ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 25(2) 2026, 39–50

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2026.5601

RESEARCH PAPER

Received: 18.09.2025
Accepted: 22.01.2026
Online first: 10.04.2026
Issue published: 29.04.2026

EFFECTS OF ALGINATE ENCAPSULATED DIVALENT IONS (Zn^{2+}, Cu^{2+} AND Ca^{2+}) ON MUSTARD (*Brassica juncea* (L.) Czern.) SEED GERMINATION AND SEEDLING GROWTH

Belén Reig Vañó[1], Beata Kowalska[2], Magdalena Szczech[3], Robert Maciorowski[4], Magdalena Olkiewicz[5], Marta Giamberini[6], Bartosz Tylkowski[7]

ABSTRACT

Encapsulation of agrochemicals allows growers to precisely control the conditions under which the active ingredient is released. Since zinc, copper, and calcium ions are essential micronutrients in crop production, the goal of our work was to incorporate them into alginate-based capsules, and to investigate the impact of their release on seed germination and seedling growth. Oriental mustard (*Brassica juncea* (L.) Czern.) was selected as a model crop. Among the tested ions, Cu^{2+} exhibited the greatest increase, followed by Zn^{2+}, whereas Ca^{2+} showed the smallest increase, and its concentration declined over time when calcium-based capsules were applied. Performed studies demonstrate that released cations from the capsules into soil solution significantly affected seeds germination and biomass of mustard sprouts in laboratory tests. The release of Cu^{2+} and Zn^{2+} negatively influenced radicle development, with Cu^{2+} almost completely suppressing radicle elongation, and Zn^{2+} exhibiting a progressive inhibitory effect with increasing incubation time. Although, Ca^{2+} stimulated radicle elongation, it did not significantly affect total sprout and cotyledon biomass.

Keywords: essential micronutrients, oriental mustard, zinc, copper, calcium

INTRODUCTION

Nowadays the United Nations projects that the world's population could grow to around 8.5 billion in 2030 and 9.7 billion in 2050. It is predicted to reach a peak of around 10.4 billion people during the 2080s, and to remain at that level until 2100 [Gerland et al. 2022]. Thus, it is widely recognized that global agricultural productivity must increase to feed a rapidly growing world population. Therefore, sustainable crop production needs to be secured and enhanced.

[1] Department of Chemical Engineering, Universitat Rovira and Virgili, Av. Països Catalans 26, Campus Sescelades, 43007 Tarragona, Spain, https://orcid.org/0000-0001-6402-4499
[2] The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland, https://orcid.org/0000-0001-7067-3233, corresponding author: beata.kowalska@inhort.pl
[3] The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland, https://orcid.org/0000-0001-8408-6937
[4] The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland, https://orcid.org/0000-0003-1059-4239
[5] Eurecat, Centre Tecnològic de Catalunya, Chemical Technologies Unit, Marcellí Domingo 2, 43007 Tarragona, Spain, https://orcid.org/0000-0002-9849-6715
[6] Department of Chemical Engineering, Universitat Rovira and Virgili, Av. Països Catalans 26, Campus Sescelades, 43007 Tarragona, Spain, https://orcid.org/0000-0001-8278-3552
[7] Department of Chemical Engineering, Universitat Rovira and Virgili, Av. Països Catalans 26, Campus Sescelades, 43007 Tarragona, Spain; Eurecat, Centre Tecnològic de Catalunya, Chemical Technologies Unit, Marcel·lí Domingo 2, 43007 Tarragona, Spain; Faculty of Health Science, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Sklodowskiej Curie 9, 85-094 Bydgoszcz, Poland, https://orcid.org/0000-0002-4163-0178

Encapsulation technology, protected in 1953 and commercialized in 1956 by Dr. Green and Dr. Schleicher from the USA National Cash Register Company, is considered as one of the most interdisciplinary technologies invented in the last decades [Marturano et al. 2019]. The market for encapsulated technologies keeps on growing in volume thanks to the expanding range of applications [Marturano et al. 2018, Wong et al. 2023, Woźniak-Budych et al. 2024]. Companies such as BASF, Syngenta or Ceradis have issued patent for encapsulation technologies focused on agriculture applications [Scher et al. 2003, Krieken et al. 2019, Staff et al. 2022]. The purpose of encapsulation is to enable a faster and more effective use of materials for extremely targeted delivery of active ingredients to specific places. It is achieved by protection of the load from aggressive environments and/or by controlled release within the time frame desired for the application [Bhatia 2020, Tylkowski et al. 2020, García-Carrasco et al. 2023].

Alginate, due to its outstanding properties, such as: biocompatible, biodegradable, water solubility (which minimalized the environmental problems associated with organic solvents), gelification at room temperature (which reduce the risk of the degradation of thermosensitive compounds), as well as due to its readily available and relatively inexpensive cost, has been used for seeds coating [Chin et al. 2021, 2022] as well as for agriculture capsules development [Lambrese et al. 2024]. The capsules have been especially applied to protect beneficial microorganisms or biostimulants from environmental degradation [Jiménez-Arias et al. 2023], as well as to control the release of active ingredients [Fan et al. 2022], for example, to control the release of pesticides [Du et al. 2023], herbicides [Artusio et al. 2021], or fungicides [de Castro Spadari et al. 2017]. As it is disclosed in the US Patent 5204111A [Handjani et al. 1993] alginate capsules can be produced by comprises slowly introducing an aqueous alginate solution into crosslinking solution of a bivalent metal salt. Cations employed as crosslinking agents can be arranged according to their affinity to alginate, as follows: Mn < Zn, Ni, Co < Fe < Ca < Sr < Ba < Cd < Cu < Pb [Ching et al. 2017, Reig-Vano et al. 2021]. Vinceković et al. [2017] reported encapsulation of *Trichoderma* species, as promising alternative to standard plant protection, in the matrix of alginate-based capsule crosslinked by copper cations. While Shaban and El-Komy [2001], and Qi et al. [2023] have investigated encapsulation of *Trichoderma* species in alginate capsules crosslinked with calcium cations. Furthermore, both copper and calcium ions have been used for encapsulation of plant growth regulators [Kudasova et al. 2021], or to reduce the environmental impact of copper fungicides in vineyards [Ortega et al. 2024]. Moreover, fertilizer encapsulation through alginate crosslinking with copper and zinc has been studied by Ekanayake and Godakumbura [2021]. There are a limited number of studies on the effect of alginate capsules containing Ca^{2+}, Zn^{2+} or Cu^{2+} cations on the parameters of crop seedlings. Therefore, the described research was undertaken with using *Brassica juncea* (L.) Czern. as a model plant.

The term mustard refers to a group of plants of the *Brassicaceae* (*Cruciferae*) family, which belongs to the genus *Brassica*. Mainly three species of mustard are cultivated worldwide for their gastronomic value: oriental mustard (*Brassica juncea*, used in our studies), yellow or white mustard (*Sinapis alba* L.), and black mustard (*Brassica nigra* L.). Usually, mustard plants are consumed as edible oils, condiments, sauces, fermented vegetables, or salad greens. These plants have been reported for their high nutritional value and richness in bioactive compounds such as glucosinolates, polyphenols, dietary fiber, β-carotene, and ascorbic acid [Devkota et al. 2020, Ramzan et al. 2023, Rahman et al. 2024]. Moreover, we decided to select mustard because it shows good promise as a rapid and short-duration vegetable crop which could be applied as a crop model. Indeed, it has been considered as a crop model for climate change assessment [Boomiraj et al. 2010], and as a crop model during investigation of have metals capture from contaminated soils [Nepal et al. 2024] . The aims of our work were to prepare alginate-based capsules containing Zn^{2+}, Cu^{2+} or Ca^{2+} ions in their structures and to evaluate the effect of these ions release on mustard seed germination and seedling growth. The experiments were conducted in laboratory conditions, using soil solution with released Zn^{2+}, Cu^{2+} or Ca^{2+} ions as well as soil with the alginate capsules.

MATERIALS AND METHODS

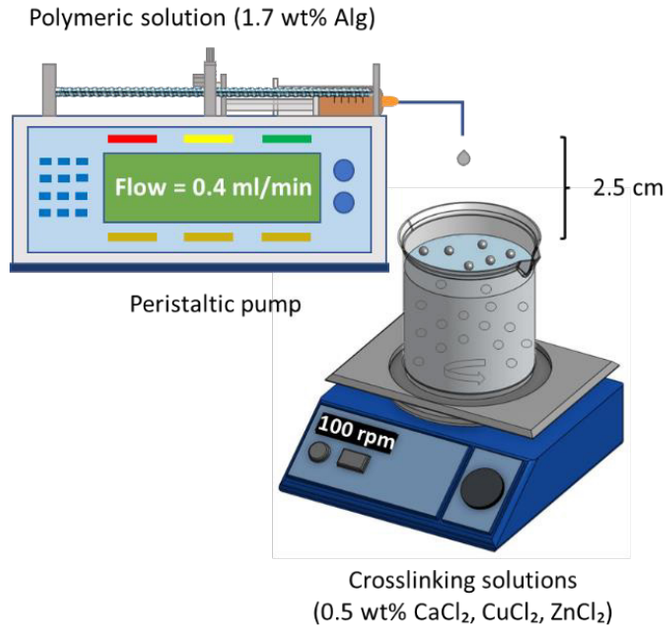
Sodium alginate was purchased from PanReac AppliChem (Barcelona, Spain). Calcium chloride anhydrous (granular, 7.0 mm, >93.0%), zinc chloride (reagent grade, >98%), braunsterican 0.5 × 16 mm BL/BL 25 G 4 5/8 needles and Norm-Ject syringes were purchased from Sigma-Aldrich (Barcelona, Spain). Copper (II) chloride dihydrate (reagent grade, >98%) was purchased from Fisher Scientific (Barcelona, Spain). Mustard *B. juncea* was provided from Kampol (Poland), and it was chosen as a test plant.

Microcapsules preparation

Three types of alginate-based microcapsules: CCa, CZn and CCu were prepared by means of a ionic gelation process during which sodium alginate was crosslinked with Ca^{2+}, Zn^{2+} or Cu^{2+} cations, respectively. As shown in

Figure 1, to fabricate the microcapsules, first 1.7 g of sodium alginate was dissolved in 98.3 g of Milli-Q water. Then, 10 g of obtained solution was extruded dropwise into 200 g of a crosslinking bath containing 0.5 wt% of aqueous solution of one of the salts: $CaCl_2$, $ZnCl_2$ or $CuCl_2$. The encapsulation process was performed at room temperature (22 ±2 °C) using a peristaltic pump (KDS 100 Legacy Syringe Pump, KD Scientific, Holliston, MA, USA) with extrusion rate set-up at 0.4 mL min^{-1}. The distance between the needle's tip and the crosslinking bath was maintained at 2.5 cm. The crosslinking solution was gently stirred, by means of a magnetic stirrer set-up at 100 rpm, during whole encapsulation process which included alginate solution extrusion, and capsules maturation periods. After 48 h of capsules maturation time [Reig-Vano et al. 2021], formed microcapsules were filtered out from the crosslinking bath, washed with Milli-Q water, and finally dried in an oven at 40 °C for 24 h.

Figure 1. Microcapsules preparation set-up



Morphology of fabricated microcapsules was observed by Leica DMS1000 Low-to-Mid Range Magnification Digital Microscope (Leica Microsistemas S.L.U., Barcelona, Spain) and by Environmental Scanning Electron Microscopy (ESEM) using a FEI ESEM Quanta 600 (FEI, Eindhoven, Netherlands) in low vacuum at an intensity of 15 kV.

Microcapsules effect on mustard germination

To investigate the effect of the encapsulated cations on the mustard germination, the mustard seeds were treated with one of 10 different types of soil solutions: SS-0; SS-CCa-3d; SS-CCa-6d; SS-CCa-9d; SS-CZn-3d; SS-CZn-6d; SS-CZn-9d; SS-CCu-3d; SS-CCu-6d; or SS-CCu-9d. In the acronyms:

"SS" means soil solution. It was prepared by thoroughly mixing of 6 kg of the soil with 6 L of water and its incubation for 24 h at ambient temperature. The solution was filtered through filter paper to remove soil particles. SS-0 refers as a control. The soil used in the experiments was sandy-loam soil collected in the National Institute of Horticultural Research (Skierniewice, Poland) and contains: $N\text{-}NO_3$ – 53 mg; P – 64 mg; K – 107 mg; Mg – 131 mg and Ca – 705 mg per 1 L of soil.

"CCa", "CZn" and "CCu" refer to the type of microcapsules, which were used for Ca^{2+}, Zn^{2+} and Cu^{2+} cations release from the CCa, CZn, CCu microcapsules, respectively. These releases were carried out by mixing 50 mL of SS-0 with 0.5 g of corresponding microcapsules and incubated at 25 °C.

"3d", "6d", "9d" corresponds to number of days during which the SS-0 was incubated with the microcapsules. As a matter of examples "SS-CCu-9d" means that this solution was obtained by mixing 0.5 g of CCu microcapsules with 50 mL of SS-0, and then incubated for 9 days. After this period the soil solution was used in the mustard germination test. Each soil solutions were prepared in triplicate.

The tests were performed in Petri plates, lined with disks of filter paper soaked with soil solution added with the capsules. The soil solution "SS" was prepared as described above. Then solution was divided into 50 ml falcon tubes (50 ml of the solution per tube) and was added with 0.5 g of the capsules containing Ca, Zn or Cu cations. Soil solution without capsules (SS-0) served as a control. Each treatment was made in triplicates. The tubes were incubated in dark for 3; 6 and 9 days at 25 °C. After 3 days of incubation, and then after 6 and 9 days, the soil solution with capsules in falcon tubes was vortexed and 2 ml was poured into Petri plate (90 mm diam.) lined with sterile cellulose filter paper. Six plates were prepared for each type of the soil/capsule solution, for each time of incubation. Fifteen seeds of *B. juncea* were placed on the saturated filter paper in each plate. The plates with the seeds were incubated in the growth chamber (Sanyo MRL-351H, Japan) for 14 h in the light at 20 °C, and 10 h in dark at 18 °C, for 10 days. After incubation, mustard sprouts were counted to evaluate germination. Then the sprouts were extracted from the plates to measure: radicle length and weight, shoot weight, and total weight. The test was repeated once at the same conditions.

The effect of the capsules added to soil on mustard seedling growth

The same soil, as for preparation of the soil solution for germination tests, was used in the experiment on the effect of cations release from the capsules on the growth of mustard seedlings. For this experiment the soil was mixed with perlite 3–6 mm (Biovita, Poland) at the volume ratio 3 : 1, and distributed into plastic containers (11 × 17 × 5 cm), 600 g of the soil mixture per one container. The capsules (6 g; 1%) were added to the soil in each container, and thoroughly mixed. The soil without capsules was prepared as a control. Then, water was added to the soil in each container, to obtain approx. 60% of moisture. Closed containers were incubated at ambient temperature for 7 and 14 days.

The next step of this experiment was performed with the use of Phytotoxkit (Tigret, Poland), which is intended for phytotoxicity screening of chemicals, leachates in soil or in other solid, bulk materials. The evaluation is carried out in special, transparent, plastic plates (21 × 15.5 × 0.5 cm), divided to two chambers, one of which is filled with soil sown with test plant seeds. Construction of the plates allows direct observation and measurement of the length of the testing plants.

After one week of incubation, 180 g of the soil with the capsules was taken from each container and distributed into three Phytotoxkit plates (60 g of soil per one plate). Ten seeds of *B. juncea* were then sown into each plate. The plates were incubated in the growth chamber (14 h in the light at 20°C, and 10 h in dark at 18°C). The same was repeated after 14 days of soil incubation in the containers. The plates were incubated for 10 days. Thereafter, mustard seedlings were extracted carefully and following parameters were measured: radicle length and weight, shoot weight, total plant weight. The tests were repeated once at the same conditions.

Chemical analysis

The soil solutions incubated with studied capsules, as well as the bulk soil added with these capsules, were subjected to chemical analysis. For soil solutions, three samples (20 ml) of each solution were taken after 3, 6 and 9 days of incubation and analyzed for the content of available forms of calcium, zinc and copper. For bulk soil mixed with the capsules, the samples (50 g) were taken after 7, and then after 14 days of incubation, and studied for available forms of elements mentioned above. The samples for each treatment were analyzed in triplicates. Determination of Ca, Zn and Cu in soil solution was directly performed using an ICP-OES plasma spectrometer model Optima 2000 DV (Perkin-Elmer), with wavelengths: 327.393 nm for Cu; 317.933 nm for Ca, and 206.200 nm for Zn. This method is widely applied in the network of Chemical and Agricultural Stations in Poland for diagnostic purposes. To measure calcium content, the soil was extracted with acetic acid solution (0.03 N), at a volume ratio of soil : acetic acid solution 20 g : 200 ml, and mixed on a rotary stirrer for 30 min. After filtration the calcium concentration was measured by ICP at the wavelength described above. For copper and zinc, the soil was extracted with Lindsey solution based on EDTA and citric acid at a ratio of vol. 25 : 100 ml. After mixing on a rotary stirrer and filtration, these elements contents were determined with ICP at mentioned wavelengths.

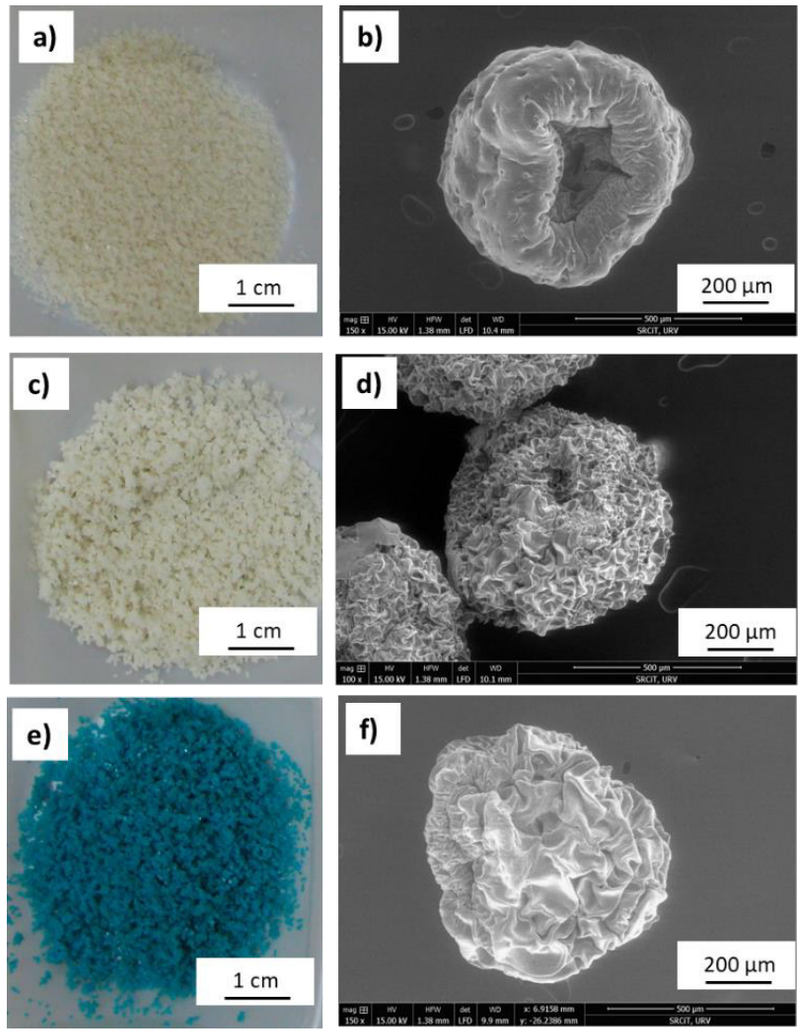
Statistical analyses

Data from the experiments were tested for normality of distribution and homogeneity of variance using Shapiro-Wilk and Lavene tests, respectively. An analysis of variance was then performed in a two-factor model with the variance due to the repetition of experiments extracted. If significance was found for the effects tested, the means were compared using Duncan's test at $\alpha = 0.05$. Calculations were performed in the statistical package Statistica v. 13 (Dell Inc. 2016).

RESULTS AND DISCUSSION

By applying the external ionotropic gelation method, three types of microcapsules formed by alginate, and calcium, zinc or copper cations were successfully fabricated. Figure 2a, c, and e shows the digital images of CCa, CZn and CCu microcapsules, respectively, while Figure 2b, d and f shows their ESEM micrographs.

Figure 2. Digital images (a, c and e) and ESEM micrographs (b, d and f) of CCa (a, b), CZn (c, d) and CCu (e, f) microcapsules



From the digital micrographs it seems that the microcapsules are aggregated, and several of them fuse together to form clumps, which could affect their stability and eventually leads to caking of particles. However, their deeper investigation by ESEM demonstrate that they are well separated. The difference in their outer morphology is remarkable. The surface of the calcium-crosslinked microcapsules (Figure 2b) is smooth and uniform, while the surfaces of the capsules crosslinked by zinc and copper cations (Figure 2d and 1f, respectively) are extremely rough and irregular. Reig-Vano et al. [2021] reported that this difference can be attributed to a different crosslinking mechanism.

Kinetics release of cations from the capsules structure into soil solution and bulk soil

In all experiments with mustard, concentrations of the cations released from the capsules were studied, according to the time of their incubation in soil solution or in bulk soil. The content of available calcium, zinc and copper ions did not change in the soil solution as well as in the soil during whole time of incubation (Tables 1 and 2).

When the capsules with Ca, Zn or Cu were added to the soil solution (after 3 days of incubation), the concentration of the respective cations significantly increased by 53%, 926%, and 232%, respectively (Table 1). After next 3 days of incubation, no further increase in concentrations of these cations in the amended solutions was noted. However, significant increase of studied cations concentrations was recorded after 9 days. It was also observed that application of capsules with Zn^{2+} also resulted in growth of the concentration of available Cu^{2+}, and opposite, application of Cu-capsules caused increased amount of available Zn^{2+} (Table 1).

McBride and Bouldin [1984] carried out studies based on ion-selective electrode data, and they reported that at least 99.5% of Cu in soil was in an organically complexed form, while Hazra et al. [1987] reported that more than 84% of total Zn in soils occurs as structurally lattice bound, about 13% as sesquioxide bound, 1.6% as organically complexed, and approximately 1% as exchangeable and water soluble forms. According to literature [Shaheen et al. 2013, Yu et al. 2023] pH value of the soil system is a very important parameter, directly influencing sorption/desorption, precipitation/dissolution, complex formation, cation exchange capacity and oxidation-reduction reactions. In general, maximum retention of cationic metals by soil active sides occurs at pH > 7. Indeed, Gong and Donahoe [1997] reported that Cu and Zn became mobile from the soil to soil solution by decreasing its pH value. Thus, very low concentrations of Cu and Zn cations detected in SS0 soil solution (0.02 and 0.01 mg L^{-1}, respectively) could be explained by its pH value of 7.7. At this pH values Cu and Zn cations can be retented by soil active sides. While, in soils solutions enriched with zinc and copper capsules (SS-CZn and SS-CCu) pH values decreased from pH 7.7 to 7.1 and 6.3 respectively. It seems that these pH value changes caused Cu and Zn cations released from the soil, and as consequence they were detected in the investigated soil solutions. Similar increase of soluble Cu^{2+} and Zn^{2+} concentration in soil solution was noted after application of Ca-capsules (soil solution pH 7.2). However, addition of Zn- and Cu-capsules to the soil solutions reduced availability of Ca presented in the stock solution, ~32% and ~36%, respectively, after 9 days of incubation. In the case of Zn, the reduction of Ca availability was significant during the first 3 days of incubation. This phenomena could be explained by Ca and Zn ions exchangeability.

Table 1. Concentration of soluble elements in soil solutions incubated for 3, 6 and 9 days

Treatment	Ca (mg L^{-1})			Zn (mg L^{-1})			Cu (mg L^{-1})		
	Days of soil solutions incubation								
	3	6	9	3	6	9	3	6	9
SS0	110.7 c	112.0 c	113.0 c	0.01 c	0.01 c	0.01 c	0.02 c	0.01 c	0.01 c
SS-CCa	168.3 b	170.0 b	200.7 a	0.36 c	0.06 c	0.37 c	0.20 c	0.02 c	0.16 c
SS-CZn	90.7 e	88.5 e	77.2 f	92.60 b	94.80 b	123.20 a	0.84 c	0.80 c	0.66 c
SS-CCu	106.7 d	104.0 d	71.6 g	0.08 c	0.07 c	0.08 c	46.50b	50.00b	73.20 a

SS0 – incubated stock soil solution (control); SS-CCa, SS-CZn, SS-CCu soil solutions incubated with microcapsules of Ca, Zn, and Cu, respectively. Means followed by the same letter do not differ significantly according to DMRT at $p \geq 0.05$

Table 2. Concentration of elements available for plants in soil incubated for 7 and 14 days with the capsules

Treatment	Ca (mg kg^{-1})		Zn (mg kg^{-1})		Cu (mg kg^{-1})	
	Days of incubation in soil					
	7	14	7	14	7	14
S0	837.3 c	768.0 d	11.6 c	10.5 c	2.2 c	3.0 c
S-CCa	1006.7 a	877.3 b	8.7 c	9.5 c	1.5 c	2.0 c
S-CZn	828.0 c	705.7 e	1369.0 b	1501.7 a	2.1 c	1.9 c
S-CCu	823.0 c	760.7 d	9.2 c	9.8 c	914.9 b	977.4 a

SS0 – incubated stock soil solution (control); SS-CCa, SS-CZn, SS-CCu soil solutions incubated with microcapsules of Ca, Zn, and Cu, respectively. Means followed by the same letter do not differ significantly according to DMRT at $p \geq 0.05$

In bulk soil, just like in the soil suspension, incubation time was not the factor changing the concentration of available cations. Addition of the capsules to the soil mixture with perlite resulted in significant growth of soluble cations amount compared to control soil (S0) not supplemented with the capsules (Table 2). For copper, after

7 days of soil incubation, the concentration of this element increased more than 400-times compared to control, and successively increased after 14 days of incubation. To the lower extend increased concentration of soluble zinc (about 100-times after 7 days), but also in the case of this ion, progressed availability was observed with time of incubation. On the contrary, calcium concentration increase, although significant after the addition of capsules, was lower compared to Cu and Zn. Moreover, in the case of calcium added with the capsules, its availability decreased with time of incubation, and was significantly lower after 14 days than after 7 days.

Effect of encapsulated cations on mustard seeds germination and sprouts biomass

Cu, Zn and Ca, released from the capsules into soil solution significantly affected seeds germination and biomass of mustard sprouts in laboratory tests. Seed germination was the most influenced by copper, especially after treatment with the nine-day-old incubated soil solution (Figure 3a), where mustard germination was inhibited by about 50% compared to control. Similar effects were observed by Rather et al. [2020] who exposed *B. juncea* to 3.0 mM copper aqueous solution for 3 days, as well as by Gautam et al. [2016] who investigated the changes in the safflower (*Carthamus tinctorius* L.) growth during its exposure to different concentrations of copper (25, 50 and 100 µM) for 20 days. In our studies we also observed that in excess of its permissible concentration, copper cations can lead to a significant reduction of seed germination and seedling growth, while zinc and calcium did not affect mustard germination significantly. Similar effect was observed in order to total sprouts weight, which was significantly reduced by copper released form the CCu capsules (Figure 3c). Zhao et al. [2022] and Xin et al. [2020] also indicated, that high concentration of copper can have negative impact on seeds germination and plant growth. While, in the studies of Bączek-Kwinta et al. [2020] it was found that zinc unaffected germination of broccoli (*Brassica oleracea* L. var. *botrytis italica*), sunflower (*Helianthus annuus* L.), and pea (*Pisum sativum* L.) seeds.

In our studies, total biomass of the sprouts was not affected by zinc and calcium released from CZn and CCa capsules. However, the cations had different effects on the ratio of radicle to cotyledons weight. The strongest effect of the cations released to soil solution was observed in the case of sprout's radicles. Copper and zinc released from CCu and CZn capsules, greatly reduced mustard radicles, especially their length (Figure 3c and d, respectively). In the case of copper, radicle development was almost completely reduced regardless of the incubation time of soil solution with the CCu capsules. Zinc also reduced the radicle development, but its effect was enhanced proportionally with increased incubation time of CZn capsule-soil solution by: 38%, 70%, 94% for 3, 6 and 9 days of capsules incubation, respectively. The results are consistent with those obtained by Ivanova et al. [2010] and Wen et al. [2024] who shown that copper and zinc have influenced on the parameters of plants.

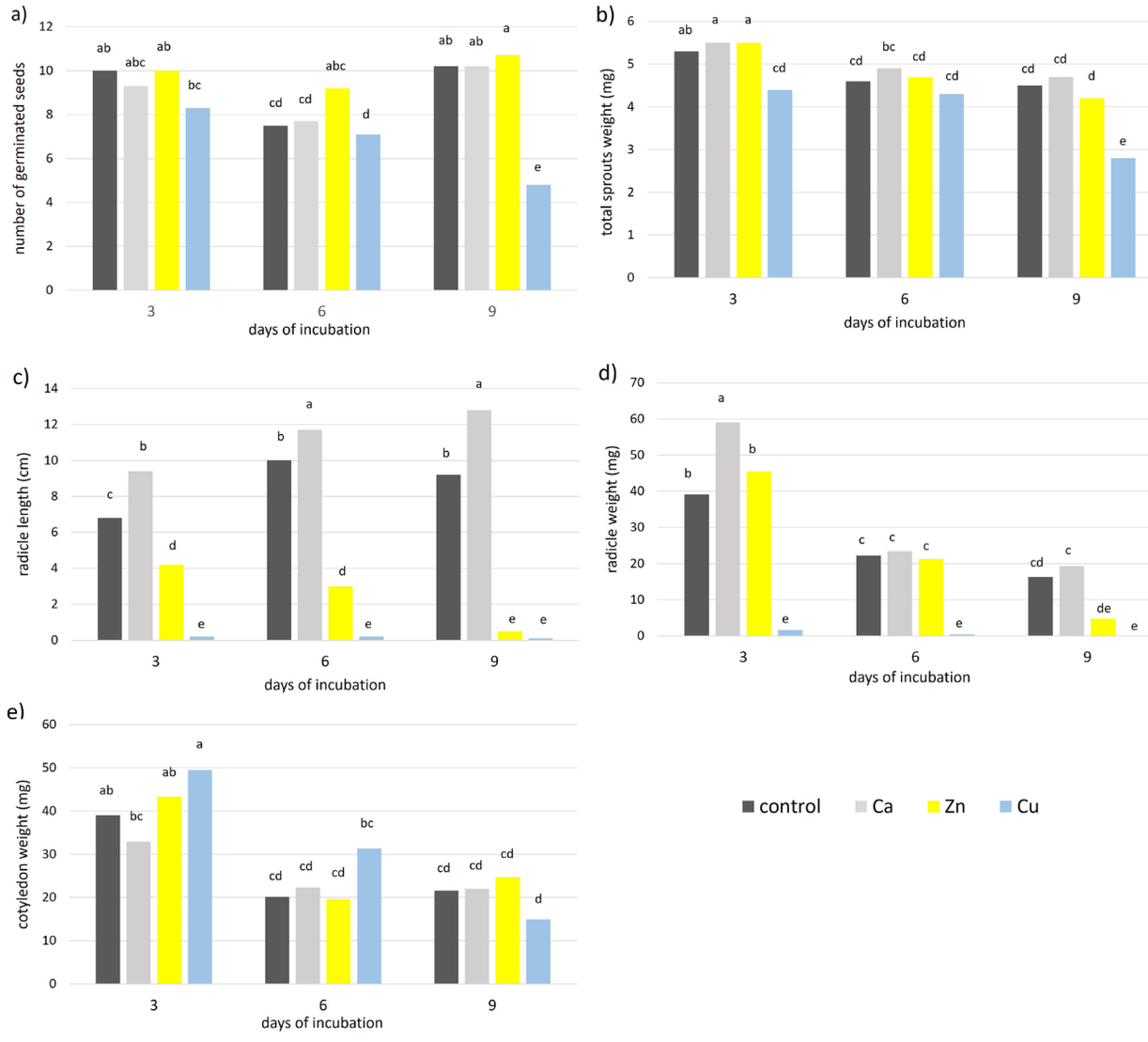
In opposite, calcium significantly stimulated sprouts radicle elongation (Figure 3c), and its effect was positively proportional to the time of its release from the CCa capsules. When the soil solution incubated with the CCa capsules for 9 days was used, the mustard radicles were 39% longer compared to control sprouts. Actually, Li et al. [2014] reported that the treatment of wheat seeds (*Triticum aestivum* L.) with aqueous solution of zinc sulfate heptahydrate $ZnSO_4 \cdot 7H_2O$ (concentration range from 0.5 mM to 3 mM) did not affect seed germination, but reduced root length, while Takahashi et al. [1992] reported that Ca^{2+} at 10 or 20 mM, significantly stimulated root elongation in *P. sativum* and *Zea mays* L. seedlings.

A different response to the ions applied with capsules was found on cotyledons. In this case, copper released to the soil solution stimulated wight of this part for 27% and 55% after 3 and 6 days of incubation, respectively, compared to control (Figure 3e). However, the differences were not significant. After 9 days of incubation in soil solution, concentration of copper released from the capsules was phytotoxic. Zinc and calcium, released from the CZn and CCa capsules, had no significant effect on cotyledons biomass.

The effect of capsules added to soil on mustard seedling growth

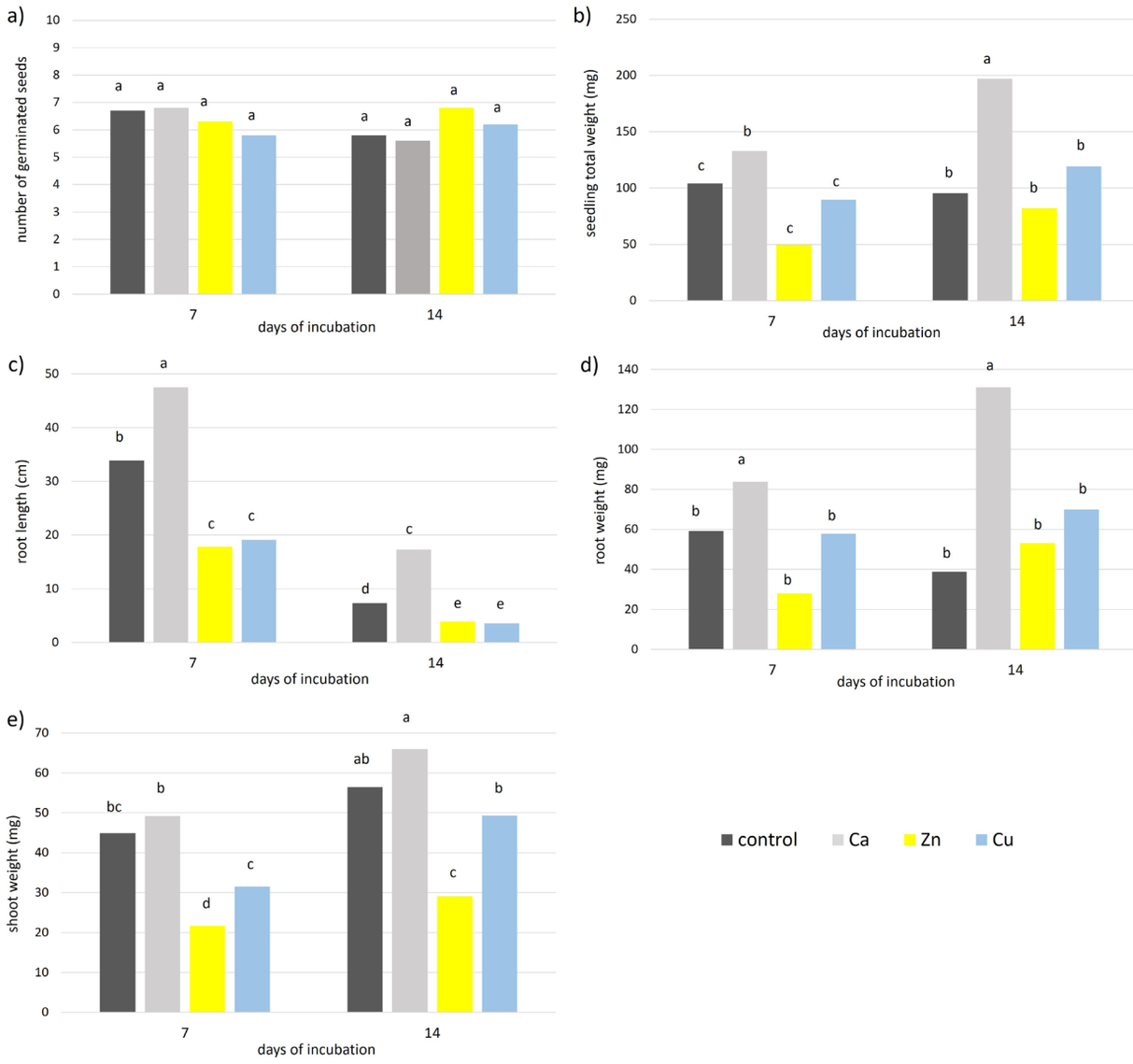
The cations released from the capsules to the soil had not significant effect on mustard seeds germination in Phytotoxkit tests. The germination was comparable for all treatments in both incubation times (Figure 4a). However, the seedling weight was significantly enhanced by calcium released to the soil from the capsules (Figure 4b). Stronger, positive effect of calcium on mustard growth was observed in soil incubated for 14 days, where the seedlings from soil with Ca-capsules were by 106% bigger than in control. This positive effect of calcium was mostly related to the greater roots development, both length and weight (Figure 4c and 4d). The average length of the roots in soil amended with Ca-capsules was 29% and 58% higher than in control for plants grown in soil incubated for 7 and 14 days, respectively. Similarly, the weight of mustard roots treated with released Ca was 29% and 70% greater than for control plants grown in soil incubated for 7 and 14 days, respectively. Moreover, calcium released from the capsules stimulated also the growth of the above-ground parts of mustard seedlings (Figure 4e), however, the differences were not significant.

Figure 3. The effect of the ions released from capsules into soil solution on mustard sprouts development: germination (a); total weight (b); radicle length (c); radicle weight (d); cotyledon weight (e). The same letters above the bars indicate that means are not significantly differentiated according to DMRT at $p \geq 0.05$



In the case of copper and zinc, these elements released to the soil during incubation, significantly decreased roots elongation (Figure 4c). However, these cations did not affect significantly the weight of the young roots (Figure 4d). In those experiments, zinc also significantly inhibited development of the above-ground parts of the seedlings (Figure 4e), what resulted in decreased total seedlings weight (Figure 4b). Copper had no marked influence on mustard seedling growth, regardless the duration of the capsules incubation in the soil. Mariano-da-Silva et al. [2025] investigated the effects of different copper concentrations on the germination and seedling development of canola (*Brassica napus* L. var. *oleifera*). Canola is an oilseed crop developed through genetic improvement of rapeseed, which originated from a spontaneous interspecific cross between wild cabbage (*Brassica oleracea* L.) and mustard (*Brassica rapa* L. syn. *campestris*). The authors reported that copper levels exceeding approximately 30–90 mg Cu kg^{-1} soil, depending on species and soil conditions, inhibit *Brassica* growth, reducing chlorophyll content, photosynthetic efficiency, nitrate reductase activity, and water relations, while increasing oxidative stress markers. Moreover they demonstrated that in case of *B. juncea*, exposures to 30–90 mg Cu kg^{-1} resulted in pronounced reductions in growth parameters and key biochemical processes. Similarly, *Brassica napus* L. seedlings exposed to 200–800 mg L^{-1} Cu exhibited significant declines in germination, root and shoot elongation,

Figure 4. The effect of the ions released from capsules on mustard seedling growth in treated soil: seeds germination (a); total seedlings weight (b); root length (c); root weight (d); shoot weight (e). The same letters above the bars indicate that means are not significantly differentiated according to DMRT at $p \geq 0.05$



and dry mass accumulation. Long et al. [2003] reported that zinc toxicity thresholds in *Brassica* typically range from 170–270 mg DTPA-Zn kg^{-1} soil, levels above which biomass and yield begin to decline. In *Brassica chinensis* L. (pakchoi, Chinese cabbage), shoot biomass dropped when soil-extractable Zn exceeded ~170 mg kg^{-1}, and phytotoxic effects in edible tissues were observed at total soil Zn concentrations of 224–413 mg kg^{-1}. The authors indicated that across heavier soils, other *Brassica* vegetables showed similar declines in growth and photosynthesis under elevated Zn.

CONCLUSIONS

The incubation time of alginate-based capsules in soil did not significantly affect the concentration of available cations. Among the tested ions, Cu^{2+} exhibited the highest increase, followed by Zn^{2+}, whereas Ca^{2+} concentration not only displayed the lowest increase, but also declined over time when calcium-based capsules were applied. The release of Cu^{2+} exerted the strongest impact on plant performance, significantly inhibiting seed germination and reducing sprout biomass. In contrast, Ca^{2+} and Zn^{2+} did not significantly affect mustard germination nor total biomass of the sprouts. The release of Cu^{2+} and Zn^{2+} from the capsules hindered radicle development. Cu^{2+} almost

completely inhibited radicle development, while Zn^{2+} had a progressive inhibitory effect with increased incubation time. In contrast, Ca^{2+} stimulated radicle elongation. Although Cu^{2+} increased cotyledon weight, prolonged incubation (9 days) led to phytotoxic effects associated with elevated Cu^{2+} concentrations. Neither Ca^{2+} nor Zn^{2+} significantly affected cotyledon biomass.

The findings of this study suggest that alginate-based encapsulated ions have potential applications in controlled nutrient delivery; however, ion-specific responses and phytotoxic thresholds must be carefully considered. These results justify further evaluation using economically important vegetable crops. Future research should focus on the development of alginate-based capsules crosslinked with zinc, copper, and calcium, with diameters smaller than 1 mm, to assess how capsule size influences cation release dynamics in soil and subsequent plant growth responses.

SOURCE OF FUNDING

Polish Ministry of Education and Science, task ZM/2/2024-4.2.24 (2024–2028).

AUTHOR CONTRIBUTIONS

M.O., B.T., M.G., M.S., B.K. – methodology, B.T., M.S., M.G. – conceptualization; B.T., M.G., M.O., R.M. – data curation; B.R. – formal analysis; B.R., B.K., M.S. – investigation; M.O., B.T., M.G., M.S., B.K – methodology; B.T. – project administration; B.T., M.G., B.K., M.S. – resources; B.T., M.G. – supervision; B.T., M.S. – visualization; B.R., B.T., M.G., M.O., M.S. – writing – original draft preparation; B.T., B.K., M.S. – writing – review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors ensure that they have neither professional nor financial connections related to the manuscript sent to the Editorial Board. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Artusio, F., Casà, D., Granetto, M. et al. (2021). Alginate nanohydrogels as a biocompatible platform for the controlled release of a hydrophilic herbicide. Processes, 9, 1641. https://doi.org/10.3390/pr9091641

Bączek-Kwinta, R., Baran, A., Simlat, M. et al. (2020). Enrichment of different plant seeds with zinc and assessment of health risk of Zn-fortified sprouts consumption. Agronomy, 10, 937. https://doi.org/10.3390/agronomy10070937

Bhatia, M. (2020). A review on application of encapsulation in agricultural processes. In: S.H., Sonawane, B.A., Bhanvase, M., Sivakumar (eds.), Encapsulation of active molecules and their delivery system. Elsevier, 131–140. https://doi.org/10.1016/B978-0-12-819363-1.00008-9

Boomiraj, K., Chakrabarti, B., Aggarwal, P.K. et al. (2010). Assessing the vulnerability of Indian mustard to climate change. Agric. Ecosyst. Environ., 138, 265–273. https://doi.org/10.1016/j.agee.2010.05.010

de Castro Spadari, C., Lopes, L.B., Ishida, K., (2017). Potential use of alginate-based carriers as antifungal delivery system. Front. Microbiol., 8. https://doi.org/10.3389/fmicb.2017.00097

Chin, J.M., Lim, Y.Y., Ting, A.S.Y. (2021). Biopolymers for biopriming of *Brassica rapa* seeds: a study on coating efficacy, bioagent viability and seed germination. J. Saudi Soc. Agric. Sci., 20, 198–207. https://doi.org/10.1016/j.jssas.2021.01.006

Chin, J.M., Lim, Y.Y., Ting, A.S.Y. (2022). Biopriming *Pseudomonas fluorescens* to vegetable seeds with biopolymers to promote coating efficacy, seed germination and disease suppression. J. Saudi Soc. Agric. Sci., 21, 493–505. https://doi.org/10.1016/j.jssas.2022.02.002

Ching, S.H., Bansal, N., Bhandari, B. (2017). Alginate gel particles – a review of production techniques and physical properties. Crit. Rev. Food Sci. Nutr., 57, 1133–1152. https://doi.org/10.1080/10408398.2014.965773

Devkota, K., dos Santos, C.F., Blochtein, B. (2020). Mustard plants distant from forest fragments receive a lower diversity of flower-visiting insects. Basic Appl. Ecol., 47, 35–43. https://doi.org/10.1016/j.baae.2020.05.005

Du, Y., Zhang, Q., Yu, M. et al. (2023). Sodium alginate-based composite microspheres for controlled release of pesticides and reduction of adverse effects of copper in agricultural soils. Chemosphere, 313, 137539. https://doi.org/10.1016/j.chemosphere.2022.137539

Ekanayake, S.A., Godakumbura, P.I. (2021). Synthesis of a dual-functional nanofertilizer by embedding ZnO and CuO nanoparticles on an alginate-based hydrogel. ACS Omega, 6, 26262–26272. https://doi.org/10.1021/acsomega.1c03271

Fan, C., Liu, Y., Dang, M. et al. (2022). Polysaccharides synergistic boosting drug loading for reduction pesticide dosage and improve its efficiency. Carbohydr. Polym., 297, 120041. https://doi.org/10.1016/j.carbpol.2022.120041

García-Carrasco, M., Valdez-Baro, O., Cabanillas-Bojórquez, L.A. et al. (2023). Potential agricultural uses of micro/nano encapsulated chitosan: a review. Macromol, 3, 614–635. https://doi.org/10.3390/macromol3030034

Gautam, S., Anjani, K., Srivastava, N. (2016). In vitro evaluation of excess copper affecting seedlings and their biochemical characteristics in *Carthamus tinctorius* L. (variety PBNS-12). Physiol. Mol. Biol. Plants., 22, 121–129. https://doi.org/10.1007/s12298-016-0339-1

Gerland, P., Hertog, S., Wheldon, M. et al. (2022). World Population Prospects 2022: Summary of results. United Nations, New York.

Gong, C., Donahoe, R.J. (1997). An experimental study of heavy metal attenuation and mobility in sandy loam soils. Appl. Geochem., 12, 243–254. https://doi.org/10.1016/S0883-2927(96)00049-2

Handjani, R.-M., Kauffmann, M., Huguenin, F. (1993). Process for the preparation of alginate capsules, apparatus for producing said capsules and cosmetic compositions containing said capsules. United States Patent, US5204111A.

Hazra, G.C., Mandal, B., Mandal, L.N. (1987). Distribution of zinc fractions and their transformation in submerged rice soils. Plant Soil., 104, 175–181. https://doi.org/10.1007/BF02372530

Ivanova, E.M., Kholodova, V.P., Kuznetsov, V.V. (2010). Biological effects of high copper and zinc concentrations and their interaction in rapeseed plants. Russ. J. Plant Pathol., 57, 864–873.

Jiménez-Arias, D., Morales-Sierra, S., Silva, P. et al. (2023). Encapsulation with natural polymers to improve the properties of biostimulants in agriculture. Plants, 12, 55. https://doi.org/10.3390/plants12010055

Krieken, W.M.V. der, Rutten, W.B.A.H., Jans, C.G.J.M. (2019). Polyelectrolyte complexes for biocide enhancement. United States Patent, US10342228B2.

Kudasova, D., Mutaliyeva, B., Vlahoviček-Kahlina, K. (2021). Encapsulation of synthesized plant growth regulator based on copper (II) complex in chitosan/alginate microcapsules. Int. J. Mol. Sci., 22, 2663. https://doi.org/10.3390/ijms22052663

Lambrese, Y.S., Illanes, C.O., Ochoa, N.A. (2024). Advancing bacterial endophyte encapsulation in alginate for sustainable agriculture: method comparisons, morphology and viability assessment. J. Clean. Prod., 457, 142473. https://doi.org/10.1016/j.jclepro.2024.142473

Li, M., Yang, X.W., Tian, X.H. et al. (2014). Effect of nitrogen fertilizer and foliar zinc application at different growth stages on zinc translocation and utilization efficiency in winter wheat. Cereal Res. Commun., 42(1), 81–90. https://doi.org/10.1556/CRC.2013.0042

Long, X.X., Yng, X.E., Ni, W.Z. et al. (2003). Assessing zinc tresholds for phytotoxicity and potencial diatery toxicity in selected vegetable crops. Commun. Soil Sci. Plant Anal., 35(9–10), 1421–1434. https://doi.org.sabidi.urv.cat/10.1081/CSS-120020454

Mariano-da-Silva, S., Mottin, F.M., Ducatti, R.D.B. et al. (2025). Effects of copper toxicity on seed germination and seedling growth of *Brassica napus* L. var. *oleifera*. Rev. Gest. Soc. Ambient. – RGSA, 19(2), e010913. https://doi.org/10.24857/rgsa.v19n2-009

Marturano, V., Bizzarro, V., De Luise, A. et al. (2018). Essential oils as solvents and core materials for the preparation of photo-responsive polymer nanocapsules. Nano Res., 11, 2783–2795. https://doi.org/10.1007/s12274-017-1908-5

Marturano, V., Kozlowska, J., Bajek, A. et al. (2019). Photo-triggered capsules based on lanthanide-doped upconverting nanoparticles for medical applications. Coord. Chem. Rev., 398, 213013. https://doi.org/10.1016/j.ccr.2019.213013

McBride, M.B., Bouldin, D.R. (1984). Long-term reactions of copper(II) in a contaminated calcareous soil. Soil Sci. Soc. Am. J., 48, 56–59. https://doi.org/10.2136/sssaj1984.03615995004800010010x

Nepal, A., Antonious, G.F., Bebe, F.N. et al. (2024). Heavy metal accumulation in three varieties of mustard grown under five soil management practices. Environments, 11, 77. https://doi.org/10.3390/environments11040077

Ortega, P., Sánchez, E., Montornes, J.M. et al. (2024). Design and evaluation of microencapsulation technology to reduce the environmental impact of copper fungicides in vineyards. Crop Prot., 176, 106502. https://doi.org/10.1016/j.cropro.2023.106502

Qi, Q., Fan, C., Wu, H. et al. (2023) Preparation of *Trichoderma asperellum* microcapsules and biocontrol of cucumber powdery mildew. Microbiol. Spectr., 11, e05084-22. https://doi.org/10.1128/spectrum.05084-22

Rahman, M., Khatun, A., Liu, L. et al. (2024). *Brassicaceae* mustards: phytochemical constituents, pharmacological effects, and mechanisms of action against human disease. Int. J. Mol. Sci., 25, 9039. https://doi.org/10.3390/ijms25169039

Ramzan, T., Shahbaz, M., Maqsood, M.F. et al. (2023). Phenylalanine supply alleviates the drought stress in mustard (*Brassica campestris*) by modulating plant growth, photosynthesis, and antioxidant defense system. Plant Physiol. Biochem., 201, 107828. https://doi.org/10.1016/j.plaphy.2023.107828

Rather, B.A., Mir, I.R., Masood, A. et al. (2020). Nitric oxide pre-treatment advances seed germination and alleviates copper-induced photosynthetic inhibition in Indian mustard. Plants, 9, 776. https://doi.org/10.3390/plants9060776

Reig-Vano, B., Tylkowski, B., Montané, X. et al. (2021). Alginate-based hydrogels for cancer therapy and research. Int. J. Biol. Macromol., 170, 424–436. https://doi.org/10.1016/j.ijbiomac.2020.12.161

Scher H.B., Shirley I.M., Chen J. et al. 2003. Novel microcapsules. European Patent Office, EP1292386A2.

Shaban, G.M., El-Komy, H.M.A. (2001). Survival and proliferation of alginate encapsulated *Trichoderma* spp. in Egyptian soil in comparison with allyl alcohol soil fumigation. Mycopathologia, 151, 139–146. https://doi.org/10.1023/A:1017968429632

Shaheen, S.M., Tsadilas, C.D., Rinklebe, J. (2013). A review of the distribution coefficients of trace elements in soils: influence of sorption system, element characteristics, and soil colloidal properties. Adv. Colloid Interface Sci., 201–202, 43–56. https://doi.org/10.1016/j.cis.2013.10.005

Staff, R.H., Mecfel-Marczewski, J., Semar et al. (2022). New microcapsules for agricultural applications. United States Patent Publication Aplication, US20220217976A1.

Takahashi, H., Scott, T.K., Suge, H. (1992). Stimulation of root elongation and curvature by calcium 1. Plant Physiol., 98, 246–252. https://doi.org/10.1104/pp.98.1.246

Tylkowski, B., Olkiewicz, M., Montane, X. et al. (2020). 11. Encapsulation technologies in agriculture. In: B., Tylkowski, M., Giamberini, S., Fernandez Prieto (eds.), Microencapsulation. De Gruyter, 287–302. https://doi.org/10.1515/9783110642070-011

Vinceković, M., Jurić, S., Đermić, E. et al. (2017). Kinetics and mechanisms of chemical and biological agents release from biopolymeric microcapsules. J. Agric. Food Chem., 65, 9608–9617. https://doi.org/10.1021/acs.jafc.7b04075

Wen X., Xu P., Tang Y. et al. (2024). Effect of copper on nitrogen uptake, transportation, assimilation processes, and related gene expression in Chinese cabbage [*Brassica campestris* L. ssp. *Chinensis* (L.)] under various nitrate-to-ammonium ratios. Fron. Plant Sci., 15, 1427720. https//doi.org/10.3389/fpls.2024.1427720

Wong, L.W., Fahimizadeh, M., Lee Tan, J.B. et al. (2023). Chapter 11. Polyurea microcapsules as effective carriers for biomedical and agricultural applications. In: P., Pasbakhsh, D., Mohotti, K., Palaniandy et al. (eds.), Polyurea. Elsevier, pp. 191–202. https://doi.org/10.1016/B978-0-323-99450-7.00019-8

Woźniak-Budych, M., Staszak, K., Wieszczycka, K. et al. (2024). Microplastic label in microencapsulation field – consequence of shell material selection. J. Hazar. Mater., 465, 133000. https//doi.org/10.1016/j.hazmat.2023.133000

Xin, X., Zhao, F., Rho, J.Y. et al. (2020). Use of polymeric nanoparticles to improve seed germination and plant growth under copper stress. Sci. Total Environ., 745, 141055. https://doi.org/10.1016/j.scitotenv.2020.141055

Yu, H., Li, C., Yan, J. et al. (2023). A review on adsorption characteristics and influencing mechanism of heavy metals in farmland soil. RSC Advances, 13, 3505. https://doi.org/10.1039/d2ra07095b

Zhao, X., Guo, Y., Papazoglou, E.G. (2022). Screening flax, kenaf and hemp varieties for phytoremediation of trace element-contaminated soils. Ind. Crops Prod., 185, 115121. https://doi.org/10.1016/j.indcrop.2022.115121