

Effect of *Fucus* extract and biomass enriched with Cu(II) and Zn(II) ions on the growth of garden cress (*Lepidium sativum*) under laboratory conditions

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Highlights

- *Fucus vesiculosus* is a valuable raw material for agriculture.
- *Fucus vesiculosus* extract can act as biostimulants of plant growth.
- *Fucus vesiculosus* enriched with microelements via biosorption can be used as a fertilizing material providing plants with these nutrients.
- *Fucus* products can biofortify edible plants in essential microelements.
- *Fucus* extracts can enhance plant length and chlorophyll content.

Abstract

In the present study, brown seaweed - *Fucus vesiculosus* was used as a raw material for the preparation of bio-products for sustainable agriculture. Biosorption was applied to produce fertilizer additives with microelements. *F. vesiculosus* was enriched with Cu(II) and Zn(II) ions. Biostimulants of plant growth were obtained by seaweed extraction with potassium hydroxide.

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Key words: *Fucus vesiculosus*; biosorption; extraction; fertilizer additive; biostimulant of plant growth; germination tests.

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Different doses of enriched *F. vesiculosus* (1, 2, 4, 6 mg/per Petri dish) and concentrations of seaweed extract (2.5, 5 and 10%) were examined in germination test on garden cress (*Lepidium sativum*). The effect of both algal products on the plant length, RGB parameters in leaves and biomass multielement composition was examined. Results showed that the biomass enrichment did not influence plant length and RGB parameters. Taking into account these two parameters, the best results were obtained in the group treated with natural *F. vesiculosus* (higher than in the control group, treated with distilled water). Enriched *F. vesiculosus* biofortified garden cress with Cu and Zn. In the case of *Fucus* extract, 5% concentration increased plant length and the value of RGB parameters when compared with the control group. Also this extract concentration resulted in elevated content of micro- and macroelements in *Lepidium sativum*. Seaweed extract is recommended for further research as biostimulant of plant growth.

Introduction

Seaweeds washed ashore on beaches can cause ecological and recreational problems, both for local inhabitants and tourists. At the same time this biomass constitutes a valuable raw material, which can be utilized for agricultural purposes (Balina *et al.*, 2016; Bikovens *et al.*, 2017; Weinberger *et al.*, 2020). One of the most abundant and widely distributed genus of seaweeds is *Fucus*, which belongs to brown seaweeds (class - *Phaeophyceae*) (Catarino *et al.*, 2018). This group of macroalgae is known to contain minerals (especially iodine), proteins, amino acids, lipids and fatty acids, including n-6 and n-3 (Mæhre *et al.*, 2014; Paiva *et al.*, 2014; Latique *et al.*, 2017; Catarino *et al.*, 2018). The main pigments are chlorophyll *a* and *c*, β -carotene and fucoxanthin and several other xanthophylls. The storage product is laminaran (β -1,3-glucopyranoside, predominantly) and mannitol. The cell wall is composed of cellulose, alginic acid and sulphated mucopolysaccharide (fucoidan) (Davis *et al.*, 2003). Brown seaweeds are also a source of plant hormones (auxins, gibberellins, cytokinins), betaines, sterols and vitamins (Khan *et al.*, 2009; Latique *et al.*,

2017). These properties cause that the most common brown seaweeds - *Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp., *Sargassum* spp., *Ecklonia* spp. - are increasingly used in agriculture (Hong *et al.*, 2007). Dried or fresh seaweeds and liquid extracts to an increasing extent are employed by horticulturists, gardeners, farmers as organic fertilizers and soil conditioners (Zodape, 2001; Rathore *et al.*, 2009; Bulgariu, 2020). Seaweed extracts, which are applied as biostimulants of plant growth have achieved a broader use and market than seaweed and seaweed meal - today there are several algal products and brands available on the market (Khan *et al.*, 2009). Biostimulants are defined as *materials, other than fertilizers, that promote plant growth when applied in small quantities* and are also referred to as *metabolic enhancers* (Zhang and Schmidt, 1997). Nevertheless, all of the seaweed products are known to have a positive effect on: i) the soil health (soil structure, moisture retention, microbes in rhizosphere); ii) plant growth and health (root development, mineral absorption, shoot growth and photosynthesis, crop yield, vegetative propagation); and iii) resistance to the environmental biotic and abiotic stress (Zodape, 2001; Khan *et al.*, 2009).

In this study, two *Fucus*-based products for the application in agriculture are proposed. Dried and milled biomass of *Fucus* sp. can be used as a fertilizing material because it contains many nutrients, especially micro- and macroelements (Truus *et al.*, 2001; Rupérez, 2002; Sharma *et al.*, 2012; Villares *et al.*, 2013; Mæhre *et al.*, 2014; Paiva *et al.*, 2014; Balina *et al.*, 2016; Catarino *et al.*, 2018), which can be supplied to plants. The content of microelements in the algal biomass, which are essential for plants, can be increased by the application of biosorption process. This process relies on the passive binding of metal ions from the aqueous solution by the non-living biomass (Davis *et al.*, 2003). The biomass of *Fucus* sp. is characterized by good biosorption properties (amount of metal ions bound by the dry biomass), although this process is mainly used to remove toxic metal ions from wastewaters (Rincón *et al.*, 2005; Ahmady-Asbchin *et al.*, 2008; Ahmady-Asbchin *et al.*, 2009; Ahmady-Asbchin and Mohammadi, 2011; Romera *et al.*, 2008; Chaudhuri *et al.*, 2009; Brinza *et al.*, 2020). In this study, biosorption was used to obtain enriched with microelements *Fucus vesiculosus*. There are some examples in the literature of the use of algal biomass loaded with metal ions to improve plant growth, as well as soil quality (Tuhy *et al.*, 2014, 2015; Bădescu *et al.*, 2017). This approach can lead to the biofortification of edible plants with important microelements. Copper and zinc ions were chosen for biosorption by *Fucus vesiculosus*, since these trace minerals are important for biochemical functions and are necessary for health maintenance. Additionally, deficiencies of both elements in soil is a well-studied and wide-spread problem in agriculture worldwide. This results from the increase in cultivation intensity (removal of higher quantities of microelements from the soil) associated with the growing demand for higher crop yields with better quality (Ronen, 2007).

The second examined preparation is extract from *Fucus vesiculosus* obtained with the use of potassium hydroxide. This macroalga is used to extract biologically active compounds such as polysaccharides [alginate (Truus *et al.*, 2001; Rioux *et al.*, 2007), fucoidan (Rioux *et al.*, 2007; Rodriguez-Jasso *et al.*, 2014; Bikovens *et al.*, 2017), laminaran (Rioux *et al.*, 2007)], antioxidants, *e.g.* phenols (Farvin and Jacobsen, 2013; Tierney *et al.*, 2013; Heffernan *et al.*, 2014), plant hormone, *e.g.* indole-3-acetic acid (Sharma *et al.*, 2012). Extracts derived from *Fucus* species have various biological activities such as antioxidative (Farvin and Jacobsen, 2013; Tierney *et al.*, 2013; Heffernan *et al.*, 2014; Bikovens *et al.*, 2017), antifungal, *e.g.* against *Fusarium culmorum*

and *Fusarium oxysporum* (Tyśkiewicz *et al.*, 2019), stimulation of plant growth (Sharma *et al.*, 2012; Latique *et al.*, 2013; Bikovens *et al.*, 2017; Latique *et al.*, 2017; Mzibra *et al.*, 2021). Polysaccharides and plant hormones are also known to stimulate plant growth and their natural defence responses (Zodape, 2001; Khan *et al.*, 2009; Mzibra *et al.*, 2021).

The research hypothesis assumed that *Fucus* products would stimulate plant growth and biofortify plants with microelements without phytotoxic effect. Therefore, *Fucus vesiculosus* enriched with Cu(II) and Zn(II) and *Fucus vesiculosus* extract were examined in germination tests on garden cress (*Lepidium sativum*). The effect of these products on the average length of plants, RGB parameters in their leaves, as well as the multielement composition of the cultivated plants was determined. It was compared which of the preparations had a better effect on the growth and composition of *Lepidium sativum* and which may be recommended for further research.

Materials and methods

Seaweed

Fucus vesiculosus L. commercially available brown macroalga (in the dried and ground form) was obtained from FLOS Department Confectioning Herbs, Poland (<http://www.flos.pl/en/>).

Biosorption process

The biosorption of Zn(II) and Cu(II) ions by *Fucus vesiculosus* was carried out in a stirred tank reactor (Biotron LiFlus LiFlus GX) at room temperature for 2 hours (3 grams of the biomass were mixed with 3 L of metal ions solution). The content of the biomass in the solution was 1 g of dry mass (d.m.) L⁻¹ according to previous studies (Michalak and Chojnacka, 2010). The stock solution of Zn(II) and Cu(II) ions (300 mg L⁻¹) was prepared by dissolving appropriate amounts of ZnSO₄·7H₂O and CuSO₄·5H₂O (Avantor Performance Materials Poland S.A.) in distilled water. pH of initial solutions was adjusted to 5 with 0.1 M HCl or/and NaOH (with the use of pH meter Mettler Toledo equipped with an electrode InLab413 with compensation of temperature, SevenMulti, Switzerland). The solution was then filtered using Whatman No.1 filter paper and the concentration of metal ions in the solution before and after biosorption was determined by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry). The biosorption capacity (calculated as a difference of metal ions concentration in the solution before and after biosorption process) of *Fucus vesiculosus* towards Cu(II) ions was 45.0 mg g⁻¹ of d.m. and Zn(II) ions - 10.3 mg g⁻¹. The obtained enriched biomass was air-dried and used later in germination tests as a fertilizing material.

Extraction process

Fifty grams of milled seaweed was mixed with 500 mL of 1M KOH (Avantor Performance Materials Poland S.A.). The mixture was autoclaved for 30 minutes and then filtrated using Whatman No.1 filter paper. The pH of the extract was adjusted to 7, using 85% H₃PO₄ (Avantor Performance Materials Poland S.A.). The light brown extract was obtained and was treated as a 100% seaweed extract. For the germination tests, appropriate solutions were prepared - 2.5, 5, 10, 25 and 50%.

Germination tests - phytotoxicity tests

Utilitarian properties of enriched with Zn(II) and Cu(II) ions *Fucus vesiculosus* and *F. vesiculosus* extracts were examined in germination tests on edible garden cress (*Lepidium sativum*) according to the international norm (International Seed Testing Association). The aim of these studies was also to demonstrate the potential toxicity of the tested preparations. Plastic dishes (diameter - 85 mm) with the cotton (approximately 5 g) soaked with deionized water were prepared. On each dish, 50 seeds were placed in rows at equal distances from each other. Experiments were performed in four replicates for each experimental group, in the standardized conditions - isolated box with adjustable lighting and temperature (temperature fluctuations $\pm 4^{\circ}\text{C}$) - Jacobsen apparatus (Laborset, Łódź, Poland). Before germination tests, the dishes with seeds were put into the fridge for stratification (3 days). Every day (8 days) small volume (2 mL) of distilled water was added to the dish in order to compensate the loss of evaporation.

Four doses of each enriched *F. vesiculosus* were applied: biomass that contained 1, 2, 4, 6 mg of Cu and Zn, separately (calculated on the basis of biosorption capacity). The selection of these doses was based on previous studies (Tuhy *et al.*, 2014). Additionally, the group with the natural biomass of *Fucus vesiculosus* also was tested. The amount (weight) of the biomass added to the dish was equal as for the highest tested dose - 6 mg. This means that the content of Cu in the natural *F. vesiculosus* per dish was 0.0003 mg, whereas the content of Zn per dish was 0.0209 mg. The seaweed biomass (natural and enriched) was evenly distributed over the dishes. The control group was watered only with distilled water. For testing algal extracts in the cultivation of *Lepidium sativum*, different initial concentrations were prepared: 2.5, 5, 10, 25, 50 and 100%. The control group was watered only with distilled water. General scheme of germination tests is presented in Figure 1. After germination tests, aerial parts of plants were measured, RGB analysis of garden cress leaves was performed and the content of micro-and macroelements in the cultivated plants was determined.

Analytical methods

RGB analysis

RGB based image analysis (intensity of the three primary colours red (R), green (G) and blue (B)) was used as a rapid and non-invasive method to determine the content of chlorophyll in the leaves of garden cress. Microscopic images of plants (seven measurements of randomly selected leaves in each group from each repetitions) were taken using a microscope (Carl Zeiss Axio Scope. A1; Oberkochen, Germany). The analysis of RGB parameters was examined using RGB model in computational application AxioVisionRelease 4.8.

Multielement composition

The content of elements in the raw and enriched biomass of *Fucus vesiculosus*, extract, as well as in the cultivated plants was determined by ICP-OES Vista MPX, Varian (Victoria, Australia). The samples were analysed in three repeats (the reported results of analyses were arithmetic mean, the relative standard deviation was $<5\%$). The samples of algal biomass and cultivated plants (about 0.5 g) prior to ICP-OES analysis were digested with concentrated nitric acid (5 mL, 69% Supra pure grade from Merck, Darmstadt, Germany) in Teflon bombs in microwave oven Milestone Start D (USA). After mineralization, samples were diluted with demineralized water (Millipore Simplicity) to 50 g.

Statistical analysis

The results were elaborated statistically by *Statistica* ver. 13.0 (TIBCO Software Inc., Tulsa, OK, USA). Normality of distribution of experimental results was assessed by Shapiro-Wilk test. Homogeneity of variances was evaluated with Brown-Forsythe test. On this basis, statistical test was selected, which was used to investigate the significance of differences between the tested groups. The differences between groups were examined with one-way analysis of variance (ANOVA) using Tukey test (for normal distribution and homogeneous variances) or Kruskal-Wallis test (for non-normal distributions). Results were considered significantly different when $P < 0.05$.

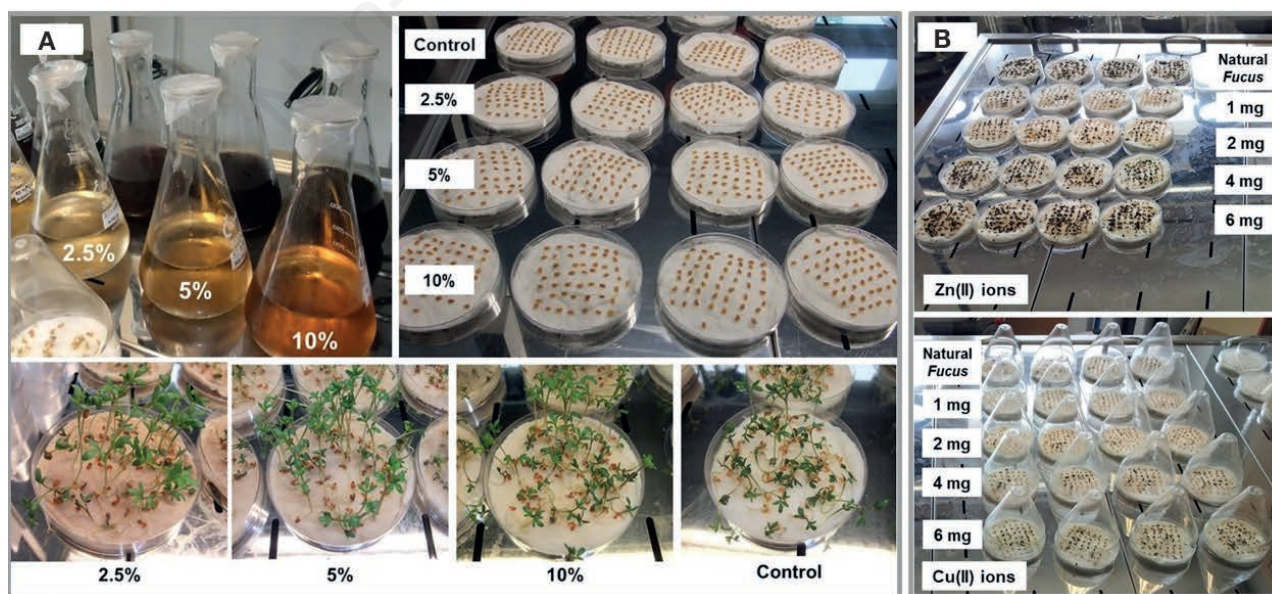


Figure 1. General scheme of germination tests with (A) *Fucus* extract, (B) *Fucus* biomass enriched with Zn(II) and Cu(II) ions.

Results and discussion

Fucus vesiculosus as a raw material for agriculture

Biomass of *Fucus* species, rich in micro- and macroelements (Table 1), can serve as their source for plants. Marine macroalgae can accumulate metal ions from the surrounding environment and their content can be higher than in terrestrial plants (Balina *et al.*, 2016; Catarino *et al.*, 2018). As can be seen from Table 1, multi-element composition of *Fucus* seaweeds varies depending on the geographic location, season, as well as algae species within the same phylum. Potassium and sodium are the major macroelements in this genus - the first one ranged from 9.76 to 52.4 g kg⁻¹, the second one from 6.30 to 54.7 g kg⁻¹. Other macroelements in the *Fucus* biomass are calcium (1.18-21.5 g kg⁻¹) and magnesium (1.63-9.94 g kg⁻¹). Among microelements, copper, zinc, iron and manganese are present in the *Fucus* biomass. The content of these elements (besides *Fucus vesiculosus* and *F. serratus* from Strangford Lough, Northern Ireland with much higher microelements levels; Sharma *et al.*, 2012) ranged from 1.8 to 45.2 mg kg⁻¹ for Cu, from 42 to 490 mg kg⁻¹ for Fe, from 34 to 238 mg kg⁻¹ for Mn (with the exception of *F. vesiculosus* - 1680 mg kg⁻¹; Balina *et al.*, 2016), and from 26 to 114 mg kg⁻¹ for Zn. Toxic metal ions in the *Fucus* biomass are randomly studied. The range of micro- and macroelements in the tested in the present study *Fucus vesiculosus* agreed with previous reports.

Due to the rich chemical composition (not only minerals, but also other biologically active compounds), seaweeds are proposed as soil amendments, which ameliorate plant growth and development (Zodape, 2001; Salcedo *et al.*, 2020). Bikovens *et al.* (2017) recommended *Fucus vesiculosus*, as well as the post extraction residue after isolation of biomolecules as valuable organic fertilizers for sustainable agriculture, which are not phytotoxic and promote the plant growth.

In this study, we propose to use in the cultivation of garden cress not only natural *Fucus vesiculosus*, but also its biomass enriched with Cu(II) and Zn(II) ions and *F. vesiculosus* extract. It is worth adding that the properties of seaweed extracts are examined much more often in the literature than the properties of the seaweed itself in terms of agricultural use.

Enriched with microelements *Fucus vesiculosus* as a fertilizing material

In this study, the biomass of *Fucus vesiculosus* was additionally enriched with Cu(II) and Zn(II) ions using biosorption process. The biosorption capacity of alga towards Cu(II) ions was 45.0 mg g⁻¹, whereas for Zn(II) ions - 10.3 mg g⁻¹. There are several examples in the literature of the use of *Fucus* biomass as a biosorbent of metal ions. The biosorption of Cu(II) ions was examined for example by Rincón *et al.* (2005), Chaudhuri *et al.* (2009), Ahmady-Asbchin and Mohammadi (2011) for *Fucus vesiculosus* and biosorption capacity ranged from 27.3 mg g⁻¹ to 117 mg g⁻¹, by Ahmady-Asbchin *et al.* (2008) and Ahmady-Asbchin *et al.* (2009) for *Fucus serratus* (102-110 mg g⁻¹) and by Romera *et al.* (2008) for *Fucus spiralis* (63.6 mg g⁻¹). In the case of Zn(II) ions, *Fucus vesiculosus* bound 31.7 mg g⁻¹ (Chaudhuri *et al.*, 2009), *Fucus serratus* - 46.3 mg g⁻¹ (Ahmady-Asbchin *et al.*, 2008) and *Fucus spiralis* - 65.4 mg g⁻¹ (Romera *et al.*, 2008). The value of biosorption capacity depends on many experimental conditions, such as pH, initial concentration of metal ions in the solution, biomass content in the solution, temperature, *etc.* (Michalak and Chojnacka, 2010) and they should be taken into account when comparing biosorption

Table 1. Mineral composition of *Fucus* sp.

Element	Trus <i>et al.</i> , 2001 <i>Fucus vesiculosus</i> Kakumäe Bay, the Baltic Sea, North Estonia	Machre <i>et al.</i> , 2014 <i>Fucus vesiculosus</i> Norway, Sommarøy	Paiva <i>et al.</i> , 2014 <i>Fucus spiralis</i> São Miguel Island, Azores Archipelago	Sharma <i>et al.</i> , 2012 <i>Fucus vesiculosus</i> Strangford Lough, Northern Ireland	Sharma <i>et al.</i> , 2012 <i>Fucus serratus</i> Strangford Lough, Northern Ireland	Rupérez, 2002 <i>Fucus vesiculosus</i> Commercial biomass (Algamar CB, Spain)	Vilareset <i>et al.</i> , 2013 <i>Fucus spiralis</i> Bay of San Simón (Galicia, Spain)	Vilareset <i>et al.</i> , 2013 <i>Fucus vesiculosus</i> Bay of San Simón (Galicia, Spain)	Balhaet <i>et al.</i> , 2016 <i>Fucus vesiculosus</i> Gulf of Riga, the Baltic Sea (Latvia)	Present study <i>Fucus vesiculosus</i> Commercial biomass
Microelements (mg kg ⁻¹ d.m.)										
Cu	≤10-20	1.8	92	n.a.	800	600	<0.5	45.2	17.2	12.7
Fe	280-320	92	34	n.a.	1500	1300	42	448	398	490
Mn	400-420	34	26	n.a.	n.a.	n.a.	55	145	238	1680
Zn	250-310	36	26	n.a.	n.a.	n.a.	37	70.5	114	89
I	130-160	130	n.a.	n.a.	700	300	n.a.	n.a.	n.a.	n.a.
Macroelements (g kg ⁻¹ d.m.)										
Ca	≤12-5.4	12.0	n.a.	1.18	14.1	13.9	9.38	6.80	6.90	21.50
K	≤20-7.0	n.a.	n.a.	9.76	30.4	46.3	43.2	39.5	52.4	11.0
Mg	4.30-6.00	7.40	n.a.	1.63	8.60	8.80	9.94	8.80	7.90	9.30
Na	0.460-0.510	n.a.	n.a.	14.3	37.0	37.0	54.7	39.4	37.8	6.30
P	n.a.	0.840	n.a.	n.a.	1.80	2.30	n.a.	2.40	3.00	1.40
S	n.a.	n.a.	n.a.	n.a.	31.3	15.4	n.a.	12.5	10.1	28.2
Other elements Al (mg kg ⁻¹ d.m.)										
As	n.a.	327	41	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	201±30
Cd	n.a.	n.a.	1.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	13.5
Cr	n.a.	1.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.7
Hg	0.0063-0.0074	0.011	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.96±0.145
Ni	9.2-13.2	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	9.6
										n.a.
										2.81±0.44

d.m., dry matter; n.a., not available.

capacity values. In the literature it was shown that microelements bound by the biomass via biosorption can be easily released to the soil and therefore can be bioavailable to plants (Tuhy *et al.*, 2014; Bădescu *et al.*, 2017; Izydorczyk *et al.*, 2020). Bădescu *et al.* (2017) performed desorption experiments using green seaweed - *Ulva* sp. enriched with Zn(II) ions and inorganic salt (*e.g.* CaCl₂). The Authors indicated that this microelement was released from the loaded biomass to the soil. Tuhy *et al.* (2014) demonstrated that the biomass (seaweeds, seaweed post extraction residue, peat, bark and spent mushroom substrate) enriched via biosorption with Zn(II) ions was characterized by higher bioavailability of this element to garden cress than conventional fertilizers - inorganic salt and chelate. The use of the enriched biomass as a fertilizing component with microelements was suggested.

Effect of the enriched *Fucus vesiculosus* on the *Lepidium sativum* shoot length and RGB parameters of leaves

For the enriched with Zn(II) and Cu(II) ions seaweeds (different doses: natural biomass of *Fucus vesiculosus* and 1, 2, 4 and 6 mg of a given element in the form of enriched biomass), the length of plants (N=20 from each group performed in four replicates) and RGB parameters (N=7 from each group performed in four replicates) were determined. The obtained results are presented in Table 2. In the group treated with Cu-*F. vesiculosus*, statistically significant difference was found between natural *F. vesiculosus* and 6 mg Cu (P=0.0290; Kruskal-Wallis test). The length of plants in the group with natural *F. vesiculosus* was almost two times higher than in the group with the highest dose of Cu applied in the form of the enriched biomass (6 mg). Generally, the length of plants decreased with the increase in Cu dose. Additionally it was observed that the length of plants in the group with natural *F. vesiculosus* was by 37.5% higher than in the control group treated with distilled water.

In the groups treated with Zn-*F. vesiculosus*, statistically significant difference was found between the group - 6 mg Zn and the control group (H₂O) (P=0.0456; Tukey test) and was by 34.4% higher in the *Fucus* group. In the case of the application of the biomass enriched with Zn(II) ions, the plant length increased with the increase in Zn dose. These differences can result from the fact

that copper is more toxic than zinc, even 5-6 times (Ivanova *et al.*, 2010).

The effect of the enriched *F. vesiculosus* on the RGB parameters in the cultivated *Lepidium sativum* is shown in Table 2. On the basis of leaf colour it is possible to assess the nutrient status and plant health (Ali *et al.*, 2012). The highest values of RGB parameters were obtained for the group treated with natural *F. vesiculosus*. The enriched with Cu(II) ions *F. vesiculosus* had lower effect on RGB parameters than natural *F. vesiculosus*. High concentrations of Cu can have inhibitory effect on enzymes responsible for chlorophyll production (Shakya *et al.*, 2008). Parameter R (red) was by 57.8% higher in the garden cress treated with natural *F. vesiculosus* than in the control group with distilled water, G by 49.7% higher and B by 10.7% higher. It was also noted that RGB parameters in the leaves of garden cress increased with the increase in Cu dose, but were lower than in the group with natural *F. vesiculosus*. The values of these parameters can be correlated with chlorophyll content in plant (Ali *et al.*, 2012; Tuhy *et al.*, 2014). In the literature there are many models to examine the relationship between R, G and B values and the true value of foliar chlorophyll content measured in the laboratory (destructive and non-destructive methods) (Ali *et al.*, 2012). Using the formula: G/(R+G+B) proposed by Suzuki *et al.* (1999) to estimate the chlorophyll content it was found that the highest content of chlorophyll in garden cress leaves was in the group treated with natural *F. vesiculosus*.

In the case of Zn-*F. vesiculosus*, the relationships between Zn doses and values of RGB parameters were not so evident as for Cu-*F. vesiculosus*. RGB parameters in the garden cress from the group treated with natural *F. vesiculosus* were lower than in the control group with distilled water, whereas the highest values were obtained for the enriched with Zn(II) ions *F. vesiculosus* used at a dose of 1 mg - R parameter was higher by 2% than in the control group and B - 2.2 times higher (G value was lower by 34.6%). RGB parameters in the group Zn-*F. vesiculosus* (1 mg) were the highest of all doses tested (2, 4 and 6 mg). However, after the application of G/(R+G+B) formula, the highest content of chlorophyll in garden cress among tested experimental groups was found for natural *F. vesiculosus*.

Table 2. Effect of *Fucus vesiculosus* biomass (natural and enriched) on plant length and RGB parameters of garden cress leaves (mean±SD).

Group/microelement dose	Plant length (cm; ±SD)		RGB analysis			
			R	G	B	G/(R+G+B)
Control	H ₂ O	3.2±1.2 ^b	69.6±7.9 ^{abe}	119±6 ^{ab}	21.5±4.5 ^{ab}	0.566
Natural <i>F. vesiculosus</i>	*	4.4±1.2 ^a	110±15 ^{efg}	178±19 ^{ef}	23.8±4.3	0.571
<i>F. vesiculosus</i> enriched with Cu(II) ions	1 mg Cu	3.1±1.2	60.9±5.5 ^{gh}	83.5±6.8 ^{fg}	19.5±2.5 ^f	0.509
	2 mg Cu	2.7±0.8	62.3±25.3 ^f	88.1±32.0 ^e	23.1±4.6	0.508
	4 mg Cu	3.1±1.0	91.8±13.1	116±13	24.0±7.3	0.500
	6 mg Cu	2.6±1.0 ^a	99.7±17.1 ^h	133±17 ^g	32.5±9.8 ^f	0.502
Natural <i>F. vesiculosus</i>	**	4.2±1.2	52.4±4.5	77.7±6.8 ^c	13.1±2.1 ^{ac}	0.543
<i>F. vesiculosus</i> enriched with Zn(II) ions	1 mg Zn	3.2±1.2	71.0±11.6 ^{cd}	83.3±12.2 ^d	46.5±13.1 ^{cde}	0.415
	2 mg Zn	3.6±0.9	29.7±3.1 ^{ac}	40.3±5.3 ^{acd}	12.8±3.5 ^{be}	0.487
	4 mg Zn	3.8±1.2	40.9±7.0 ^{bd}	51.5±9.4 ^b	14.8±1.0 ^d	0.480
	6 mg Zn	4.3±1.2 ^b	50.6±8.8	71.0±13.1	18.4±2.7	0.507

^{a-b}Differences statistically significant for P<0.05; *the content of Cu in the natural *F. vesiculosus* per dish was 0.0003 mg; **the content of Zn in the natural *F. vesiculosus* per dish was 0.0209 mg.

Effect of the enriched *Fucus vesiculosus* on the micro- and macroelement content in the cultivated *Lepidium sativum*

The multielement composition of the cultivated garden cress is presented in Table 3. The content of Cu in the garden cress treated with Cu-*F. vesiculosus* increased with the increase in Cu dose. In the case of Fe, Zn and Mn, their content in garden cress was lower in all experimental groups treated with the enriched biomass when compared with the natural *F. vesiculosus* and the control group with distilled water. This can indicate inhibitory effect of copper on the accumulation of these microelements in garden cress. It was shown, that the natural *F. vesiculosus* was responsible in the highest extent for the enrichment of garden cress with Mn, Fe and Zn (their content was higher by about 5 times, 4 times and 2 times, respectively as compared with the control group).

Generally, all tested macroelements had lower contents in garden cress groups treated with the *Fucus* enriched with Cu(II) ions when compared to natural *F. vesiculosus* and the control group. The only exception was calcium, which was averagely 18.3% higher than in the control group. Garden cress cultivated with the addition of natural *F. vesiculosus* had higher content of macroelements than the control group - K - 6.7 times, Ca - 5.4 times, Mg - by 36.1%, S - by 22.5%, Na - by 21.5%.

The content of zinc in garden cress increased with the increase in the dose of enriched biomass. In the group Zn-*F. vesiculosus* (6

mg) it was higher by almost 6 times than in the group with natural *F. vesiculosus* and by 8 times than in the control group. In the case of microelements - Cu, Fe, Mn - their content in all experimental groups treated with enriched with Zn(II) ions biomass was comparable to the content in the control group, but much lower than in the group treated with natural *F. vesiculosus*, especially for Cu.

Similar observations have been made for macroelements. The content of Ca, K, Mg, P, S in Zn-*F. vesiculosus* treated groups was comparable to the control group and much smaller than in the group with natural *F. vesiculosus*. The sodium content in Zn-*F. vesiculosus* groups was averagely 2 times lower than in the control group and 4 times lower than in the group with natural *F. vesiculosus*. In the case of Ca and Na it was observed that with the increase in zinc dose, the content of these macroelements decreased in the biomass. Similar results were obtained by Tuhy *et al.* (2014), who tested seaweeds collected from the Baltic coast enriched with Zn(II) ions via biosorption on garden cress. Enriched seaweeds (applied at a dose of 4 mg of Zn/Petri dish) increased the content of zinc in garden cress 16.5 times as compared with the control group. This could be an effective approach for the biofortification of food into selected minerals. The biomass enriched with micronutrients can act as controlled-release fertilizers (Izydorczyk *et al.*, 2020), the effectiveness of which has been confirmed in field trials - for the post extraction residues of alfalfa and goldenrod (Izydorczyk *et al.*, 2020) and post extraction residue of microalga *Spirulina platensis*, both enriched with Cu, Zn and Mn

Table 3. The multielement composition of the cultivated with enriched biomass *Lepidium sativum*: Cu-*F. vesiculosus* and Zn-*F. vesiculosus* (mean±SD; N=3).

Element	Control	Cu doses in the form of enriched <i>F. vesiculosus</i>					
		Natural <i>F. vesiculosus</i>	1 mg	2 mg	4 mg	6 mg	
Microelements (mg kg ⁻¹ d.m.)	Cu	8.69±1.30	13.4±2.0	123±18	404±61	1344±269	2955±591
	Fe	111±17	444±39	103±15	118±18	134±20	128±19
	Mn	32.8±4.9	169±25	23.4±3.5	27.6±4.1	30.4±4.6	31.8±4.8
	Zn	285±43	581±87	233±35	174±26	190±28	253±38
Macroelements (g kg ⁻¹ d.m.)	Ca	1.82±0.36	9.93±2.0	2.25±0.45	1.95±0.39	2.35±0.47	2.08±0.42
	K	10.8±2.2	72.2±14.4	11.4±2.3	10.2±2.0	8.34±1.67	10.9±2.2
	Mg	4.70±0.94	6.40±1.28	4.82±0.96	4.56±0.91	4.61±0.92	5.39±1.08
	Na	8.14±1.63	9.89±1.98	6.078±1.21	5.78±1.16	6.17±1.23	4.89±0.98
	P	7.18±1.43	6.42±1.28	6.81±1.36	7.05±1.41	6.73±1.35	7.67±1.53
	S	14.3±2.9	17.5±3.5	13.8±2.8	13.8±2.8	13.7±2.7	14.8±3.0
Other elements (mg kg ⁻¹ d.m.)	Cd	<LOD	<LOD	0.00198±0.00039	<LOD	<LOD	<LOD
	Cr	4.05±0.20	<LOD	<LOD	<LOD	<LOD	<LOD
	Ni	14.0±2.1	<LOD	<LOD	248±37	23.5±3.5	0.556±0.083

Element	Control	Zn doses (mg) in the form of enriched <i>F. vesiculosus</i>					
		Natural <i>F. vesiculosus</i>	1 mg	2 mg	4 mg	6 mg	
Microelements (mg kg ⁻¹ d.m.)	Cu	8.69±1.30	278±42	7.83±1.17	10.3±1.5	7.97±1.20	9.15±1.37
	Fe	111±17	296±44	110±16	123±18	117±18	119±18
	Mn	32.8±4.9	48.4±7.3	28.3±4.2	30.7±4.6	29.9±4.5	28.8±4.3
	Zn	285±43	399±60	882±132	1304±261	2212±232	2269±454
Macroelements (g kg ⁻¹ d.m.)	Ca	1.82±0.36	5.57±1.11	2.81±0.56	2.01±0.40	1.84±0.37	1.69±0.34
	K	10.8±2.2	54.6±10.9	10.9±2.2	12.5±2.5	13.1±2.9	11.6±2.3
	Mg	4.70±0.94	8.99±1.80	5.10±1.02	5.07±1.01	5.04±1.01	5.13±1.03
	Na	8.14±1.63	17.0±3.4	4.88±0.90	4.63±0.92	4.106±0.82	3.85±0.77
	P	7.18±1.43	9.67±1.93	6.75±1.35	6.79±1.36	6.72±1.34	6.86±1.37
	S	14.3±2.9	22.5±4.5	13.4±2.7	15.4±3.1	14.9±3.0	15.4±3.1
Other elements (mg kg ⁻¹ d.m.)	Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Cr	4.05±0.20	0.283±0.042	<LOD	1.41±0.21	0.232±0.035	0.987±0.148
	Ni	14.0±2.1	3.34±0.50	1.80±0.27	8.84±1.33	<LOD	<LOD

d.m., dry matter; <LOD, below limit of detection.

(Tuhy *et al.*, 2015). Besides Zn, the remaining elements in garden cress were on a comparable level as in the control group. This can indicate that zinc could inhibit the accumulation of other micro- and macroelements. In the work of Zhao *et al.* (2012), who examined the effect of Cu, Mn and Zn on *Phytolacca americana* growth and elemental accumulation it was shown that Zn was an antagonist of Fe, Mn and Cu.

The enrichment of garden cress with microelements was observed mainly for natural *F. vesiculosus* - the content of Cu was about 32 times higher than in the control group, Fe - about 2.7 times higher, Mn - by 47.6% and Zn - by 40%. For macroelements, the following values were obtained - for K - about 5 times higher than in the control group, Ca - 3 times higher, Mg and Na - 2 times higher, S - by 57.5% and P - by 34.7%. This confirms the assumptions of many authors that *Fucus* biomass can be used as a source of micro- and macroelements for plants (Truus *et al.*, 2001; Zodape *et al.*, 2011; Sharma *et al.*, 2012; Mæhre *et al.*, 2014; Paiva *et al.*, 2014; Balina *et al.*, 2016).

***Fucus vesiculosus* extract as a biostimulant of plant growth**

Extract (100%) obtained by autoclaving *Fucus vesiculosus* with KOH, contained the following microelements - Cu $0.079 \pm 0.011 \text{ mg L}^{-1}$, Fe $6.49 \pm 0.97 \text{ mg L}^{-1}$, Mn $0.859 \pm 0.129 \text{ mg L}^{-1}$, Zn $2.25 \pm 0.34 \text{ mg L}^{-1}$, macroelements - Ca $308 \pm 46 \text{ mg L}^{-1}$, K $29.4 \pm 5.9 \text{ g L}^{-1}$ (high due to extraction with KOH), Mg $232 \pm 35 \text{ mg L}^{-1}$, P $5.30 \pm 1.05 \text{ g L}^{-1}$ (pH adjustment with H_3PO_4), S $1.40 \pm 0.28 \text{ g L}^{-1}$, as well as Na $2.62 \pm 0.52 \text{ g L}^{-1}$ and Al $1.35 \pm 0.20 \text{ mg L}^{-1}$. Toxic metal ions such as Cd, Cr, Ni and Pb were below limit of detection in the examined *Fucus* extract. The extraction percentage of micro- and macroelements from the raw *Fucus vesiculosus* was below 10% (S - 8.0%, Na - 7.4%, Zn - 6.3%, Cu - 3.3%, Mg - 2.9%, Fe - 1.6%, Mn - 1.3%). Potassium and phosphorus were not included since compounds of these elements participated in the extraction process. For comparison, Latique *et al.* (2017) determined multi-element composition of extract obtained from *Fucus spiralis* via boiling of fresh ground biomass with distilled water for one hour. This extract contained Ca $51.2 \pm 2.8 \text{ mg L}^{-1}$, K $142.9 \pm 5.4 \text{ mg L}^{-1}$ and Na $72.5 \pm 0.1 \text{ mg L}^{-1}$ and these values were lower than for the extract examined in the present study.

In the literature, the examples of the application of *Fucus* extracts in the plant cultivation are scarce. Sharma *et al.* (2012) evaluated the efficacy of aqueous extracts produced from *Fucus serratus* and *Fucus vesiculosus* (homogenization with water and adjusted to pH 3 with acetic acid or 9 with potassium hydroxide) as plant biostimulants for mung bean (*Vigna mungo*) and pak choi (*Brassica rapa chinensis*). The alkaline extracts stimulated significantly higher dry matter yield of the mung bean plants. Latique *et al.* (2017) found that extract produced from fresh *Fucus spiralis* enhanced seed germination and improved the growth of wheat seedlings under salt stress. Mzibra *et al.* (2021) examined the

extract obtained from *Fucus spiralis*, which accelerated the germination of tomato seeds, improved the biomass yield and biosynthesis of chlorophyll. In the work of Latique *et al.* (2013) it was showed that *Fucus spiralis* extract (25%) obtained by boiling enhanced the vegetative growth of bean plant. Bikovens *et al.* (2017) indicated positive effect of *Fucus vesiculosus* extract on the root elongation of oat. In the present study, several concentrations of the *Fucus vesiculosus* extract (2.5, 5, 10, 25, 50, 100%) were examined in the germination tests. It was observed that higher concentrations of extract (25, 50 and 100%) had toxic effect on plants. Not all seeds germinated, plant growth was much weaker than in the control group watered with distilled water (data not shown). Therefore, higher dilutions of the initial extract were prepared - 2.5, 5 and 10% and tested in the present study.

Effect of *Fucus vesiculosus* extract on the *Lepidium sativum* shoot length and RGB parameters of leaves

The effect of *Fucus vesiculosus* extract on the length of *Lepidium sativum* and RGB parameters of garden cress leaves is presented in Table 4. In all tested experimental groups, the average length of cultivated plants (E 10%: $4.8 \pm 0.1 \text{ cm}$, E 5%: $5.3 \pm 0.7 \text{ cm}$ and E 2.5%: $5.2 \pm 0.4 \text{ cm}$) was higher than in the control group ($4.6 \pm 0.4 \text{ cm}$) by 4.3%, 15.2% and 13.0%, respectively. However, differences between examined groups were not statistically significant. These data are consistent with the previous results obtained for KOH extract produced from green seaweed *Enteromorpha* sp. It was found that *Lepidium sativum* treated with 2.5% seaweed extract (among tested concentrations - 2.5, 5 and 10%) was by 15% longer than in the control group with distilled water (Michalak *et al.*, 2014). *Fucus spiralis* extracts tested in concentrations - 6, 12.5, 25, 50 and 75% on bean plants - increased the shoot length, but only till the 25% concentration (Latique *et al.*, 2013). Latique *et al.* (2017) tested extract from *Fucus spiralis* on wheat and showed that seedlings length increased with the increase in extract concentration (5, 10, 25%) and decreased for 50%, but for all concentrations was higher than in the control group. The potential of seaweed extracts to enhance plant growth may be attributed to the presence of micro- and macroelements or other growth promoting substances like phytohormones (Rathore *et al.*, 2009). For each seaweed extract, preliminary studies should be carried out to determine the optimal concentration of the preparation so that it is not phytotoxic to plants. As demonstrated in the examples presented, the lower concentrations of the extracts stimulated plant growth. This confirm the statement of Crouch and van Staden (1993) that seaweed extracts are bioactive at low concentrations (diluted as 1:1000 or more).

Table 4 presents also results of RGB analysis of the garden cress leaves. All tested concentrations of algal extracts increased the values of RGB parameters in leaves. The best results were obtained for 2.5% extract - R value was by 40.5% higher than in the control group, G value - by 44.1% higher and B - by 96.9%

Table 4. Effect of *Fucus vesiculosus* extract on plant length and RGB parameters of garden cress leaves (mean \pm SD).

Group/concentration of extract		Plant length (cm; \pm SD)	RGB analysis			
			R	G	B	G/(R+G+B)
Control	H ₂ O	4.6 \pm 0.4	88.5 \pm 12.5 ^a	120 \pm 15 ^{ab}	6.4 \pm 2.0	0.558
<i>Fucus vesiculosus</i> extract	2.5%	5.2 \pm 0.4	124 \pm 20 ^{ab}	172 \pm 20 ^{ac}	12.6 \pm 3.1 ^a	0.557
	5%	5.3 \pm 0.7	109 \pm 7	168 \pm 6 ^{bd}	12.4 \pm 4.1	0.580
	10%	4.8 \pm 0.1	91.2 \pm 12.0 ^b	131 \pm 12 ^{cd}	4.7 \pm 2.1 ^a	0.578

^{a-d}Differences statistically significant for P<0.05.

higher. It was also noticed that RGB parameters decreased with the increase in the concentration of extract. The application of G/(R+G+B) formula allows to state that the content of chlorophyll in garden cress was comparable in all experimental groups. Seaweeds and products derived therefrom, due to their natural high content of pigments and other biologically active compounds, in most cases increased the level of chlorophylls and carotenoids in the cultivated plants, for example polysaccharide enriched extracts from *Fucus spiralis* in the cultivation of tomato (Mzibra *et al.*, 2021) or *Fucus spiralis* extract (obtained by boiling) on bean (Latique *et al.*, 2013) or on wheat (Latique *et al.*, 2017).

The use of seaweed extracts in agriculture as biostimulants of plant growth requires finding an application for the obtained post extraction residue. Bikovens *et al.* (2017) proposed to apply the solid residual biomass of *F. vesiculosus* after extraction of active compounds as organic fertilizer. This will guarantee an effective biorefinery approach for algae processing.

Effect of *Fucus vesiculosus* extract on the micro- and macroelement content in the cultivated *Lepidium sativum*

The multielement composition of *Lepidium sativum* treated with *Fucus vesiculosus* extract is presented in Table 5. In the case of microelements (Cu, Fe, Mn, Zn), only 5% extract increased the content of all of them in garden cress (by 1.8 times, by 9.1%, 5.2% and 3.8%, respectively) when compared to the control group. For macroelements, the content of Ca, S, P and K was higher in groups treated with all extracts concentrations than in the control group. The high content of K and P resulted from the experimental conditions of the extract production and their content in garden cress increased with the increase in extract concentration (for 10% E the content of K was 2.8 times higher than in the control group and for P by 28.3%). The content of Ca in the group treated with 2.5% extract was by 36.8% higher than in the control, whereas the content of S in the group with 5% extract - increase by 11.6%). Summarizing the obtained results, 5% extract can be recommended for further experiments.

In the previous work, it was found that algal extracts obtained from green macroalga (*Enteromorpha* sp.) with KOH can act as biostimulants of plant growth. The content of microelements (B,

Cu, Fe, Mn, Zn) was also higher in all experimental groups (concentrations: 2.5, 5 and 10%) than in the control group treated with distilled water (Michalak *et al.*, 2014). Zodape *et al.* (2011) showed that the foliar application of extract from seaweed - *Kappaphycus alvarezii* on tomato increased the content of macro (13.2-67.5%) and micro (23.8-42.6%) elements in fruits over the control. Seaweed extracts are known to improve nutrient uptake by roots and in the leaves (Khan *et al.*, 2009; Rathore *et al.*, 2009; Zodape *et al.*, 2011).

Conclusions

This paper presents results from the germination tests on garden cress conducted under laboratory conditions, in which two *Fucus*-based products were tested. In the case of seaweed enriched with Cu(II) and Zn(II) ions, no significant effect on plant length and RGB parameters was observed. This biomass can be recommended for the biofortification of plants with a given microelement. In the group treated with Zn-*F. vesiculosus* (6 mg), the content of zinc was almost 6 times higher than in the group with natural *F. vesiculosus* and 8 times higher than in the control group and for Cu-*F. vesiculosus* (6 mg) - 220 and 340 times higher, respectively. Much better effects in terms of plant length, chlorophyll content, biofortification in all micro- and macroelements were observed for the natural, unenriched *Fucus vesiculosus*. The *F. vesiculosus* extract had a more favourable effect on plant length, chlorophyll content and multielement composition of garden cress. The highest length and RGB parameters, as well as plant biofortification in Cu, Fe, Mn, Zn, Ca, S, P and K were determined for 5% extract. The length of plants treated with 5% extract was by 20% higher than for the natural *F. vesiculosus* used as a powder. On the other hand, the overall content of micro- and macroelements was higher in garden cress after application of raw *F. vesiculosus*, rather than algal extract. Therefore, *Fucus* can be directly used in agriculture, in the absence of toxic metals in the biomass. Nevertheless, *Fucus* extract is recommended for subsequent research. It is also necessary to conduct further studies on the application of the obtained post extraction residue in agriculture.

Table 5. The multielement composition of cultivated with *Fucus vesiculosus* extract (E) *Lepidium sativum* (mean±SD; N=3).

Element		Control	E 2.5%	E 5%	E 10%
Microelements (mg kg ⁻¹ d.m.)	Cu	7.64±1.15	6.66±1.00	13.6±2.0	7.97±1.20
	Fe	121±18	111±17	132±20	116±17
	Mn	34.3±5.1	33.8±5.1	36.1±5.4	32.0±4.8
	Zn	131±20	134±20	136±20	120±18
Macroelements (g kg ⁻¹ d.m.)	Ca	2.25±0.45	3.08±0.62	2.66±0.53	1.91±0.38
	K	16.2±3.2	36.5±7.3	37.8±7.5	45.6±9.1
	Mg	4.97±0.99	4.58±0.92	4.61±0.92	4.38±0.88
	Na	5.84±1.17	4.84±0.97	5.26±1.05	4.75±0.95
	P	7.68±1.54	8.33±1.67	9.12±1.82	9.86±1.97
	S	9.93±1.99	10.4±2.1	11.1±2.2	10.7±2.1
Other elements (mg kg ⁻¹ d.m.)	Cd	<LOD	<LOD	<LOD	<LOD
	Cr	<LOD	0.194±0.029	0.729±0.109	<LOD
	Ni	3.76±0.45	0.127±0.016	<LOD	<LOD

d.m., dry matter; <LOD, below limit of detection.

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