Industrial Crops & Products 185 (2022) 115128

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Micronutrient-controlled-release protein-based systems for horticulture: Micro vs. nanoparticles

Mercedes Jiménez-Rosado *, Victor Perez-Puyana, Antonio Guerrero, Alberto Romero

Departamento de Ingeniería Química, Universidad de Sevilla, 41012 Sevilla, Spain

A R T I C L E I N F O

Keywords:
Micronutrients
Controlled release
Protein-based materials
Horticulture
Nanoparticles

A B S T R A C T

Fertilization is an increasingly common practice in horticulture. Nevertheless, the conventionally used fertilization method is ineffective, and it generates contamination problems due to excess nutrients. Therefore, new technologies, such as nanofertilization or controlled-release systems of fertilizers, are currently being tested. Thus, the main objective of this work was to develop controlled-release systems for micronutrients, using soy protein as raw material. Different micronutrients (zinc, copper, iron, and manganese) were evaluated, as well as their incorporation in the form of micro and nanoparticles. The mechanical and functional properties (water uptake capacity, biodegradability, and micronutrient release) of the systems, as well as their use in crops, were studied to assess their viability. The results showed the great potential of these systems to incorporate micronutrients into crops, especially when combined with nanotechnology, improving the benefits of conventional fertilization.

1. Introduction

Horticulture is defined as that branch of agriculture dedicated to the growth of plants for human consumption, medicine, and esthetic use (Stabenow et al., 2014). According to the latest studies carried out by Faostat (Food and Agriculture Organization of the United Nations, 2022), total horticulture production in 2020 was 329.86 million tons, which is an increase of about 2.93% with respect to 2019. This high demand has led to the need to create a massive plantation system, which is associated with the disadvantage of excessive soil depletion (Wainwright et al., 2014). This degradation makes the soil incapable of effective recycling of nutrients and energy, thus both have to be supplied by humans (Hazell and Wood, 2008).

There are many research lines aimed at solving this problem, providing alternatives such as using conservation tillage (Aqsa, 2021) or stopping the contamination of the soil with anthropogenic waste (Bullock, 1992). Nevertheless, the most commonly used method to avoid soil depletion is fertilization, as it is the fastest method (Hazell and Wood, 2008). The conventional use of fertilizers usually leads to an excess of nutrients, due to their low assimilation by the plants. This fact makes this method ineffective, since a large quantity of fertilizer is necessary to perceive an improvement in the soil (Kondraju and Rajan, 2019). For this reason, it is necessary to find an alternative that helps introducing nutrients into plants and improves the assimilation efficiency of the conventional method.

A possible alternative that could solve this drawback is the use of controlled-release materials that allow the nutrients to be supplied gradually. There are commercial plastics that are added to the soil to store and release micronutrients in a controlled manner: Nutricorte from Projar (Projar, 2020) and Multicote from Haifa (Haifa, 2020). However, these materials have problems due to the poor biodegradability of the plastics used, which compromises their use in horticulture (Manzano et al., 2019). Substituting these materials for bio-based bioplastics would provide clear advantages in terms of zero toxicity and high biodegradability, which makes them an attractive proposal for incorporating essential nutrients for the health and development of plants in horticulture (Akalin and Pulat, 2020a; Pimenta et al., 2022; Pulat and Yoltay, 2017).

Nutrients are elements needed by plants. Among them, although needed in small quantities, micronutrients are very important for the growth and development of plants (Alloway, 2008). There are seven essential micronutrients in horticulture: Iron (Fe), Copper (Cu), Boron (B), Zinc (Zn), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), and Chlorine (Cl). It is very important to meet the needs of these micronutrients in crops to have a satisfactory production of high quality materials (Alloway, 2008). The deficiency of these micronutrients

* Corresponding author.
E-mail address: mjimenez42@us.es (M. Jiménez-Rosado).

https://doi.org/10.1016/j.indcrop.2022.115128
Received 21 February 2022; Received in revised form 2 May 2022; Accepted 22 May 2022
Available online 27 May 2022

depends on the cultivation area, although, generally, the most frequent deficiencies are found for Zn, Fe, Cu, and Mn (López-Rayo et al., 2016). Zn is necessary to produce growth hormone and the elongation of internodes. In addition, it activates the enzymes responsible for certain proteins, it is used in the synthesis of chlorophyll and in the conversion of starches to sugars (plant energy) (Silva and Uchida, 2000). Cu activates certain enzymes involved in lignin synthesis and is essential for various enzyme systems (Smeets et al., 2009). It is also necessary in the process of photosynthesis and is crucial for plant respiration (Ishka et al., 2022). The role of Fe is key, as it is involved in the synthesis of chlorophyll and participates in other enzymatic, and metabolic processes without which plants cannot carry out their life cycle (Rahman et al., 2020). Finally, Mn participates in chlorophyll, vitamin, ATP, and lignin syntheses, nitrate assimilation, hormonal activation, and cell division (Schmidt and Husted, 2019). Therefore, it is very important to cover the deficiencies of these micronutrients without harming the production yield. Normally, to cover these deficiencies, micronutrients are incorporated into the soil as chelates or sulfate salts, with the latter being the most widely used. However, the high solubility of chelates and sulfates makes them inefficient (the amount assimilated by the plant is much lower than those that has to be incorporated into the soil), especially in irrigated crops (Ferrandon and Chamel, 1988). On the other hand, the use of nanoparticles (nanofertilization) is currently being investigated, as they can improve nutrient assimilation by plants (Zulfiqar et al., 2019). Furthermore, the nanoparticles also influence some metabolic processes in plants, helping them to mobilize nutrients for absorption. Finally, the cost of their application is usually lower, since they are required in small quantities (Seleiman et al., 2020).

In this way, the main objective of this work was to evaluate the use of soy protein-based matrices as controlled-release systems of micronutrients. In addition, the use of micro and nanoparticles was compared as micronutrient suppliers. To this end, Zn, Fe, Cu, and Mn sulfates (microparticles) and oxides (nanoparticles) were incorporated into soy protein-based systems. Later, the mechanical and functional (water uptake capacity, biodegradability, and micronutrient release) properties of the different systems were compared. Finally, the systems were evaluated in their use in crops. Thus, the novelty of this work is the combination of two potential technologies: controlled release biodegradable systems and nanoparticles, generating a symbiosis between both.

2. Material and methods

2.1. Materials

Soy protein isolate (SPI) was used as matrix to form the controlled release systems. This material, which has 91 wt% protein, was supplied by Protein Technologies International (Belgium). Glycerol, which was provided by Panreac Química S.A. (Spain) was utilized as plasticizer to develop the systems.

The micronutrients selected in this work were zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn). They were incorporated as micro and nanoparticles. The used microparticles were sulfates of each micronutrient supplied by Panreac Química S.A., which are often used to make up for their deficiencies in horticulture. On the other hand, the nanoparticles were micronutrient oxides which were synthetized in the lab by chemical colloidal precipitation (Chen et al., 2008). In this way, microparticles has a size of 500–1000 µm while nanoparticle size is 35 ± 2 nm (optimal nanoparticle size for plants (Eichert et al., 2008)).

2.2. Processing of controlled-release systems

The systems were obtained following the protocol described by Jiménez-Rosado et al. (2021c). This process consists of 5 different stages: mixing, injection molding, dehydrothermal treatment, immersion and freezing-dried.

Firstly, the raw materials (SPI, glycerol, and micro/nanoparticles) were homogenized in a Polylab QC rotating mixer (ThermoHaake, Germany). In order to compare the different systems, the percentage of micronutrient incorporated was always kept fixed at 3.63 wt% (maximum percentage of Zn that can be incorporated into the matrices by means of zinc sulfate (Jiménez-Rosado et al., 2018)), incorporating the amount of micro or nanoparticles necessary to achieve said percentage (Table 1). In addition, the SPI/glycerol ratio remained constant at 1:1. It is worth mentioning that a mixed system (mix) where the micronutrient percentage was supplied by a mixture of Zn, Fe, Mn, and Cu was also performed. That is, the percentage of 3.63 wt% is completed in equal parts with Zn, Fe, Cu, and Mn (0.90 wt% of each one). The raw materials were mixed for 10 min at 50 rpm under adiabatic conditions (temperature was always lower than 35 °C) to obtain homogenized blends.

During the injection molding, the obtained blends were subjected to 40 °C in the pre-chamber, from where they were injected at a pressure of 600 bars for 20 s into a rectangular-shape (60x10x1 mm^3) mold at 90 °C. This is kept in the mold during the holding (300 s) exerting a pressure of 200 bars to prevent the recoiling of the blend. Finally, the bioplastic matrices were demolded. These injection conditions were optimized in previous works (Jiménez-Rosado et al., 2018 for microparticles and Jiménez-Rosado et al., 2021b for nanoparticles).

Later, the bioplastic matrices were subjected to a dehydrothermal treatment at 50 °C for 24 h in a conventional oven to reinforce them. Subsequently, they were immersed in 300 mL of ethanol for 24 h in a closed vessel to remove the plasticizer (glycerol). Lastly, they were freeze-dried (0.01 mbar and −80 °C for 24 h) in LyoQuest equipment (Testlar, Spain) to obtain the matrices.

2.3. Characterization of controlled-release systems

2.3.1. Mechanical properties

Mechanical properties of the systems are important to verify their correct behavior during transport/storage and when they are buried in the cultivation soil. To this end, they were evaluated in dynamic compression mode. These measurements were performed in a dynamic-mechanical analyzer RSA3 (TA Instrument, USA) with a parallel plate tool (diameter: 8 mm). Frequency sweep tests were carried out from 0.1

Table 1
Content of the different matrices processed.

System		SPI (wt%)	Glycerol (wt%)	Salt (wt%)	Micronutrient (wt%)
Ref		50.0	50.0	–	–
Microparticles	Zn	45.0	45.0	10.0 ($ZnSO_4{\cdot}H_2O$)	3.63 (Zn^{2+})
	Fe	41.0	41.0	18.0 ($FeSO_4.7H_2O$)	3.63 (Fe^{2+})
	Cu	43.0	43.0	14.0 ($CuSO_4.5H_2O$)	3.63 (Cu^{2+})
	Mn	44.5	44.5	11.0 ($MnSO_4{\cdot}H_2O$)	3.63 (Mn^{2+})
	Mix	43.3	43.3	2.5 ($ZnSO_4{\cdot}H_2O$) 4.5 ($FeSO_4.7H_2O$) 3.5 ($CuSO_4.5H_2O$) 2.9 ($MnSO_4{\cdot}H_2O$)	0.90 (Zn^{2+}) 0.90 (Fe^{2+}) 0.90 (Cu^{2+}) 0.90 (Mn^{2+})
Nanoparticles	Zn	47.75	47.75	4.5 (ZnO)	3.63 (Zn^{2+})
	Fe	47.65	47.65	4.7 (FeO)	3.63 (Fe^{2+})
	Cu	47.75	47.75	4.5 (CuO)	3.63 (Cu^{2+})
	Mn	47.65	47.65	4.7 (MnO)	3.63 (Mn^{2+})
	Mix	47.7	47.7	1.1 (ZnO) 1.2 (FeO) 1.1 (CuO) 1.2 (MnO)	0.90 (Zn^{2+}) 0.90 (Fe^{2+}) 0.90 (Cu^{2+}) 0.90 (Mn^{2+})

to 20 Hz maintaining a constant strain (in the linear viscoelastic range), and temperature (25 ± 1 °C). The elastic modulus (E') of each system was evaluated with the change of frequency.

2.3.2. Water uptake capacity

One of the advantages of SPI matrices is their high water absorption capacity (Cuadri et al., 2017), which could serve as an extra reservoir of water to reduce the frequency of need for irrigation. This property was evaluated by immersing the system in 300 mL of distilled water for 24 h (ASTM D570-98, 2005). The water uptake capacity (WUC) was calculated with Eq. (1):

$$WUC \quad (\%) = \frac{Wet \quad matrix \quad weight - Dried \quad matrix \quad weight \quad after \quad immersion}{Dried \quad matrix \quad weight \quad after \quad immersion} \cdot 100 \tag{1}$$

In addition, soluble matter loss (SML) was also evaluated following Eq. (2):

$$SML \quad (\%) = \frac{Dried \quad matrix \quad weight - Dried \quad matrix \quad weight \quad after \quad immersion}{Dried \quad matrix \quad weight} \cdot 100 \tag{2}$$

2.3.3. Release analysis

The micronutrient release in a controlled manner is the main objective of these systems. It was determined using two different protocols. Firstly, it was evaluated in water following the protocol proposed by Essawy et al. (Essawy et al., 2016). To this end, the systems were immersed in 300 mL of distilled water. The micronutrient release was determined through the conductivity it caused in the medium at different time points, using a EC-Metro BASIC 30 device (Crison, Spain). On the other hand, release was also evaluated in soil following the protocol proposed by González et al. (González et al., 2015). In this way, systems were buried in soil in glass tubes (height: 40 cm, diameter: 20 mm) and irrigated with 20 mL of water every 24 h (simulating intensive horticultural irrigation of 20 L water/m^2 soil). The conductivity of the leachates collected in each irrigation was evaluated, as in the previous case, at different time points.

Conductivity is directly related to the release of salt. In this way, when it remains constant, it is because all the salt has been completely released. It is worth mentioning that a reference matrix (without any added salt) was also measured and used as a baseline in the different systems, subtracting its conductivity to only evaluate the release of the salt. In this way, the percentage of salt release was calculated, considering 100% release the maximum conductivity value, and represented over time.

2.3.4. Biodegradability

It is important that the systems present a correct biodegradability; in other words, they must be disintegrated after their use without generating toxic waste. In this way, the systems were buried in soil (2:1 farmland:compos) at room temperature and 70–80% RH. Thus, the samples were degraded by the soil microorganisms. The systems were unearthed at different days to evaluate them visually. The biodegradation time was estimated as the time after which the systems cannot be unearthed (no pieces larger than 1 mm were found).

2.3.5. Crop evaluation

Finally, the capacity of these systems to incorporate micronutrients in crops was evaluated. In this sense, lettuce (*Lactuca sativa*) and Italian sweet pepper (*Capsicum annum*) crops were used. Firstly, the seeds were germinated for 3 weeks to select similar specimens for testing. The germinated plants were transplanted into 2-liter pots, using a ratio 6:2:1 of commercial farmland, sand and vermiculite, respectively. Each plant was subjected to a different treatment, having 4 replicates of each treatment. For the treatments with the different matrices, one matrix was buried at 2 cm by the roots of the plant, using a single matrix per plant. In addition, a positive control (with conventional fertilization of each micronutrient which consists of pouring each microparticulate salt directly into the soil at a concentration of 5 g/m^2 every 14 days) and a negative control (without any fertilization) were also included as references in order to compare the effect of the matrices with conventionally fertilized and non-fertilized crops. All the plants were watered until saturation every two days, noting the volume of water needed. Finally, the different crops were evaluated after 40 (lettuce) and 70 (Italian sweet pepper) days. For this, they were unearthed and subsequently analyzed to measure their fresh weight, size (height x width, h x w), foliage (number of leaves), and color (measured by a Konika Minolta CM-700D spectrocolorimeter, obtaining chroma, tone, and clarity). Furthermore, the micronutrients assimilated by each plant were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). To this end, the plants were first acidly digested and, later, analyzed in an ICP SpectroBlue TI spectrometer (Spectro, Germany).

2.4. Statistical analysis

At least three replicates of each measure were performed (four in plants). Statistical analyses were performed using SPSS 18 (Windows), establishing a confidence level of 95% ($p < 0.05$). The results are presented as mean values and standard deviations.

3. Results and discussion

3.1. Mechanical properties

Fig. 1 shows the mechanical properties of the systems at compression mode with different micro (Fig. 1A) and nanoparticulate (Fig. 1B) micronutrients. A system without any micronutrient has been included as reference (Ref). As can be seen, all of them present a similar profile, where the elastic modulus (E') increases slightly with frequency. These results could indicate some instability in the systems when repeated compression forces are applied in short periods of time (high frequencies), such as during transport. Therefore, care should be taken in these actions to prevent the systems from being damaged. Furthermore, the loss tangent (viscous modulus / elastic modulus) of the systems is 0.2–0.3, which highlights their strong solid character.

The incorporation of any micronutrient significantly increases the E' values. This could be due to the reinforcement produced by

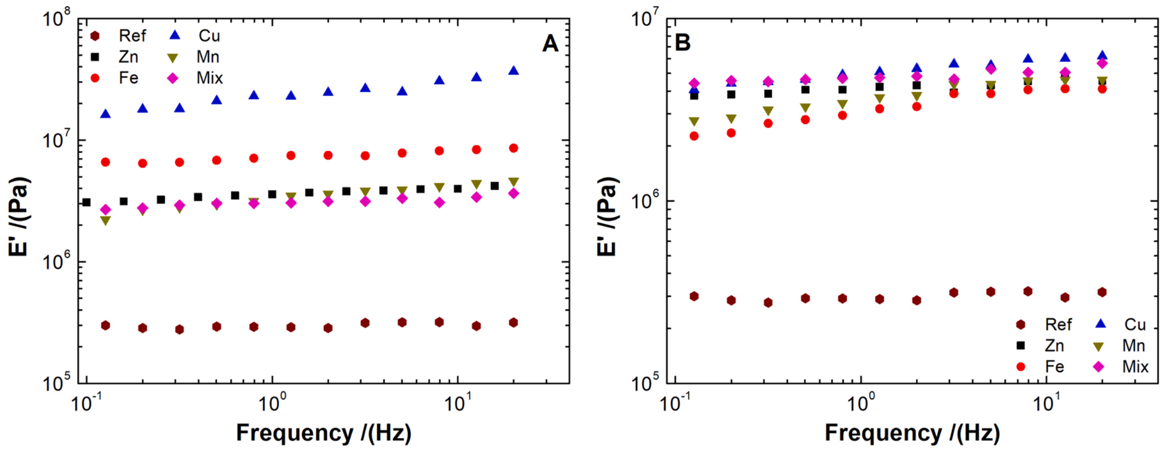

Fig. 1. Mechanical tests of systems with different (A) micro and (B) nano particles. A matrix without micro or nanoparticles has been included as reference (Ref).

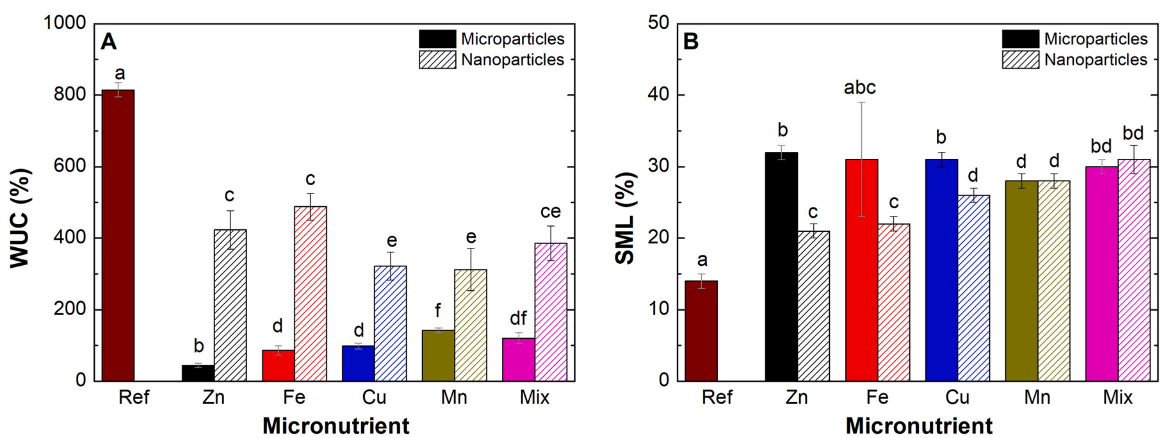

Fig. 2. (A) Water uptake capacity (WUC) and (B) soluble matter loss (SML) of systems with different micro and nanoparticles. A matrix without micro or nanoparticles has been included as reference (Ref). Different letter indicates significant differences ($p < 0.05$).

incorporating the salt (either micro or nanoparticulate). Nevertheless, the behavior is different for micro and nanoparticles. Thus, microparticles generate different reinforcements, which could be due to possible interactions between the metals (Zn, Fe, Cu or Mn) and the soy protein. In this way, copper achieves the highest E' values. However, these interactions seem not to manifest when nanoparticles are incorporated. Thus, the nanoparticulate systems do not show significant differences in E' values. In this way, nanoparticles act as a filler material unrelated to protein. This may be due to the strong interaction of oxides that do not split ionically to generate electrostatic interactions with protein chains.

3.2. Water uptake capacity

Fig. 2 shows the water uptake capacity (Fig. 2A) and soluble matter loss (Fig. 2B) of the systems with different micro and nanoparticles included. A system without any micronutrient has been included as reference (Ref). As can be seen, nanoparticles allow a better water uptake capacity (WUC) than microparticles, regardless of the

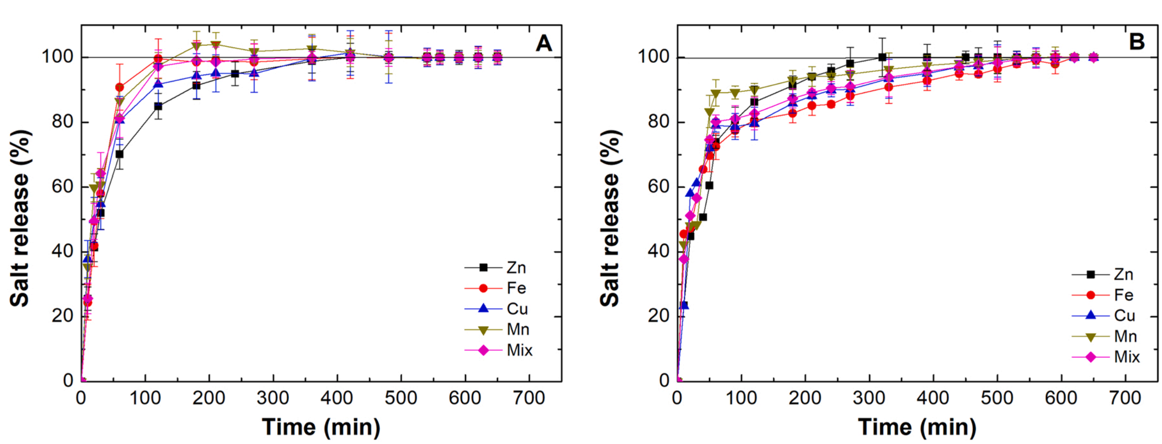

Fig. 3. Release profile in water of systems with different (A) micro and (B) nanoparticles.

micronutrient incorporated (c.a. 400 vs. 100% for nano and microparticulate systems, respectively). This behavior could be due to the higher electrostatic interactions generated by microparticulate salts, which inhibit systems to absorb water (Judawisastra et al., 2017). In this way, microparticles generated a conductivity in water of 150–300 μS/cm, while nanoparticles generated 20–60 μS/cm. These results show the better ability of the systems with nanoparticles incorporated to be used as a water reservoirs. This water reservoir allows rain or irrigation water that the plant has not taken to be stored and slowly provided when needed, which would allow it to stay longer without watering. Therefore, it would be an added value for these systems. Nevertheless, all the systems present lower WUC than reference system (without any salt), possibly because their structure is more compact by having salts as fillets.

Regarding the soluble matter loss (SML), all the systems present a higher SML than reference system, which indicates that they lost the salts incorporated a part of the soluble proteins. Although no great differences are observed, the systems that incorporated nanoparticles generally presented lower SML than those that incorporated microparticles, possibly due to the lower solubility of nanoparticles which are more difficult to release in water.

3.3. Release analysis

3.3.1. Release in water

The salt release analyses in water are presented in Fig. 3 for the systems with microparticles (Fig. 3A) and nanoparticles (Fig. 3B). Thus, the salt release was represented over time. All the systems presented the same release profile in water: a first quick release followed by a downhill drop until the release was completed. Nevertheless, the maximum release time only depends on the particle size, being similar for each micronutrient. Thus, microparticles generated a quicker release than nanoparticles (maximum release time: 450 and 530 min for micro and nanoparticles, respectively), possibly due to the higher solubility of sulfates (microparticles) than oxides (nanoparticles) (de Romaña et al., 2003). It is worth mentioning that microparticulate oxides cannot be used as fertilizers, due to their insolubility, that is, they cannot dissociate to be incorporated into plants (Wellburn, 1990). On the other hand, nanoparticulate oxides can be assimilated by plants due to their smaller size and surface instability (Rastogi et al., 2017).

3.3.2. Release in soil

Fig. 4 shows the salt release in soil over time of the different systems. Nevertheless, due to the low conductivity values produced by systems with nanoparticles incorporated, as well as their insolubility (which hinders their dragging with water), the results obtained by nanoparticles

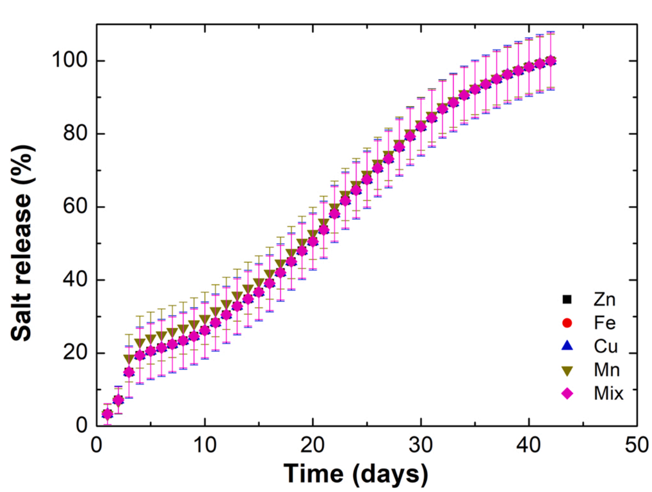

Fig. 4. Release profile in soil of systems with different microparticles.

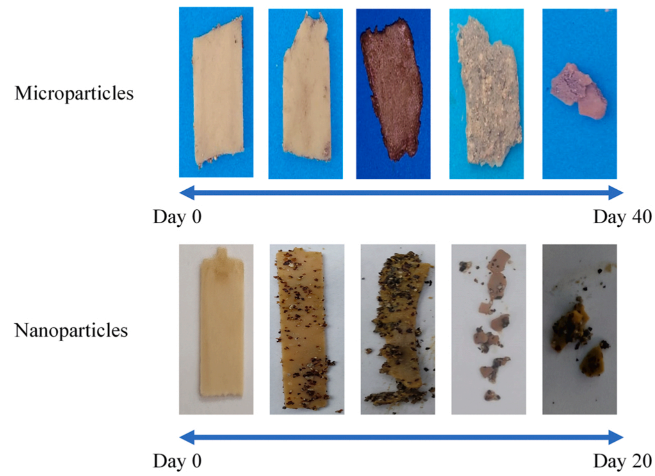

Fig. 5. Biodegradability of systems with different micro and nanoparticles.

systems do not have significant differences with respect to the target realized (sample only with land without any system incorporated). This effect can be seen from two points of view. On the one hand, it can be an advantage, since it does not contaminate groundwater. On the other hand, it could be a drawback if it is not assimilated by plants, causing contamination due to excess of nutrients in the soil.

Regarding the systems with microparticles incorporated, all the systems presented a similar profile where the first days (10 days) a slower release is observed, possibly because the salt has not yet reached the end of the leaching column. Then the release is faster until it decreases to reach the maximum release, assimilating to the profile observed in water, although in a longer time. Thus, the controlled release lasted for 40 days, being slower than in water as was previously predicted (Akalin and Pulat, 2020b). This release time is suitable for short cycle crops, such as lettuce, pepper, zucchini, and strawberries, since it could adapt throughout their growth.

3.4. Biodegradability

The visual appearance of the systems with micro and nanoparticles during biodegradation analysis is shown in Fig. 5. As can be seen, systems with nanoparticles biodegrade in less time (20 days) than systems with microparticles (40 days). This behavior could be due to the surface instability of the nanoparticles, causing their support medium (proteins) to degrade in order to seek a certain stability (Abdullah et al., 2020). It is worth mentioning that both matrices with micro and nanoparticles also biodegrade in less time than reference systems (without micronutrients) which need 60 days to biodegrade completely. This behavior has also been observed in a previous work (Jiménez-Rosado et al., 2021b).

On the other hand, a similar value was obtained when comparing biodegradation time with the maximum soil release time of systems with microparticles incorporated. Therefore, controlled release occurred throughout system biodegradation, possibly because the micronutrients interact with the protein-based matrix. If a similar behavior is predicted in systems with nanoparticles, the nutrients release is faster (20 days). However, as was previously mentioned, these would not be leached, thus they would still be assimilable by the plants.

3.5. Crop evaluation

Table 2 shows the data obtained from the evaluation of lettuce fertilized with the different systems. In addition, the visual appearance of the lettuce treated with the systems with different micro and nanoparticulate micronutrients can be observed in Fig. 6. Similarly, these results can be found in Table 3 and Fig. 7 for Italian sweet peppers. As can be seen, the fertilization improved the quality of both crops, since

Table 2
Micronutrient assimilation and crop physiology after using the different systems in lettuces.

Particle	Micronutrient	Micronutrient content (mg/kg)	Crop weight (g)	Number of leaves	Leaf size (h x w) cm	Color		
						Chroma	Tone	Clarity
Positive control	Zn/Fe/Cu/Mn	35.6/235.9/4.9/35.6	63.3 ± 1.4	16 ± 1	15.6 × 10.1	28.2 ± 3.0	-67.7 ± 8.8	46.6 ± 1.4
Micro	Zn	155.3	62.5 ± 5.2	14 ± 1	14.6 × 5.6	25.6 ± 1.5	-66.6 ± 2,8	42.3 ± 2.1
	Fe	263.2	92.7 ± 2.7	17 ± 1	21.0 × 7.9	27.2 ± 1.2	-66.8 ± 2.7	45.6 ± 0.7
	Cu	5.9	63.3 ± 5.5	18 ± 1	14.1 × 6.8	25.4 ± 1.2	-65.3 ± 3.2	43.8 ± 0.1
	Mn	128.7	70.9 ± 2.2	17 ± 1	22.2 × 8.2	21.8 ± 1.6	-64.9 ± 5.0	38.0 ± 0.8
	Mix	55.2/618.2/11.9/79.9	59.8 ± 0.8	21 ± 1	22.3 × 7.1	27.3 ± 1.0	-68.4 ± 1.1	44.2 ± 0.2
Nano	Zn	167.2	77.2 ± 0.7	17 ± 3	16.0 × 9.8	29.5 ± 4.1	-68.6 ± 4.8	45.8 ± 5.0
	Fe	340,5	108.2 ± 3.3	21 ± 2	20.5 × 10.1	29.7 ± 0.8	-67.9 ± 2.9	46.3 ± 0.1
	Cu	7.9	80.2 ± 2.1	19 ± 2	15.1 × 6.5	33.8 ± 1.9	-72.4 ± 5.3	52.3 ± 1.7
	Mn	135.9	90.2 ± 3.4	21 ± 1	15.4 × 7.8	26.3 ± 0.6	-67.5 ± 1.3	44.5 ± 1.6
	Mix	55.8/963.2/18.9/93.2	81.1 ± 1.4	25 ± 1	22.4 × 10.4	27.8 ± 0.4	-68.4 ± 1.1	45.4 ± 0.1
Negative control	–	15.5/74.4/4.2/25.3	8.8 ± 0.5	10 ± 1	13.0 × 4.2	26.4 ± 2.2	-67.5 ± 2.7	43.7 ± 0.8

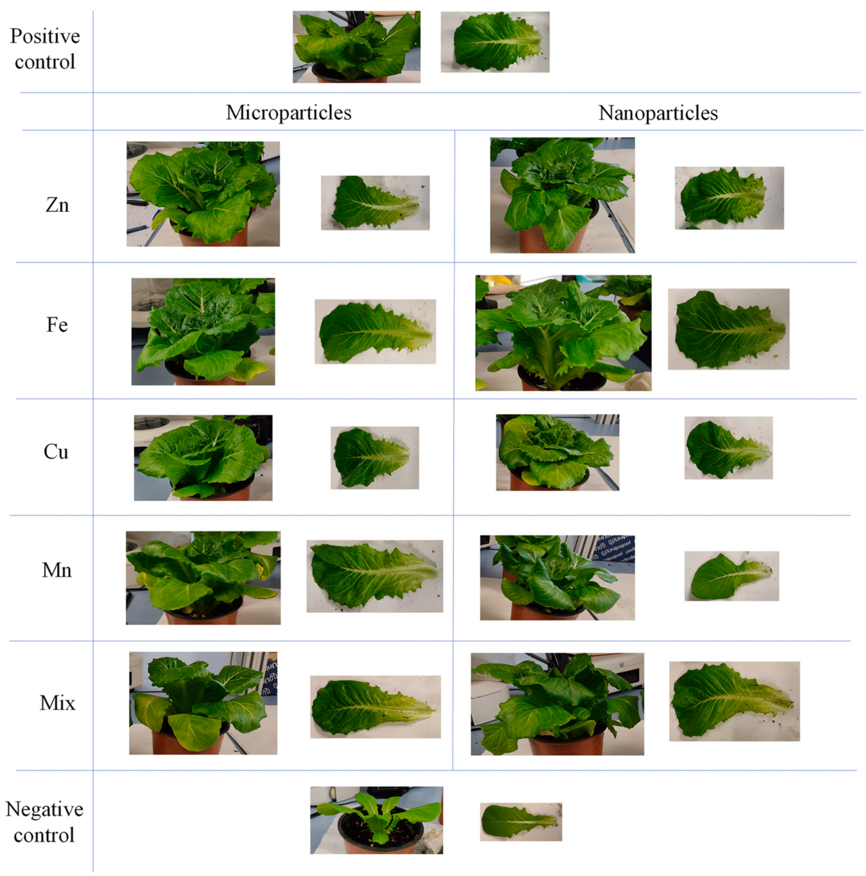

Fig. 6. Visual appearance of lettuces obtained after treatment with matrices. Positive (conventional fertilization) and negative (without fertilization) controls were also included.

negative control obtained the worst results (lowest micronutrient content, weight and dimensions).

Regarding the different fertilization methods, micronutrient content is higher in the crops fertilized with the controlled release systems than with conventional fertilization, most likely enhanced by continued exposure of the micronutrient, which allows for controlled release. Furthermore, nanoparticles are better assimilated by lettuces and peppers than microparticles. This effect could be due to the size of the nanoparticles, which can be better assimilated by the pores of the plants (Khan et al., 2019), in addition to the lower leachate, which allows it to be available for crops for a longer time. This higher micronutrient assimilation is also observed in the plant physiology. In this sense, lettuces and peppers grown with controlled release systems had higher weight and dimensions, and a greater number of leaves, being especially superior when nanoparticles are used instead of microparticles. All this can be observed in Figs. 6 and 7, where lettuces and peppers treated by nanoparticles are larger and leafier. Furthermore, all plants reflect a green color in their color parameters, which indicates their correct formation of chlorophyll, an indicator that the plant is developing correctly (Palta, 1990). However, those plants fertilized with nanoparticles showed a green color more akin to chlorophyll pigments (higher chroma and clarity, and lower tone), which shows that this fertilization favors their synthesis, allowing the plant to obtain a greater amount of energy, and therefore, a further growth, as mentioned before.

On the other hand, the mixture of micronutrient in the controlled release systems has a different behavior in lettuce and peppers. In the former, this fertilization improves the conventional method. However, the mixed system favors iron and copper assimilation, while zinc and

Table 3
Micronutrient assimilation and crop physiology after using the different systems in Italian sweet peppers.

Particle	Micronutrient	Micronutrient content (mg/kg)	Crop weight (g)	Number of leaves	Leaf size (h x w) cm	Color		
						Chroma	Tone	Clarity
Positive control	Zn/Fe/Cu/Mn	41.1/1519.9/14.1/128.8	21.1 ± 1.0	11 ± 1	12.1 × 5.2	27.6 ± 0.4	-72.8 ± 5.0	46.5 ± 0.7
Micro	Zn	71.0	28.1 ± 1.4	10 ± 1	12.6 × 6.4	23.3 ± 1.4	-64.3 ± 4.8	40.8 ± 0.9
	Fe	1945.4	15.3 ± 2.4	11 ± 1	15.9 × 7.5	24.0 ± 0.1	-71.9 ± 1.4	40.8 ± 0.9
	Cu	20.8	24.6 ± 2.9	12 ± 2	15.9 × 7.5	29.3 ± 1.3	-73.3 ± 5.4	42.6 ± 0.7
	Mn	179.6	16.1 ± 1.0	10 ± 1	12.7 × 5.1	26.9 ± 1.5	-72.4 ± 5.5	43.8 ± 0.4
	Mix	–	–	–	–	–	–	–
Nano	Zn	82.3	32.6 ± 0.7	12 ± 1	17.6 × 6.6	26.6 ± 0.2	-67.7 ± 3.0	41.9 ± 0.1
	Fe	11,150.5	40.4 ± 1.4	15 ± 1	19.3 × 9.7	28.7 ± 0.2	-73.9 ± 1.0	47.4 ± 0.2
	Cu	670.4	57.7 ± 1.3	13 ± 1	18.4 × 9.5	32.5 ± 1.9	-77.9 ± 0.3	45.5 ± 0.1
	Mn	262.4	29.5 ± 3.1	14 ± 1	16.3 × 8.0	29.0 ± 1.2	-75.0 ± 4.7	48.9 ± 1.6
	Mix	59.9/2925.0/16.8/130.5	8.9 ± 0.2	7 ± 1	9.4 × 4.2	30.7 ± 0.7	-69.8 ± 1.8	45.5 ± 0.6
Negative control	–	8.7/480.9/4.3/56.2	14.2 ± 2.9	9 ± 1	10.0 × 5.2	27.5 ± 0.1	-69,5 ± 4.0	41.9 ± 0.1

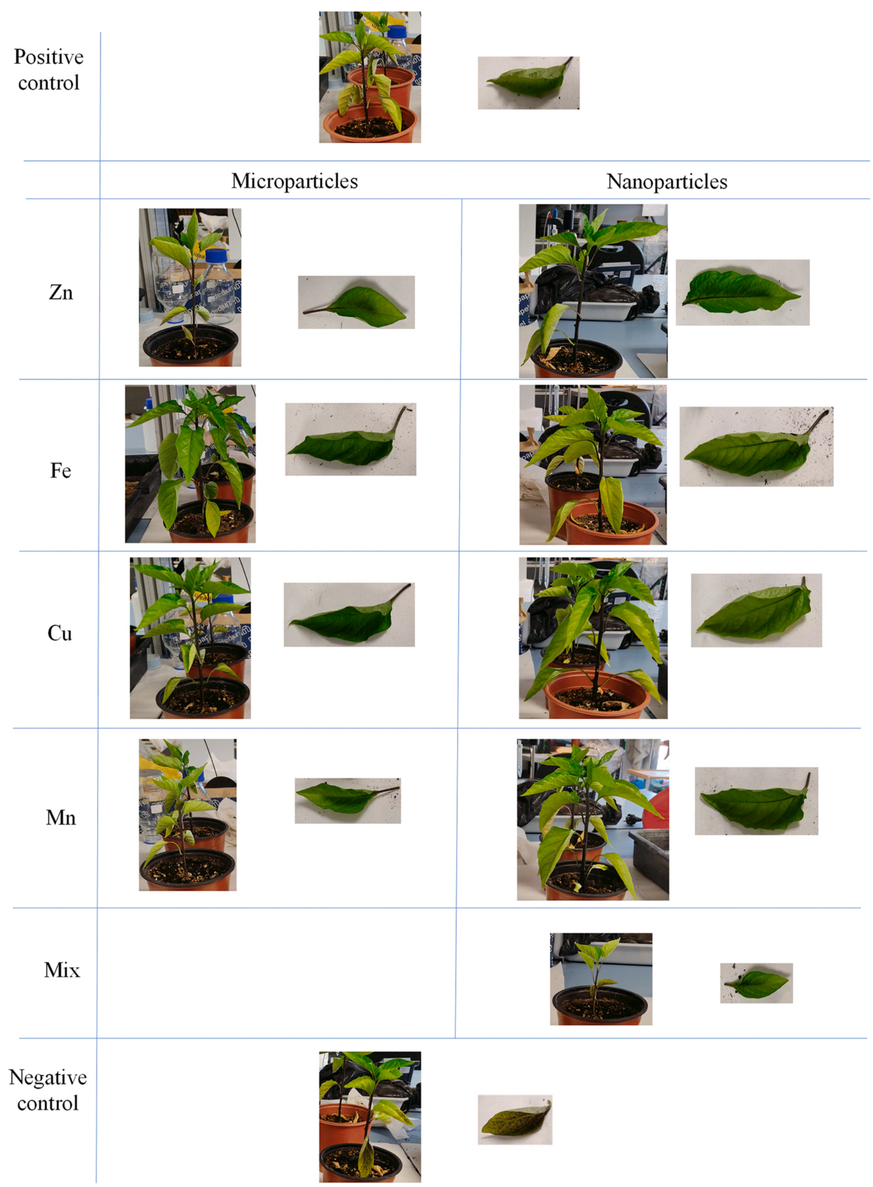

Fig. 7. Visual appearance of Italian sweet peppers obtained after treatment with matrices. Positive (conventional fertilization) and negative (without fertilization) controls were also included.

manganese are blocked, probably by the other two incorporated micronutrients. For this reason, although more nourished and larger lettuces are obtained than in conventional fertilization, there is no synergy between the different micronutrients to achieve a complete enhancement. This micronutrient blockage was observed to a greater extent in peppers, where those fertilized with microparticulate systems could not even be obtained. Similarly, peppers fertilized with nanoparticulate systems were smaller than the conventional ones.

On the other hand, the water uptake capacity shown by controlled-release systems made it possible to reduce the need for irrigation by 33% when the microparticles were incorporated. Similar results were obtained in previous studies (Jiménez-Rosado et al., 2021a). In the case of nanoparticles, the greater water uptake capacity of the systems allowed them to further reduce the need for irrigation by up to 50%, being a potential water reservoir that could increase the functionality of these systems.

4. Conclusion

To sum up, it is possible to achieve a controlled fertilization of micronutrients from soy protein-based systems, being more effective than conventional fertilization by obtaining more nourished, larger and less polluting crops, as they biodegrade into non-toxic substances after use. In this way, soy protein-based systems with suitable mechanical and functional properties could be processed with different micro and nanoparticulate micronutrients. Nevertheless, systems with nanoparticles presented better characteristics: higher water uptake capacity, slower release and longer settling time in the soil. All this allowed for a better assimilation of micronutrients in the crops, which translated into their larger size. In addition, a greater reduction of irrigation can be obtained as an added value of these systems. In this sense, this study opens the door to a novel, more efficient and less polluting fertilization that could be transferred to large-scale crops in future works.

CRediT authorship contribution statement

Mercedes Jiménez-Rosado: Conceptualization, Methodology, Formal analysis, Data curation, Writing. **Victor Perez-Puyana:** Methodology, Formal analysis, Data curation, Writing. **Antonio Guerrero:** Validation, Resources, Funding acquisition. **Alberto Romero:** Conceptualization, Validation, Resources, Writing, Supervision.

Declaration of Competing Interest

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

Acknowledgments

This work is part of the RTI20180-097100-B-C21 project which is sponsored by MCI/AEI/FEDER, EU from Spanish Government. The authors acknowledge their financial support. In addition, the authors thank MEFP from Spanish Government for the predoctoral grant of Mercedes Jiménez Rosado (FPU2017/01718), and Junta de Andalucía and Universidad de Sevilla for the postdoctoral grant of Victor Perez-Puyana (Talento Doctores, Junta de Andalucía con Fondo Social Europeo, Convocatoria 2019–20, DOC_00586). Graphical abstract created with BioRender.com.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.115128.

References

Abdullah, A.H.D., Putri, O.D., Fikriyyah, A.K., Nissa, R.C., Hidayat, S., Septiyanto, R.F., Karina, M., Satoto, R., 2020. Harnessing the excellent mechanical, barrier and antimicrobial properties of zinc oxide (ZnO) to improve the performance of starch-based bioplastic. Polym. Technol. Mater. 59, 1259–1267. https://doi.org/10.1080/25740881.2020.1738466.

Akalin, G.O., Pulat, M., 2020a. Controlled release behavior of zinc-loaded carboxymethyl cellulose and carrageenan hydrogels and their effects on wheatgrass growth. J. Polym. Res. 27, 6. https://doi.org/10.1007/s10965-019-1950-y.

Akalin, G.O., Pulat, M., 2020b. Preparation and characterization of κ-carrageenan hydrogel for controlled release of copper and manganese micronutrients. Polym. Bull. 77, 1359–1375. https://doi.org/10.1007/s00289-019-02800-4.

Alloway, B.J., 2008. Micronutrient Deficiencies in Global Crop Production. Spinger Science+Business Media, Berlin. https://doi.org/10.1007/978-1-4020-6860-7.

Aqsa, N.K., 2021. A review on concerns about soil quality and innovative methods for improving soil health. Emerg. Life Sci. Res. 7, 5–13. https://doi.org/10.31783/elsr.2021.720513.

ASTM D570-98, 2005. Standard Test Method for Water Absorption of Plastics.

Bullock, D.G., 1992. Crop rotation. CRC Crit. Rev. Plant Sci 309–326. https://doi.org/10.1080/07352689209382349.

Chen, C.C., Liu, P., Lu, C.H., 2008. Synthesis and characterization of nano-sized ZnO powders by direct precipitation method. Chem. Eng. J. 144, 509–513. https://doi.org/10.1016/j.cej.2008.07.047.

Cuadri, A.A., Romero, A., Bengoechea, C., Guerrero, A., 2017. Natural superabsorbent plastic materials based on a functionalized soy protein. Polym. Test. 58, 126–134. https://doi.org/10.1016/j.polymertesting.2016.12.024.

Eichert, T., Kurtz, A., Steiner, U., Goldbach, H.E., 2008. Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. Physiol. Plant 134, 151–160.

Essawy, H.A., Ghazy, M.B.M., El-Hai, F.A., Mohamed, M.F., 2016. Superabsorbent hydrogels via graft polymerization of acrylic acid from chitosan-cellulose hybrid and their potential in controlled release of soil nutrients. Int. J. Biol. Macromol. 89, 144–151. https://doi.org/10.1016/j.ijbiomac.2016.04.071.

Ferrandon, M., Chamel, A.R., 1988. Cuticular retention, foliar absorption and translocation of Fe, Mn and Zn supplied in organic and inorganic form. J. Plant Nutr. 11, 247–263. https://doi.org/10.1080/01904168809363800.

Food and Agriculture Organization of the United Nations, 2022. Data on Food and Agriculture [WWW Document]. URL: ⟨http://www.fao.org/⟩. (Accessed 28 March 2022).

González, M.E., Cea, M., Medina, J., González, A., Diez, M.C., Cartes, P., Monreal, C., Navia, R., 2015. Evaluation of biodegradable polymers as encapsulating agents for the development of a urea controlled-release fertilizer using biochar as support material. Sci. Total Environ. 505, 446–453. https://doi.org/10.1016/j.scitotenv.2014.10.014.

Haifa, 2020. Multicote Micro [WWW Document]. URL: ⟨https://www.haifa-group.com/es/multicote-micro-0⟩. (Accessed 17 August 2020).

Hazell, P., Wood, S., 2008. Drivers of change in global agriculture. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2007.2166.

Ishka, M.R., Chia, J.-C., Vatamaniuk, O.K., 2022. Advances in understanding of copper function and transport in plants. In: Cation Transporters in Plants. Elsevier, pp. 205–226. https://doi.org/10.1016/B978-0-323-85790-1.00021-X.

Jiménez-Rosado, M., Perez-Puyana, V., Cordobés, F., Romero, A., Guerrero, A., 2018. Development of soy protein-based matrices containing zinc as micronutrient for horticulture. Ind. Crop. Prod. 121, 345–351. https://doi.org/10.1016/j.indcrop.2018.05.039.

Jiménez-Rosado, M., Perez-Puyana, V., Guerrero, A., Romero, A., 2021a. Controlled release of zinc from soy protein-based matrices to plants. Agronomy 11, 580. https://doi.org/10.3390/agronomy11030580.

Jiménez-Rosado, M., Perez-Puyana, V., Sánchez-Cid, P., Guerrero, A., Romero, A., 2021b. Incorporation of zno nanoparticles into soy protein-based bioplastics to improve their functional properties. Polymers 13. https://doi.org/10.3390/polym13040486.

Jiménez-Rosado, Mercedes, Rubio-Valle, J.F., Perez-Puyana, V., Guerrero, A., Romero, A., 2021c. Eco-friendly protein-based materials for a sustainable fertilization in horticulture. J. Clean. Prod. 286, 124948 https://doi.org/10.1016/j.jclepro.2020.124948.

Judawisastra, H., Sitohang, R.D.R., Marta, L., Mardiyati, 2017. Water absorption and its effect on the tensile properties of tapioca starch/polyvinyl alcohol bioplastics. IOP Conf. Ser. Mater. Sci. Eng. 223. https://doi.org/10.1088/1757-899X/223/1/012066.

Khan, M.R., Adam, V., Rizvi, T.F., Zhang, B., Ahamad, F., Jośko, I., Zhu, Y., Yang, M., Mao, C., 2019. Nanoparticle–plant interactions: two-way traffic. Small 15, 1901794. https://doi.org/10.1002/smll.201901794.

Kondraju, T.T., Rajan, K.S., 2019. Excessive fertilizer usage drives agriculture growth but depletes water quality. ISPRS Ann. Photogramm. Remote Sens. Spat. Inf. Sci. IV-3/W1, 17–23. https://doi.org/10.5194/isprs-annals-IV-3-W1-17-2019.

López-Rayo, S., Nadal, P., Lucena, J.J., 2016. Novel chelating agents for iron, manganese, zinc, and copper mixed fertilisation in high pH soil-less cultures. J. Sci. Food Agric. 96, 1111–1120. https://doi.org/10.1002/jsfa.7183.

Manzano, V., García, N.L., Ramírez, C.R., D'Accorso, N., Goyanes, S., 2019. Mulch plastic systems: recent advances and applications. In: Polymers for Agri-Food Applications. Springer International Publishing, Cham, pp. 265–290. https://doi.org/10.1007/978-3-030-19416-1_14.

Palta, J.P., 1990. Leaf chlorophyll content. Remote Sens. Rev. 5, 207–213. https://doi.org/10.1080/02757259009532129.

Pimenta, I.F., Figueiredo, L.R.F., Santos, A.M.C., Oliveira, J.E., Medeiros, E.S., 2022. Development of controlled release fertilizer systems for <scp>KCl</scp> using glycerol-based polymers. J. Appl. Polym. Sci. 139, 51747. https://doi.org/10.1002/app.51747.

Projar, 2020. Nutricote: Controlled Release Fertilizer [WWW Document]. URL: ⟨https://www.projar.es/productos/productos-hortofruticultura-jardineria/fertilizantes/abonos_minerales/fertilizantes-de-liberacion-controlada/fertilizante-de-liberacion-controlada-nutricote/⟩. (Accessed 17 August 2020).

Pulat, M., Yoltay, N., 2017. Smart fertilizers: preparation and characterization of gelatin-based hydrogels for controlled release of MAP and AN fertilizers. Agrochimica 60, 249–261. https://doi.org/10.12871/00021857201641.

Rahman, R., Sofi, J.A., Javeed, I., Malik, T.H., Nisar, S., 2020. Role of micronutrients in crop production. Int. J. Curr. Microbiol. Appl. Sci. 11, 2265–2287.

Rastogi, A., Zivcak, M., Sytar, O., Kalaji, H.M., He, X., Mbarki, S., Brestic, M., 2017. Impact of metal and metal oxide nanoparticles on plant: a critical review. Front. Chem. 5, 78. https://doi.org/10.3389/fchem.2017.00078.

de Romaña, D.L., Lönnerdal, B., Brown, K.H., 2003. Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. Am. J. Clin. Nutr. 78, 279–283. https://doi.org/10.1093/ajcn/78.2.279.

Schmidt, Husted, 2019. The biochemical properties of manganese in plants. Plants 8, 381. https://doi.org/10.3390/plants8100381.

Seleiman, M.F., Almutairi, K.F., Alotaibi, M., Shami, A., Alhammad, B.A., Battaglia, M.L., 2020. Nano-fertilization as an emerging fertilization technique: why can modern agriculture benefit from its use? Plants 10, 2. https://doi.org/10.3390/plants10010002.

Silva, J., Uchida, R., 2000. Essential nutrients for plant growth. Plant Nutrition Management in Hawaii's Soils, Approaches and Application to Tropical and Subtropical Agriculture. CTAHR Editorial, Hawaii.

Smeets, K., Opdenakker, K., Remans, T., Van Sanden, S., Van Belleghem, F., Semane, B., Horemans, N., Guisez, Y., Vangronsveld, J., Cuypers, A., 2009. Oxidative stress-related responses at transcriptional and enzymatic levels after exposure to Cd or Cu in a multipollution context. J. Plant Physiol. 166, 1982–1992. https://doi.org/10.1016/j.jplph.2009.06.014.

Stabenow, Debbie, Farm Bill Act, Stabenow, Debbie, 2014. Agricultural Act of 2014. US House Represent.

Wainwright, H., Jordan, C., Day, H., 2014. Environmental impact of production horticulture. In: Horticulture: Plants for People and Places, Vol. 1. Springer Netherlands, Dordrecht, pp. 503–522. https://doi.org/10.1007/978-94-017-8578-5_15.

Wellburn, A.R., 1990. Tansley review no. 24 why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? New Phytol. 115, 395–429. https://doi.org/10.1111/j.1469-8137.1990.tb00467.x.

Zulfiqar, F., Navarro, M., Ashraf, M., Akram, N.A., Munné-Bosch, S., 2019. Nanofertilizer use for sustainable agriculture: advantages and limitations. Plant Sci. 289, 110270 https://doi.org/10.1016/j.plantsci.2019.110270.