 2014); all of them are components of SB-bags here used although in a minor amount in comparison to other organic additives (see Section 3.1); adipic acid was identified in the leachate of SB-bag too, but it did not represent  $>1.5 \%$  of all the GC-MS detectable compounds.

The root-to-shoot ratios were significantly higher than the control for the seeds treated with the 10 and 25 % dilution of SB-mulch leachate and the 10 % dilution of SB-bag leachate, suggesting that shoot growth was severely inhibited. Ratios statistically lower than the control were obtained with the 25 and 50 % dilutions of SB-bag leachate, in line with the literature (Fig. 7) (Balestri et al., 2019). The main significant difference between the leachate from SB-bag and all the other ones here tested was the concentrations of some major elements like K and Ca (10 times higher than in the other leachates:  $13.6$  and  $12.6 \text{ mg L}^{-1}$ , respectively), and some trace elements, in particular  $\text{Ba} \gg \text{Zn} \gg \text{Sr}, \text{B}, \text{Ti} > \text{Al}, \text{Cu}$



**Fig. 5.** Effect of the leachate of bioplastic materials on the shoot elongation of cress seeds: a) PLA- and PBS/PLA-based materials; b) SB-bioplastics, tested at three dilutions (10, 25, and 50 %). Sorbitol was tested at  $0.4$  and  $2 \text{ g L}^{-1}$  under the same conditions. Treatments marked with different letters were significantly different; data are reported as the mean  $\pm$  standard deviation;  $n = 3$  for the treatments and  $n = 9$  for the control.



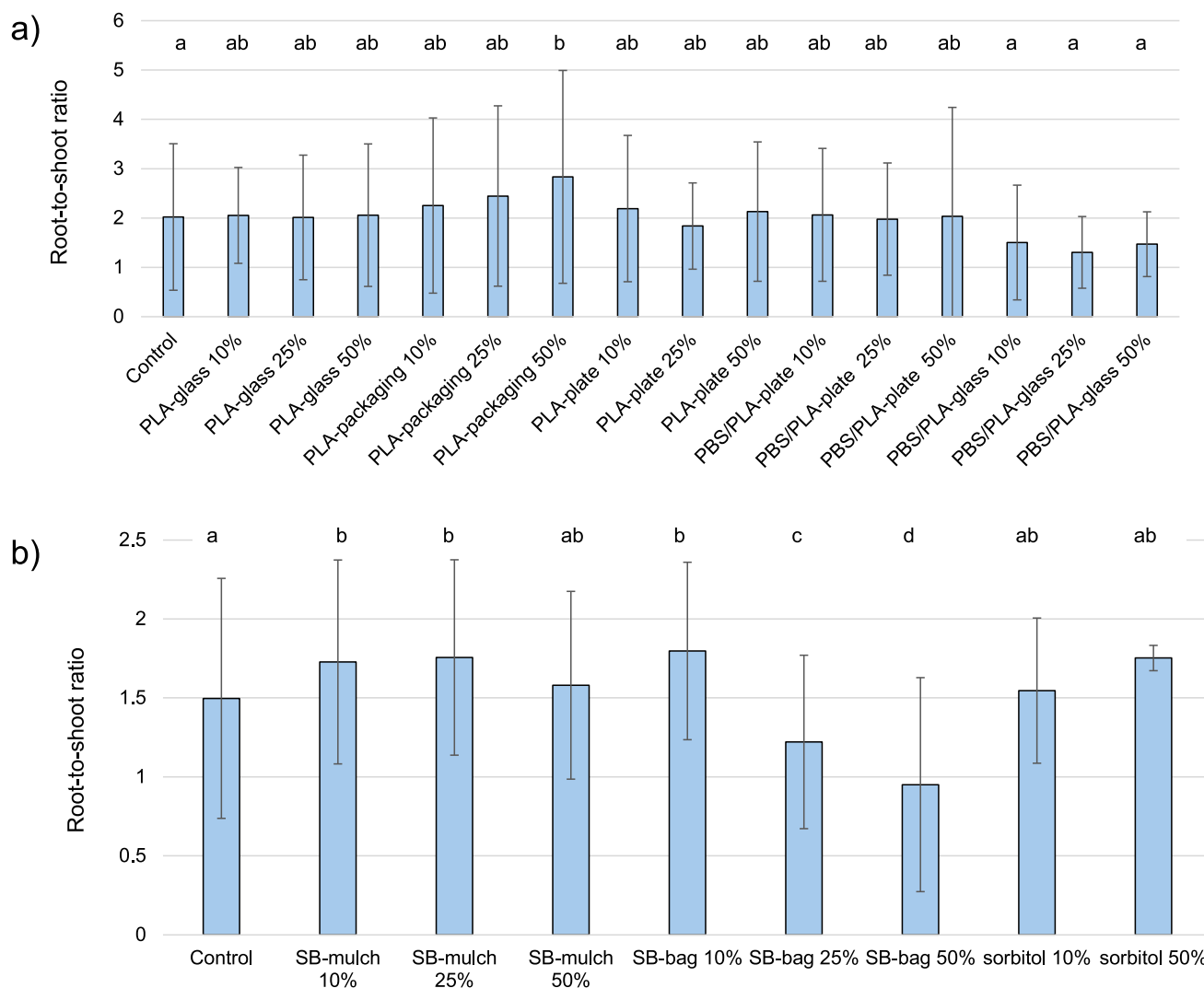
**Fig. 6.** Effect of the leachate of bioplastic materials on the root elongation of cress seeds: a) PLA- and PBS/PLA-bioplastics, and b) SB-bioplastics, tested at three dilutions (10, 25, and 50 %). Sorbitol was tested at 0.4 and 2 g L<sup>-1</sup> under the same conditions. Treatments marked with different letters were significantly different; data are reported as the mean  $\pm$  standard deviation; n = 3 for the treatments and n = 9 for the control.

and Ni (Fig. 3). K is a crucial macronutrient for various plant functions including photosynthesis and stomatal control, membrane potential regulation, reduction of reactive oxygen species (ROS) levels, and contribution to abiotic stress tolerance, while Ca is involved in growth regulation (Johnson et al., 2022). Ba is a nonessential element that has toxic effects on most organisms including plants at concentrations higher than 100  $\mu$ M; on the other hand, it can have a positive effect on germination (Sleimi et al., 2021). Zn can exert a hormetic effect on plant growth too, promoting biomass accumulation by enhancing the photosynthetic ability, the chlorophyll content, and the activities of antioxidant enzymes, especially at concentrations in the range of 10–100  $\mu$ M; however, it might cause inhibition of shoot growth at concentrations above 300  $\mu$ M (Manzo et al., 2011; Poschenrieder et al., 2013; Wei et al., 2022). B, Cu, Fe, Mn, and Mo are essential micronutrients taken up and consumed by plants in small amounts, playing an eminent role in plant growth, development, and metabolism (Tripathi et al., 2015). Sr is not primarily toxic to plants and can be adsorbed and partially substitute Ca (Isermann, 1981). Furthermore, some algae can also preferentially use Sr instead of Ca. In most cases, Ca and Sr maintain the same ratio in both the nutrient solution and plant parts, thus making the effects of Sr largely invisible to the plant (Isermann, 1981). Ni is not considered as a micronutrient. However, barley plants deprived of nickel showed significantly lower root and shoot weights compared to the control, with a reduction of 30 % (Brown et al., 1987). Additionally, the levels of Fe in plant tissues were positively correlated with the levels of nickel in the

same tissues (Brown et al., 1987). A recent study reveals that Ti has a beneficial effect on crop phenology stimulating germination, root formation, vegetative growth, as well as resistance to biotic or abiotic stress conditions. Additionally, Ti has been found to enhance the uptake of both macro and micronutrients (Bacilieri et al., 2017). Sn is rarely investigated as an inhibitor of plant growth, its uptake only occurs at concentrations above 2 mg L<sup>-1</sup>, and it is mostly retained in the root systems (Müller et al., 2015). Sn additions also resulted in no visual toxicity symptoms in plants. It is important to underline that the inhibitory/promoting effects reported above were observed on the growth of plants in the field and not in vitro tests like the ones here performed. Moreover, the concentrations of all the inorganic elements found in the leachates were far below the ones reported to have a positive or negative effect on plants. Therefore, the growth inhibition observed for SB-bag leachate at 25 % and 50 % could be attributed to the cumulative effect of more elements, organic and inorganic, occurring in the leachate.

#### 4. Conclusions

Bioplastics are characterized by a variety of organic additives and inorganic components used as plasticizers, fillers, pigments, lubricants, etc., that can be released once they enter aquatic ecosystems. The chemical profile of these additives is complex but seems to be correlated with the polymer type more than the item functionality: the rigid



**Fig. 7.** The root-to-shoot ratio of seeds exposed to leachates obtained from a) PLA- or PBS/PLA-bioplastics, and b) SB-bioplastics, tested at three dilutions (10, 25, and 50 %). Sorbitol was tested at 0.4 and 2 g L<sup>-1</sup> under the same conditions. Treatments marked with different letters were significantly different; data are reported as the mean  $\pm$  standard deviation; n = 3 for the treatments and n = 9 for the control.

bioplastic items made by PBS/PLA here analyzed had the highest concentrations of inorganic elements, while the PLA-based items, both rigid and flexible, had the lowest one; on the other hand, the two films made by starch blends had more inorganic additives than PLA films. The same held for the organic additives: some of them were peculiar to specific polymer types, like lactic acid derivatives present only in PLA and PBS/PLA-items, or sugars/polyols and N-derivatives (long-chain amides and nitriles) in SB films, while others like fatty acid derivatives and small oxygenated compounds were found in all the parent materials. The leachates from PLA items exhibited lower toxicity towards algae than the ones from SB and PBS/PLA materials, in line with a lower content of inorganic elements in the leachates themselves; a 0.5 % dilution of the leachates obtained from PBS/PLA-glass, PBS/PLA-plate and SB-bag caused significant effects on algal growth, suggesting possible concerns for aquatic photosynthetic organisms in enclosed ecosystems like lagoons, reservoirs, lakes or plastic accumulation “hotspots”. It is worth mentioning that a concentration of 0.5 % of the total leachate corresponds to a bioplastic concentration in water of 0.4 g L<sup>-1</sup>. By considering the estimation of suspended plastic concentrations in freshwater ecosystems like European rivers (0.0002–0.0054 g m<sup>-3</sup>, van Emmerik and Schwarz, 2020) or in the oceanic regions in which plastic waste accumulates like the Great Pacific Garbage Patch (the concentration of mesoplastics with a dimension of 0.5–5 cm has been estimated as

3.9 kg km<sup>-2</sup>, Lebreton et al., 2018) the order of magnitude of the tested concentrations of this study seems unrealistic. However, as stated by other authors (Capolupo et al., 2020; Nava et al., 2023), plastic concentration in enclosed ecosystems can be higher than those observed in subtropical oceanic gyres, thus information on possible adverse effects occurring in worst-case scenarios is useful (Zimmermann et al., 2019).

Evidence of toxicity of specific bioplastic items was not observed in the phytotoxicity experiments apart for SB-bag leachate: seed germination was not affected by any concentration of any tested leachate, while root and shoot elongation was significantly reduced only in the presence of a high concentration of SB-bag leachates (25 and 50 %). This suggests that leachates generated from PLA and PBS/PLA items, that could eventually enter soil ecosystems since they are biodegradable and compostable, should not arouse any particular concern for terrestrial photosynthetic organisms.

#### CRediT authorship contribution statement

**Alberto Crema:** Investigation. **Enrico Dinelli:** Writing – review & editing. **Elena Fabbri:** Writing – review & editing, Funding acquisition, Conceptualization. **Paola Galletti:** Writing – review & editing. **Nicolas Greggio:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Valentina Lastella:** Investigation. **Adriano**

**Parodi:** Investigation. **Andrea Pasteris:** Writing – review & editing. **Marta Pedrizzi:** Investigation. **Chiara Samori:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Data curation, Conceptualization.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Chiara Samori reports financial support was provided by European Union Next-Generation EU National Recovery and Resilience Plan (NRRP). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

<sup>1</sup>H NMR characterization of the PBS/PLA blend; ash content and inorganic composition of the bioplastics; inorganic composition of the leachates; percentage inhibition of the algal growth and seed germination after exposure to the leachates in comparison to the control; germinated seeds of cress with not developed roots and shoots and the root-to-shoot ratio of seeds exposed to leachates. Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.177205>.

### Data availability

Data will be made available on request.

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