Waste and Biomass Valorization (2023) 14:1097–1111
https://doi.org/10.1007/s12649-022-01922-7

ORIGINAL PAPER

Back to the Origins: Potential of Beach-Cast Macroalgae as Biofertilizer

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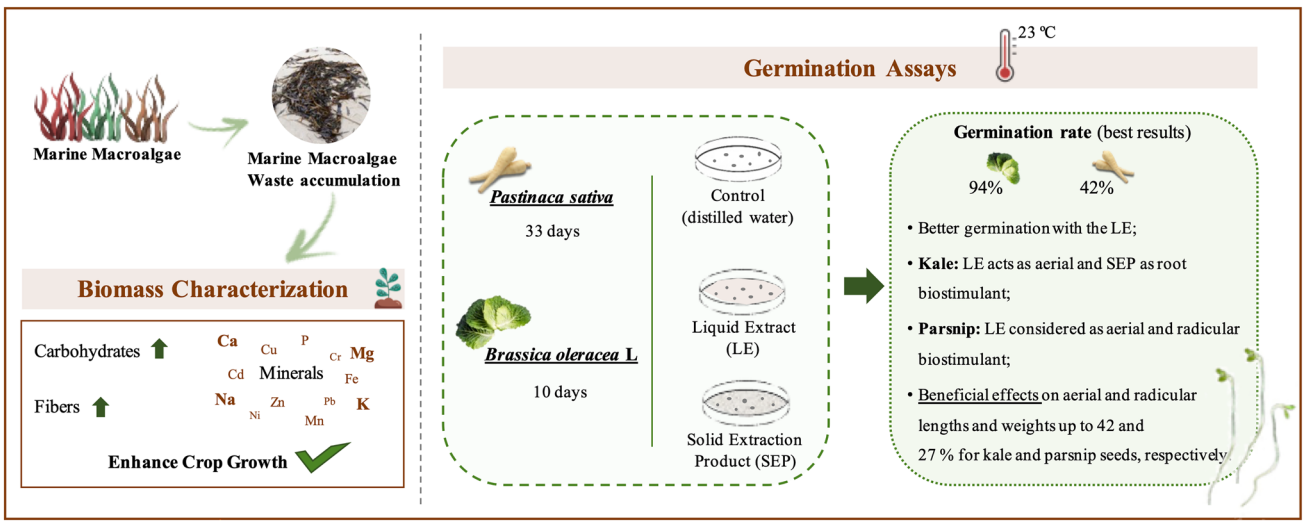
Received: 5 May 2022 / Accepted: 29 August 2022 / Published online: 18 September 2022

Abstract

In the present study, Marine Macroalgae Waste (MMW) biomass, mainly composed of *Saccorhiza polyschides*, collected from the seashore in northern Portugal, was characterized and seed germination was performed to evaluate the potential of re-exploiting this biomass for agricultural purposes. Kale (*Brassica oleracea* L) and parsnip (*Pastinaca sativa*) seeds were used in laboratory assays (23 °C; 10—33 days) to study the effect of using two types of MMW derived products: liquid (water extraction, 0.12 g/mL; diluted at 1.2 vol.%) and solid (after filtering the extract); distilled water was used as control. The germination rate and growth parameters (radicular and aerial part lengths and weights) of the seedlings were further measured. The raw biomass showed promising results for agricultural purposes due to the high carbohydrate content (69.97 ± 0.08 wt.%), mainly composed by fibers (63.4 ± 0.1 wt.%), and the presence of minerals (ash content of 18.54 ± 0.07 wt.%). High amounts of K (2242 mg/100 g), Ca (1886 mg/100 g), Na (985 mg/100 g) and Mg (546 mg/100 g) were found in the ash, with the toxic elements content being within the regulated limits. Seeds germinated better with the liquid extract than with the solid product, with significantly higher ($p < 0.05$) aerial and radicular lengths and weights compared to the control, from up to 42 and 27% for kale and parsnip seeds, respectively. The results show that the production of biostimulants, biofertilizers or soil conditioners from this resource might be feasible and that further studies should be conducted.

Graphical abstract



Keywords Marine macroalgae waste · Seed germination · Seedling development · Growth promoters · *Brassica oleracea* · *Pastinaca sativa*

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Statement of Novelty

Waste recovery might present a very relevant role towards the production of sustainable products, allowing waste treatment and contributing to positive environmental, social and economic impacts. The production of biofertilizers/biostimulants from low-cost raw materials is of high interest aiming to effectively replace the synthetic products, conventionally used. Marine Macroalgae Waste (MMW) was traditionally used as an organic soil fertilizer, but this practice fell into disuse mostly due to economic reasons; thus, studies devoted on MMW biomass are still scarce. The present study accessed the effect of using MMW derived products for seed germination, evaluating their potential to act as fertilizers/biostimulants for further use in sustainable agriculture systems.

Introduction

Marine Macroalgae Waste (MMW)—beach-cast macroalgae or drifted Marine Macroalgae (MM)—is a natural resource which was traditionally collected by coastal populations and used as a solid organic fertilizer or soil conditioner [1, 2]. Nowadays, such traditional use has fallen into disuse essentially due to the costs associated to collection and transport of the biomass, and the widespread use of synthetic, low-cost fertilizers [3]. However, presently, the replacement of conventionally used chemical fertilizers by natural/organic substances is of high relevance not only due to the economic reasons, but also due to health and environmental impacts of the former; this approach also contributes to the United Nations Sustainable Development Goals, promoting sustainable consumption and production patterns. Thus, the use of macroalgal extracts as biostimulants or biofertilizers should be explored. Furthermore, the accumulation of MMW on the beaches creates several problems related to eutrophication of coastal areas, leading to environmental, touristic (specially in summer months), health and economic issues [3, 4]. Most MMW, when removed from beaches, is not valorized, being generally disposed in landfills, generating environmental and economic impacts [3, 4].

MM can be divided into three main groups according to their pigmentation—red (Rhodophyta), green (Chlorophyta) and brown macroalgae (Ochrophyta, Phaeophyceae) [5]—being their metabolism influenced by the environment, such as water temperature and salinity, light and nutrient availability [6]. Their capacity to adapt to extreme climatic conditions, and survive, is related to their capacity to produce a wide variety of secondary metabolites [6]. The chemical composition of the seaweeds reveals their potential as a natural source of biostimulant compounds [1]. MM are rich in organic matter and inorganic nutrients (minerals and trace elements) which allow their direct use by the plant roots from the soil [1, 7]. In addition, the high fiber content supports soil moisture retention, making them a promising soil conditioner [2, 7]. However, the metals accumulation and particularly the heavy metal content which might be found in MM can restrict their use as biofertilizer. Thus, although promising, the biomass composition must be carefully analyzed to avoid environmental problems and to guarantee the product quality [1].

Seaweed extracts can have both positive or negative effects when directly applied to seeds or plants, due to its constituents and respective concentration. Furthermore, the effects are not always consistent among plant species, since they can exhibit different sensitivity to the bioactive compounds [1, 8].

The fertility level of the soil is considered a key of the agriculture success [2]. According to EU Regulation 2019/1009, biostimulants are defined as any substance, regardless its nutrient content, that improves the nutrient efficiency, tolerance to abiotic stress, crop quality traits and/or nutrient availability in the soil or rhizosphere [9]. The use of MM (including MMW) in agriculture has shown benefits in terms of nutrients to promote plant growth and increase stress (biotic and abiotic) tolerance [2, 3, 8, 10]. Literature reports several advantages of using seaweed derived products (liquid or solid) as fertilizers for plant growth, including higher germination rates, resistance to pathogens, root development, increase in leaf number and area, plant weight and plant vigor, thus demonstrating that such biomass might acts as biostimulant [8, 11–15]. The brown group of MM, which include kelp species, is the most used in agriculture, being reported its beneficial effects in several plants, such as tomato, maize, wheat, cabbage, radish and beans [11, 12, 14–19], mainly for human consumption.

According to Food and Agriculture Organization (FAO) data, in 2019 the world production of *Brassica* vegetables was around 97 million tonnes, being 27 million tonnes of cauliflower and broccoli, and 70 million tonnes of cabbage and other *Brassicas* [20]. Kale (*Brassica oleracea* L), originated from Mediterranean region or Asia Minor, is considered an important food crop, being recognized as a leafy vegetable. Kales are cultivated as an annual crop in a wide range of soils, but the best are low acidic/neutral soils (pH 6—6.5), deep and with the adequate water and air conditions [21, 22]. Seeds germinate in 4–7 days in the nursery or directly into soil [22] and seedlings develop strong main and lateral roots [21]. Depending on the variety, kale can grow under 27 °C; however, being a cool season crop, the best growth occurs between 15.5 and 18 °C [22].

Parsnip (*Pastinaca sativa*) is a root vegetable, of the carrot family, native from Eurasia (Europe and Asia) [23]. FAO reports a world production of around 10 million tonnes of

roots and tuber vegetables in 2019 [20]. Although being a biannual plant, parsnip is usually cultivated as an annual cool season crop. It is considered one of the hardest vegetables to germinate (seed germination is normally between 40 and 60% successful), being its flavour enhanced by frost. Despite having a low germination rate, parsnip is considered an important human food crop, with high interest mostly in the United Kingdom, but considered as an alternative food product throughout Europe [24]. Similarly to carrots, parsnip grows in deep, stone-free and well-drained soils. Its reproduction is from seeds which germinate better in soils at 10–29 °C, in a germination period between 10 and 21 days [23].

The use of MMW as biofertilizer for agricultural purposes is seen as a cost-effective, renewable and environmentally friendly solution that allows to replace or at least minimize the currently used chemical fertilizers [2]. Despite the existence of studies focused on fresh MM biomass, studies devoted on MMW biomass are still scarce. Thus, the aim of the present study is to access the effect of MMW derived products on seed germination, bringing back the traditional use of such biomass as fertilizer/biostimulant in sustainable agriculture systems. For this purpose, the biomass was characterized in terms of macro and micronutrients as well as contaminants to anticipate benefits and restrictions of using MMW derived products. After, two types of MMW derived products (liquid and solid) were used to grow two human food crop species (*Brassica oleracea* L and *Pastinaca sativa*), which have different characteristics and also different fertilizer requirements. The growth parameters (weight and length) were monitored and compared to a control condition (distilled water) to evaluate the beneficial effects associated with their use.

Materials and Methods

Sample Collection and Preparation

The biomass (MMW, composed of a mixture of several species) was collected in the end of the summer season (September 2020), from a beach located at northern Portugal (Vila Nova de Gaia municipality, 41º6′23.616″ N, 8º39′46.591″ W). MMW collection was performed manually on the sand, near to the water (up to 2 m), during the low tide, being further transported to the laboratory in plastic buckets.

The biomass was after thoroughly washed with tap water to remove salt, sand, and other impurities, in agreement with previous studies [25, 26]. Then, it was sun dried during few days (until completely dried by bare eye), ground (≤ 1 mm) in a Cutting Mill (Zipor, by contract), followed by a laboratory mill (Grindomix GM 200, Retsch, Germany) during 20 s at 10,000 rpm, and stored in hermetic plastic flasks at room temperature (RT) until use.

Biomass Characterization

Biomass characterization comprised a set of analysis conducted to the raw biomass, both concerning nutritional assessment and mineral profile.

Nutritional Assessment

A nutritional assessment of MMW was performed considering moisture, lipids, protein, ash, carbohydrates and dietary fibers, following appropriate standards, namely the Association of Official Analytical Chemist methods (AOAC) [27], which present an adequate accuracy for general characterization of the biomass, being used in previous studies [25, 26, 28].

Moisture content was measured using an infrared moisture analyzer (Kern DBS, Germany) at 105 °C, and the obtained values were further used to express results in dry basis.

The ash content was determined by calcination in a furnace at 550 °C during a minimum of 24 h, according to AOAC 920.153 [27].

The total lipid content was obtained by solvent extraction using petroleum ether, in a Soxhlet apparatus (100 mL), according to AOAC 991.36 [27].

Total nitrogen was obtained by the Kjeldahl method, based on AOAC 928.08 [27]. The sample (~ 1 g) was digested with sulfuric acid (96%) in an automatic digester unit (K-424, Büchi, Switzerland) coupled to a scrubber (B-414, Büchi, Switzerland). Then, ammonia was automatically distilled (Kjelflex K-360, Büchi, Switzerland) and collected in a solution of boric acid (4%), which was then subjected to volumetric titration with sulfuric acid (0.2 M). A nitrogen-to-protein conversion factor of 5 [29] was used to estimate the protein content, in consistency with previous studies [25, 26, 28].

Total carbohydrates (TC) content was calculated by difference to 100%, considering the ash, protein and lipids contents (dry basis).

The total dietary fiber was determined according to AOAC 985.29, through an enzymatic–gravimetric method [27]. The sample (~ 0.5 g) was digested with three enzymes (α-amylase, amyloglucosidase and protease) and then ethanol (96%) was added to precipitate the fibers. The solution was after filtered (CSF 6 Filtration System, Velp Scientifica, Italy) and the residue was analyzed (ash and protein) to obtain the total dietary fiber. The insoluble fiber was determined following the AOAC 991.42; the procedure was similar to the total dietary fiber determination but without the addition of ethanol (96%) after digestion, and using hot

water in the filtration step instead of ethanol (78%) [27]. The soluble fiber content was obtained by difference considering total and insoluble fiber contents.

All determinations were performed in triplicate and the results (except moisture) are expressed in dry basis.

Mineral Profile

The mineral profile of MMW was determined through dry mineralization from ash. MMW was calcinated at 550 °C and the resulting ashes were submitted to acid digestion using an aqua regia solution (1:3 V/V of nitric acid and hydrochloric acid), according to ISO 11466:1995. Digestions were performed in triplicate, with about 1 g of MMW ash and 10 mL of solution, at 90 °C in a water bath during 3 h. Then, the digestion solution was filtered, the solid residues washed, and the final volume of solution adjusted to 100 mL using distilled water.

Calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) quantification was performed by atomic flame absorption spectrophotometry (UNICAM 969 AA spectrometer), after the necessary dilutions (1:10; 1:100; 1:500), using the corresponding hollow-cathode lamp for each element. Phosphorus content was determined by UV/VIS spectrophotometry (PG instruments T80 + UV/Vis spectrophotometer, United Kingdom), following ISO 6491:1998. Results are expressed in mg of each element per 100 g of dry MMW.

Preparation of MMW Derived Products and Germination Assays

The MMW derived products were prepared as described by Sousa et al. [30]. MMW was added to a blender (Moulinex LM811D, perfect mix) with distilled water (0.12 g/mL), at program "smoothie" during 2 min. The resulting mixture was a viscous pulp, that was filtered, under vacuum, through a Buchner funnel with a nylon filter (80 mesh). The solid fraction was further used as a solid extraction product (SEP) in the germination assays. The liquid fraction was filtered again through a G2 filter; the final solution (concentrated solution) was adjusted to a final concentration of 1.2% in agreement with the solution characteristics [30, 31] and used as liquid extract (LE). The parameters pH, electric conductivity (EC) and total dissolved solids (TDS) were measured (HI98129 Combo tester, Hanna) for both the concentrated and the LE.

The germination assays were carried out according to Pacheco et al. [31]. SEP, LE and distilled water (control (C)) were tested for two different seeds: kale (*Brassica oleracea* var. capitata L.) and parsnip (*Pastinaca sativa*). Since the objective was to evaluate the effect of the products to stimulate plant growth, a negative control was used. As such, the application of distilled water as a control is justified by the fact that water was used as an extraction media and therefore would provide a direct comparison with the use of the liquid extract. In the present study, a positive control (commercial product) was not considered; however, it will be of high relevance to consider it in future studies, depending upon the results of the present study (selection of solid or liquid products).

Before use, the seeds were disinfected with sodium hypochlorite (NaClO, 2%) during 1 min and washed with distilled water 3 times (100 mL, 1 min each). Sterilized petri dishes were previously prepared with filter paper with cotton below. Then, the bioproducts were added: 70 mL for LE; 12 g for SEP; and 70 mL for control. After that, 25 disinfected seeds were placed in each petri dish which was sealed with parafilm (to avoid moisture loss) and incubated (Heraeus, Germany) at 23 °C, in darkness, until seed germination (which is expected to vary among the two species). A total of 5 replicates was performed for each condition.

At the end of the assays, the following parameters were evaluated:

- Germination rate: number of germinated seeds/total seeds × 100;
- Seedling radicular and aerial parts length: using a ruler;
- Fresh weight of the aerial and radicular parts: using an analytical scale (Kern, Germany).

Statistical Analysis

To determine if there are significant differences in the results of the seed germination assays, a statistical analysis was performed. One-way analysis of variance (ANOVA, $p = 0.05$) was used to assess differences of the results (germination rate, radicular and aerial part lengths and, radicular and aerial part weight) obtained from the three conditions tested (C, LE, SEP). Tukey's HSD test was applied aiming pairwise comparisons.

Results and Discussion

The collected MMW was a mixture of different species, mainly belonging to the brown group (~ 77%), followed by the green (~ 13%) and the red (~ 10%) ones. A higher variation of species was found in the red group. The most representative species was *Saccorhiza polyschides*, an annual kelp belonging to the brown macroalgae group. The other species identified in the brown group was *Sargassum* sp.. Considering the scarce literature devoted to the use of MMW for seed germination, comparisons were performed with the results

obtained from using fresh (non-waste) biomass (*Saccorhiza polyschides* species or brown algae) when necessary.

Biomass Characterization

The results concerning the nutritional assessment of MMW biomass are presented in Table 1.

The determined moisture content does not reflect the original water content of the biomass since it was determined after the pre-treatment step (washing and sun-drying), being used solely to correct parameters to dry basis.

Carbohydrates represent around 70%, being the greatest component of MMW followed by ash, protein, and lipids.

The obtained results are generally in the range of those obtained by the authors in previous studies [25, 28], with MMW collected in the same location but during different harvest times (different seasons or even collection years).

Considering the known composition of this type of biomass (either residual or non-residual), a lipid content lower than 1% was expected, being the obtained result in agreement with the literature [5, 25, 26, 28, 32–34].

The estimated protein content (around 11 wt.%) is directly related with the nitrogen-to-protein conversion factor used, being one of the main reasons of the differences between the literature values; in the present study a factor of 5 was considered more accurate for this kind of biomass (instead of the traditional used 6.25), according to the study of Angell et al. [29]. Despite that, the obtained protein content is in the range of those reported in the literature (8.7–15.1 wt.%) [5, 6, 25, 26, 28, 32, 33, 35–38].

It is important to highlight that there is a difference in the pre-treatment method (washing step) employed in the present study and in other published studies [5, 28, 36] since in the most of the studies the biomass was subjected to a brief cleaning with sea water (high presence of salt), and in the present study the biomass was thoroughly washed with tap water. This resulted in a low ash content of the biomass when compared with the literature (36–56 wt%) [5, 26, 28, 33, 34, 36], since the washing step allowed a more complete removal of impurities attached to the MMW. It is expected that the determined ash content is mostly related to macro- and microminerals of the biomass, as well as diminished sand and salt contents.

As expected, the carbohydrates were the most abundant constituent of the MMW biomass, being slightly higher than those reported in the literature (30–54.9 wt.%) [26, 28, 36, 38], essentially due to the performed pre-treatment steps (mostly affect the ash/mineral content of the biomass); and, according the calculation methodology (obtained by difference). The obtained carbohydrate content is in agreement with the one obtained by Pardilhó et al. [25] (69.3 wt.%), with the same pre-treatment, reinforcing that this methodology positively affected the carbohydrate content. The high carbohydrate and fiber contents reveals that the carbohydrate fraction is mainly composed by fibers (soluble and insoluble ones), with little expression of other carbohydrates, as sugars and starch, being seaweeds thus considered a rich source of fibers. The total fiber content was higher than that reported in other studies [5, 34, 39], but the relation between soluble and insoluble ones was similar to that described in the literature [39, 40]. The characterized biomass had higher expression of the insoluble fibers, however there are reported cases where soluble fibers prevail or are in a similar order of magnitude [5, 34]; this may be due to the variability and heterogeneity that occurs with such kind of biomass, considering that there are no references regarding this parameter.

Considering the scope of the present study, the high fiber and ash (minerals) contents show potential of using MMW for agricultural purposes where the residual biomass can act as soil conditioner, essentially due to being mainly composed by fibers and other polysaccharides such as alginate (present in brown algae). In fact, alginate polysaccharides can retain water up to hundreds of times of their own weight, thus increasing soil water retention (a basic property of the soils), maintaining its moisture during a long time [31, 41, 42]. MMW is also considered a nutrient and trace elements source (expressive mineral fraction, translated by its ash content), improving the soil microbiome and promoting the plant growth and defense against diseases and insects [5, 7, 42].

Several mineral elements are important for plant health and development, being essential for its metabolic pathways and metabolism. Nitrogen is essential for plant hormones, proteins, and chlorophyll production; K is vital to the plant health (immune system); Fe regulates and promotes growth; Mn, Mg and P are vital to enhance the plant photosynthetic capacity; Cu is a vital key to the plant enzymes and Ca is

Table 1 Nutritional assessment (values in wt.%) of Marine Macroalgae Waste (MMW) biomass

	wt.%
Moisture	8.06 ± 0.09
Ash	18.54 ± 0.07
Lipids	0.69 ± 0.04
Nitrogen	2.18 ± 0.03
Protein*	10.9 ± 0.1
Total carbohydrates	69.97 ± 0.08
Total fiber	63.4 ± 0.1
Insoluble fiber	50 ± 2
Soluble fiber	13 ± 2

Results (except moisture) are expressed on dry basis (g/100 g) and correspond to the sample mean value ± standard deviation; $n = 3$

*Estimated value based on Angell et al. [29]

essential for plant aerial and root development [43]. N, P and K are vital minerals for plant development [43].

The analysis of the mineral profile of MMW (Table 2) showed that the biomass contains high levels of macrominerals such as N, K, Ca, Na and Mg and microminerals like Fe, Zn and Mn. Among the macrominerals, K is the most abundant element (2242 mg/100 g) followed by N (2176 mg/100 g), Ca (1886 mg/100 g), Na (985 mg/100 g) and Mg (546 mg/100 g). The NPK ratio obtained was 21:1:21 showing high potential in terms of the presence of vital nutrients for plant development.

In terms of macrominerals, Lorenzo et al. [35] reported a different mineral profile of fresh brown seaweeds from the Atlantic Coast, where K (3745–9316 mg/100 g) presented the highest content, followed by Na (1837–4576 mg/100 g), Ca (985–1160 mg/100 g) and Mg (528–868 mg/100 g), being much higher than that obtained in the present study. The same trend is also reported by Rupérez [44], who studied the mineral profile of commercial edible seaweeds (K (4322–11,579 mg/100 g) > Na (3818–7064 mg/100 g) > Ca (931–1005 mg/100 g) > Mg (659–1181 mg/100 g)), and Michalak et al. [14] concerning the mineral profile of a brown seaweed (*Fucus* sp.) used to produce an algal compost (K (4390 mg/100 g) > Na (3515 mg/100 g) > Ca (1310 mg/100 g) > Mg (805 mg/100 g)); the last referred study presented micromineral content (Cu, Ni and Zn) close to that obtained in the present study.

Michalak et al. [4] used MMW from the Baltic Sea (composed by green and red seaweed species) obtaining the following mineral profile: Ca (1319 mg/100 g) > K (544 mg/100 g) > Na (521 mg/100 g) > Mg (382 mg/100 g). The reported values are in general about a half of those obtained in the present study, reinforcing that apart from the residual nature of the biomass, different species and geographic locations affect the biomass composition.

One of the disadvantages of using seaweeds and the resultant residual biomass might be the presence of toxic metals, since this kind of biomass is known to accumulate metals/heavy metals [1, 4], which might restrict their use for agricultural purposes. Most of the trace elements present in the MMW biomass were heavy metals (Cd, Cr, Cu, Pb, Ni) being their content in agreement with the regulated maximum acceptable limits in biofertilizers (Table 2), defined at European level in the Regulation EU 2019/1009 [9]. The metals Cr, Ni and Pb were in low concentrations (less than 0.2 mg/100 g expected; values below the lower limit established by the method). Cd concentration in the raw biomass was however just at the limit value. Considering the negative effect of this metal, Cd concentrations should be carefully monitored in the MMW derived products before recommending their use for agricultural purposes.

In the study of Michalak et al. [4] above referred, the authors also reported the Cu, Zn, Pb, Mn, Fe, Cr, and Cd contents, being a bit higher than those obtained in the present study.

The results showed that MMW is a good source of Ca, K, Mg, Na, Cu, Fe, Zn, being a possible promising raw material for biofertilizer production.

The interpretation of the obtained results regarding the mineral profile with those found in the literature should be made with comparable determination methods, which is not always possible, since the methods employed are in some cases different or even omitted in some studies. Moreover, and more importantly, the scarcity of studies performed with MMW and the variability and heterogeneity of the samples (species composition and geographic location), makes difficult a direct correlation between the obtained and the reported values.

Depending upon the pre-treatment and the parameter under study, some differences are expected between the characterization of the raw biomass and the MMW derived products. As the main objective of this study was to access the viability of using the liquid aqueous extract and the solid residue as bioproducts, such characterization was not conducted. However, although some differences are expected between the raw biomass and the MMW derived products, biomass characterization shows the potential of this resource and related products for agricultural purposes.

Table 2 Mineral profile of the of Marine Macroalgae Waste (MMW) biomass

Minerals	mg/100 g DW	Regulated limits [9] (mg/100 g DW)
Nitrogen (N)*	2176 ± 30	–
Phosphorus (P)	106 ± 2	–
Calcium (Ca)	1886 ± 62	–
Magnesium (Mg)	546 ± 17	–
Potassium (K)	2242 ± 89	–
Sodium (Na)	985 ± 11	–
Copper (Cu)	0.7 ± 0.4	≤ 60
Zinc (Zn)	4.3 ± 0.2	≤ 150
Iron (Fe)	17.3 ± 0.6	–
Manganese (Mn)	1.1 ± 0.1	–
Cadmium (Cd)	0.15 ± 0.01	≤ 0.15
Chromium (Cr)	*n.d*	≤ 0.2
Nickel (Ni)	*n.d*	≤ 5
Lead (Pb)	*n.d*	≤ 12

Results are expressed as mean value ± standard deviation; $n = 3$

n.d. non detected (below the limit established by the method)

*From the nutritional assessment of the biomass

Germination Assays

The success of the seed germination is directly related with pH and salinity (expressed as EC) of the media. The measurements of these parameters, as well as of the TDS, for concentrated and diluted LE, are presented in Table 3.

The salt quantity and pH tolerance can vary among plant species. The uptake of nutrients in plants is greatly affected by soil pH, being the ideal close to neutral values (6.5–7.5) [2, 31, 45]. When pH values are greater than 7.5 or lower than 6.5, less soluble compounds are formed due to the reaction of phosphate ions with Ca and Mg (in alkaline conditions) or Al and Fe (in acid conditions), compromising nutrient availability to plants [45]. Also, micronutrients tend to be less available when pH is above 7.5 in the soil [45].

Variations of salinity in the nutrient media, usually expressed as EC values, can affect seed germination, being this parameter considered as representing the major stress factor responsible for the inhibition of seed germination or the reduction of germination rates [31, 46]. In general, high salinity levels (high EC) inhibit seed germination due to the low osmotic potential, consequently causing a decreased water uptake. Also, high sodium and chloride ion concentrations may be toxic for seeds [46].

Only one study was found presenting quantitative data of salt tolerance for parsnip and kale crops [47]. Parsnip is considered a salt sensitive crop, which yield is affected when EC exceeds 800 µS/cm; for kale it is reported crop growth when irrigated with water with EC close to 2300 µS/cm [47]. In the present study, the concentrated solution (resulting from the filtration of MMW) has a high EC, being thus considered unusable for seeds germination. On the other hand, the LE (used in the seed germination assays) presented an EC lower than 1 000 µS/cm also presenting neutral pH (Table 3), being thus considered acceptable for both parsnip and kale seeds germination [31].

Table 3 Characterization of relevant parameters of the liquid concentrated solution and liquid extract (LE) aiming seed germination

Parameter	Concentrated solution	LE
T (°C)	21.1 ± 0.3	20.4 ± 0.4
pH	5.89 ± 0.09	6.7 ± 0.2
EC (µS/cm)	> 3999 *	182 ± 4
TDS (ppm)	> 2000 *	91 ± 1

Results are expressed as mean value ± error (difference of the maximum to the mean; $n = 2$)

LE liquid extract, EC electric conductivity, TDS total dissolved solids

*Maximum limit of the equipment

Germination of Kale Seeds

The germination of kale seeds was performed during 10 days (Fig. 1) and the first seeds germinated after 24 h from the beginning of the assay.

Through observations (visual and contact inspections) it was verified that germination occurred in all cases. The seedlings with LE were less brittle than those in the C, which may be an indicator of the presence of minerals in the former medium. In SEP assay, roots seem to be shorter but more robust.

The results concerning germination rate and growth parameters (radicular and aerial part lengths and weights), for kale seeds, after 10 days of incubation, are presented at Fig. 2.

Regarding germination rate (Fig. 2i)), no significant difference ($p > 0.05$) was found between the MMW derived products and the control. The C and SEP presented the same

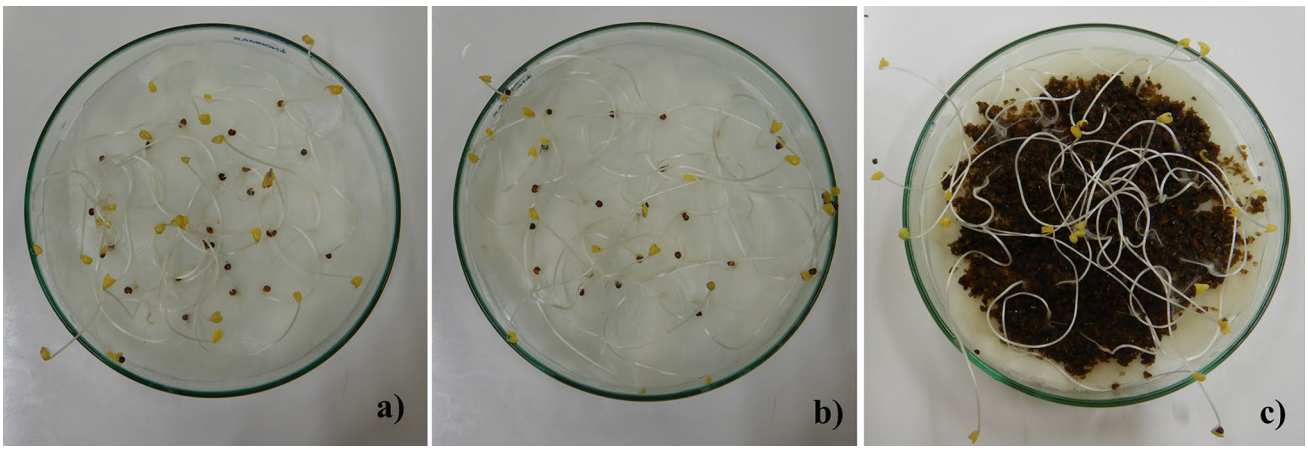
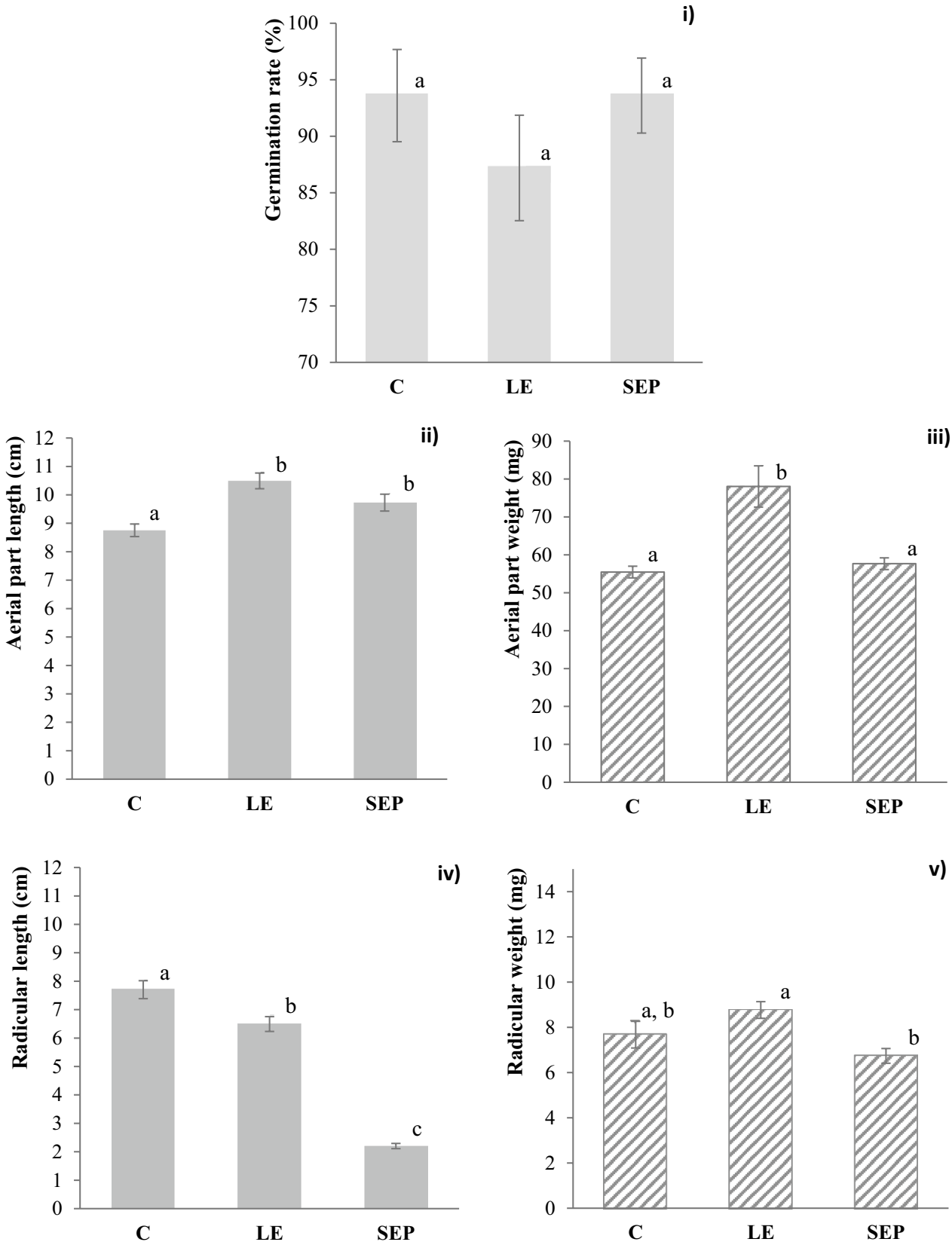


Fig. 1 Photographic record of kale seeds germination assays after 10 days of incubation: **a)** Control (C), **b)** Liquid Extract (LE), **c)** Solid Extraction Product (SEP); petri dishes—∅ = 15 cm


i)
Germination rate (%)
100
95
90
85
80
75
70
a
a
a
C
LE
SEP
ii)
Aerial part length (cm)
12
11
10
9
8
7
6
5
4
3
2
1
0
a
b
b
C
LE
SEP
iii)
Aerial part weight (mg)
90
80
70
60
50
40
30
20
10
0
a
b
a
C
LE
SEP
iv)
Radicular length (cm)
12
11
10
9
8
7
6
5
4
3
2
1
0
a
b
c
C
LE
SEP
v)
Radicular weight (mg)
14
12
10
8
6
4
2
0
a, b
a
b
C
LE
SEP

◂**Fig. 2** Germination rate (i), aerial part length (ii) and weight (iii), and radicular length (iv) and weight (v) for kale seeds germination assays (10 days) with Control (C), Liquid Extract (LE) and Solid Extraction product (SEP). Results are expressed as mean values ($n = 5$ replicates: 25 seeds each). Error bars show the standard error. Mean values with different superscripts (a–c) in the same parameter differ significantly ($p < 0.05$)

germination rate (94%), whereas in LE seed germination was 8% lower.

The use of LE and SEP had however a positive effect on the length and weight of the aerial part (Fig. 2ii, iii), presenting average values higher (using SEP—length: 9.7 cm; weight: 58 mg; using LE—length: 10.5 cm; weight: 78 mg) than those obtained with at the C (length: 8.8 cm; weight: 55 mg). The highest increase was observed using LE ($p < 0.05$), being the aerial part length around 20% longer and its weight around 42% higher than that obtained in control seedlings. Using the SEP, only 10 and 5% of improvement (compared to C) was observed for the aerial part length and weight, respectively, with significant differences being observed only for length parameter ($p < 0.05$).

Considering the radicular part, both the LE and SEP seem to cause a significant ($p < 0.05$) negative impact on the radicular length (Fig. 2iv), − 16% for LE and − 71% for SEP, when compared to C. Regarding the radicular weight (Fig. 2v), the use of SEP and LE led to different results ($p < 0.05$). Comparing with the control, with SEP it was lower (6.2 mg; − 13%), whereas with LE this parameter was higher (8.8 mg; 14%). However, both without significant differences ($p > 0.05$).

The germination rates obtained in the present study are higher than those obtained by Šamec et al. [48] for commercial *Brassica oleracea* seeds (Croatia) which used agar as medium and achieved a germination rate of 65%. In the present study, it was possible to achieve germination rates (mean values) higher than 90%, both using distilled water (C) and the SEP, which indicates that MMW can enhance the germination rate when compared with agar until 29% (using SEP). Due to the variability of the results obtained using the LE, although the mean value for germination rate was lower, the statistical analysis showed that the results were not significantly different than those obtained with SEP.

Sousa et al. [30] studied kale (*Brassica oleracea*) seed germination, during 17 days, using MMW collected from a northern Portugal seashore, comprised of a mixture of green, brown and red species. In that study, only SEP was used and a germination rate of 64% was obtained (80% for the C), both values lower than those obtained in the present study. The authors obtained a mean radicular and aerial seedling lengths of 1 and 4.5 cm, respectively, values about half of those obtained in the present study. When radicular and aerial part weights were considered, respectively with 0.5 and 9.7 mg, values much lower than those obtained in the present study were reported. On the other hand, the same author reported a germination rate of 100% using a LE obtained from MMW [49], a value higher than that obtained in the present study. In what concerns to the mean radicular and aerial part lengths (5.4 and 7.7 cm, respectively) and weights (1 and 25.9 mg, respectively), the values reported by Sousa [49] are lower than those observed in the present study, being the differences between 17 and 89%, with the highest differences found in the weight variable.

Another study, performed by Pacheco et al. [31], evaluated the impact of different brown seaweed polysaccharides in kale seeds growth. The authors obtained a germination rate between 90 and 99% and the following results for growth parameters: 3–5.5 cm for radicular length; 4–5 cm for aerial part length; 15–20 mg for radicular weigh; and 40–50 mg for aerial part weight. The results obtained by Pacheco et al. [31] are, in general, lower than those obtained in this study, but in the referred study only the polysaccharide fraction was used instead of all MMW biomass, which was the purpose of the present study. It is therefore evidenced that other components of the biomass, besides the polysaccharide fraction, can influence the germination of the seeds and subsequently the growth parameters. The literature shows in fact evidence of the presence of growth regulators in MMW biomass, which can influence the effect of MMW as biofertilizer [17, 42, 50, 51].

The analysis of the weight:length ratios, for C, LE and SEP, was performed considering aerial and radicular parts and total seedlings (Table 4). Results demonstrated that LE improved the weight:length ratio mostly in the aerial part, when compared to control, contributing for the total ratio increase of seedlings. This is an important achievement since the aerial part is the edible part of kales. On the other hand, for SEP, it was verified an increase in radicular ratio. These results show that MMW have potential for two types of biostimulant effect: LE acts as aerial biostimulant and SEP as root biostimulant.

Overall, the use of MMW derived products enhance the growth of kale seeds, with the best results being found for LE. Considering the seedlings total lengths and weights both LE and SEP improve these parameters with exception of seedlings length using SE (mainly related with the decrease of radicular length), which indicates an improvement in seedling vigor that can increase the further crop yield. Considering the edible part of the plant (aerial part), the enhancement of growth parameters in this component is considered positive to further plant development, since it can be an indicator of good root nutrient uptake.

Table 4 Kale weight:length (mg cm^{-1}) seed germination ratios, after 10 germination days

Weight:length	C	LE	SEP
Aerial part	6.3	7.4	6.0
Radicular part	1	1.4	3.0
Total seedling	3.5	5.1	5.4

C control, LE liquid extract, SEP Solid extraction product

Parsnip Seeds

As expected, germination of parsnip seeds took longer than that of kale, which can be related with seeds characteristics (e.g., the seed coat is thicker than the seed coat of kale, which difficults water flow across the membrane) [52]. The first seeds germinated after 8 and 10 days, for SEP and LE, respectively, but the germination assay lasted 33 days (Fig. 3). Through visual and contact inspections, it was verified that germination occurred in all media, but with less expression compared to kale seeds.

Results concerning the germination rate and the evaluation of growth parameters (radicular and aerial part lengths and weights), for parsnip seeds, are shown in Fig. 4.

The use of the LE had a positive effect in the germination rate of the seeds (Fig. 4i)) when compared to control (42% for LE *vs* 41% for C, an increase of + 2%); however, not significant ($p > 0.05$). On the other hand, the use of SEP had a significant negative effect on the germination (− 80%) compared to control, reducing seed germination potential (only 8% of seeds germinated); this can be related with the mineral content of the product (mostly, the EC and SEP properties), and with the seed characteristics [52].

There was an improvement in the seedling aerial part length (7.4 cm; + 3%; Fig. 4ii)) and weight (40 mg; + 18%; Fig. 4iii)) using LE, compared to control (7.2 cm; 34 mg; $p < 0.05$). When SEP was used, as occurs in the germination rate, a negative effect on the referred parameters was observed (− 17% and − 21%, for aerial part length and weigh, respectively; $p < 0.05$).

Fig. 4 Germination rate (i), aerial part length (ii) and weight (iii), and radicular length (iv) and weight (v) for parsnip seeds germination assays (33 days) with Control (C), Liquid Extract (LE) and Solid Extraction Product (SEP). Results are expressed as mean values ($n = 5$ replicates: 25 seeds each). Error bars show the standard error. Mean values with different superscripts (a–c) in the same parameter differ significantly ($p < 0.05$) ▶

The same trend was observed for the radicular length (Fig. 4iv)), with an enhancement of 3% using LE and a decrease of 38% when SEP was used, both with not significant differences ($p > 0.05$) compared to control. However, the radicular weight increased (Fig. 4v)) for both LE and SEP (27% and 20%, respectively), but with no significant differences.

Considering the total seedlings length and weight, the LE had a positive effect in both parameters (+ 2 and + 20%, respectively), while SEP had a negative effect (− 21 and − 18%, respectively), when compared to control.

No information was found in the literature concerning the germination of parsnip using MMW. The poor seed germination is reported as a problem of several cultures including parsnip [53]. For this reason, the germination standards have been reduced, being lower than in most plants; for parsnip only 60% germinating seeds is required [53]. Despite of this, the germination rates obtained in the present study, for both MMW derived products and C, were lower than such requirement.

The absence of published information does not allow the comparison of the obtained results with other authors, but some conclusions can be drawn. Although from a statistical point of view the differences are not relevant, an

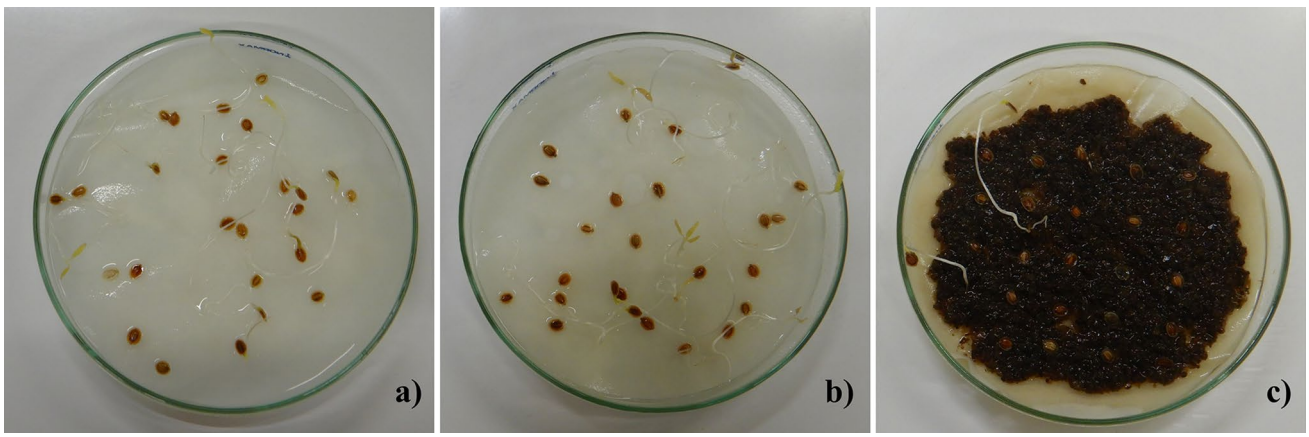
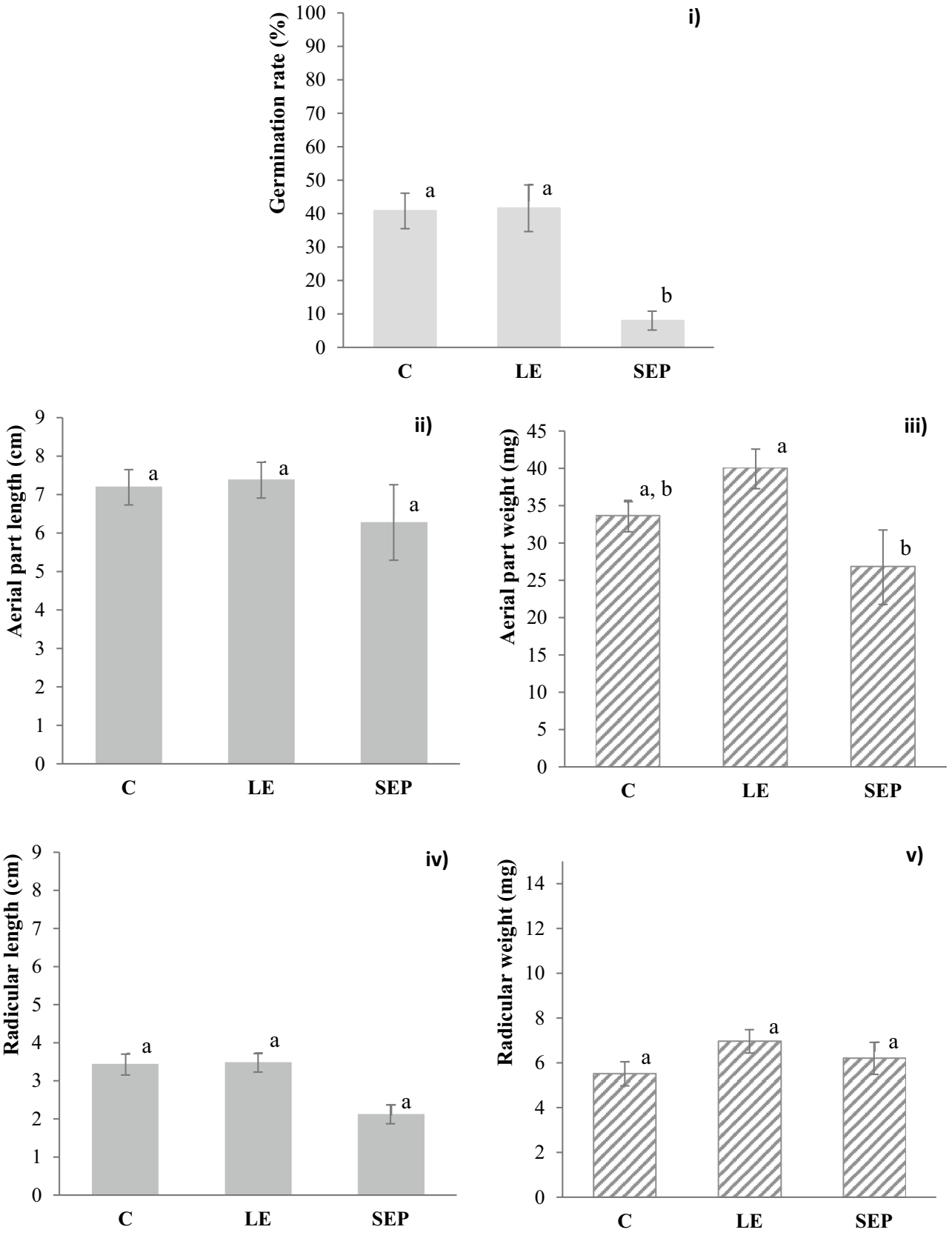


Fig. 3 Photographic record of parsnip seeds germination assays after 33 days of incubation: **a)** control (C), **b)** liquid extract (LE), **c)** solid extraction product (SEP); petri dishes—∅ = 15 cm


i)
Germination rate (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
C
LE
SEP
ii)
Aerial part length (cm)
9
8
7
6
5
4
3
2
1
0
a
a
a
C
LE
SEP
iii)
Aerial part weight (mg)
45
40
35
30
25
20
15
10
5
0
a, b
a
b
C
LE
SEP
iv)
Radicular length (cm)
9
8
7
6
5
4
3
2
1
0
a
a
a
C
LE
SEP
v)
Radicular weight (mg)
14
12
10
8
6
4
2
0
a
a
a
C
LE
SEP

improvement in radicular weight using LE was observed (27%), which indicates more seedling vigor can positively affect the crop yield; since parsnip is a root vegetable, it is essential the good development of the underground part of its seedlings.

Kaliniewicz et al. [54] studied parsnip germination on water moistened filter paper, achieving a germination rate of 57%, 15% higher than that obtained in the present study using the LE. In other study, Hendrix [55] demonstrated that seed after-ripening and/or cold seed treatment can benefit the parsnip seed germination; considering the same germination duration as in the present study, the obtained germination rate is in the range of the reported one, 30–50%.

As performed for kale seeds, an analysis of the weight:length ratios was conducted considering aerial and radicular parts as well as total parsnip seedlings (Table 5). The results demonstrated that LE improved the ratio weight/length of the parsnip, with higher expression for aerial part; the SEP improved the parsnip radicular ratio, with a little decreasing in aerial part ratio, when compared to control. However, since with SEP the germination rate is very low (8%), the LE is considered the most adequate product for future studies related with parsnip germination using MMW. These results indicate that LE can be considered as aerial and radicular biostimulant.

In general, for both types of seeds (kale and parsnip), compared with the C, LE improved better the seedling growth parameters, and the SEP worsen some of them. This can mean that the use of MMW LE in seed germination might result in a better seedling vigor, which can increase crop yields (demonstrating a potential biostimulant and/or biofertilizer activity). Lower seed germination observed with SEP can be related with the biomass mineral content, which affects conductivity of the solution and consequently seed germination; such problem can be overcome by mixing SEP with soil, to balance the mineral content.

The results of the mineral profile of MMW revealed relevant amounts of nutrients, but further studies should also consider the full characterization of the MMW derived products (focusing on Cd content, which was found to be closer to the regulated limit). Although future analysis of the aqueous extract (where better results were obtained) regarding Cd concentration are advised, considering that the extract pH was very close to neutral and that the mobility of this element to the aqueous phase is promoted at lower pH [56], it is expected that such is not a limitation for its use. The analysis of antifungal activity of the products, the use of other seed species, the transplantation of the seedlings to the soil and the implementation of germination assays using the mixture of MMW products with the soil (focusing on the use of the liquid extract) should be further taken into account. Also, a comparison with a commercial substrate/fertilizer should be considered to evaluate the benefits of using MMW; considering the results of the present study, the evaluation of a liquid substrate would be relevant.

Table 5 Parsnip weight:length (mg cm^{-1}) seed germination ratios, after 33 germination days

Weight:length	C	LE	SEP
Aerial part	4.7	5.4	4.5
Radicular part	1.6	2.0	3.0
Total seedling	3.7	4.3	3.9

C control, LE liquid extract, SEP Solid extraction product

Conclusions

The potential of Marine Macroalgae Waste (MMW) as biofertilizer was evaluated, considering the use of a liquid extract and a solid product to enhance seed germination (Kale—*Brassica oleracea* L and parsnip—*Pastinaca sativa*). Biomass was mainly composed of *Saccorhiza polyschides* and revealed a high carbohydrate content (70%, mostly fibers) and a rich mineral fraction, which can promote water retention and nutrient uptake from the soil by plants, emphasizing that MMW can be used for agricultural purposes. The toxic metal contents were in agreement with the defined limits; however, Cd content was close to the limit, thus further surveillance of this parameter is advised.

The liquid extract was, in general, a better growth promoter than the solid product, translated by a highest growth of the seedlings aerial and radicular parts (length and weight), up to 42%.

The present study contributes to the incorporation of beach-cast macroalgae into the economic sector in the form of biostimulants, biofertilizers or soil conditioners, reducing the use of synthetic substances and contributing to the circular economy. Further studies should consider a full characterization of MMW derived products (including their antifungal activity), assays in vivo and the direct comparison with commercial products.

Acknowledgements This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE); UIDB/04292/2020 (MARE); and UIDB/50006/2020 (REQUIMTE-LAQV), all funded by national funds through the FCT/MCTES (PIDDAC). The authors also acknowledge Foundation for Science and Technology for Sara Pardilhó's (SFRH/BD/139513/2018) PhD fellowship, funded by national funds and the European Social Fund (ESF). João Cotas thanks to the European Regional Development Fund through the Interreg Atlantic Area Program, under the project NASPA 523 (EAPA_451/2016).

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SP, JC and DP. The first draft of the manuscript was written by SP and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE); UIDB/04292/2020 (MARE); and UIDB/50006/2020 (REQUIMTE-LAQV), all funded by national funds through the FCT/MCTES (PIDDAC). The authors also acknowledge Foundation for Science and Technology for Sara Pardilhó's (SFRH/BD/139513/2018) PhD fellowship, funded by national funds and the European Social Fund (ESF). João Cotas thanks to the European Regional Development Fund through the Interreg Atlantic Area Program, under the project NASPA 523 (EAPA_451/2016).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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